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# A note on the usage of Likert Scaling for research data analysis

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**Abstract**

In this paper, biases on some used known method adapting Likert Scaling are shown. Then, an improvement of the method to correct or minimize the bias is presented in order to have better interpretation of the result of the analysis of the data.

**Keywords:** bias, bipolar scaling, difference, Likert Scale, psychometric scale, rating scale, statistical tool.

## Introduction

Likert scaling is a psychometric scale commonly used in questionnaires and is the most widely used scale in survey researches particularly in social science researches. Invented by a psychologist named Rensis Likert, he sought to find effective and systematic means of studying human attitudes and the factors that influence them. His research led him to develop a scale for attitude measurement which now bears his name. This term is interchangeably used with rating scale even though the two are not synonymous. If a respondent wanted to respond to a Likert questionnaire item, this respondent specifies a level of agreement to a particular statement.

Likert scale received a wide range of acceptance. It is used not only in psychology and marketing researches but also in other areas: education (Anonymous 1998), medicine (Grant et al 1999), nutrition (Lindhorst et al 2007), nursing (Seal 2007), and other fields in finance, engineering, and human study.

The scale is used either as a summated scale or as an individual scale item. When used as a summated scale, each item is summated to produce an index. Individual scale items are used in eliciting latent variables, such as in Structural Equation Modeling.

A simple statement in which the respondent is asked to evaluate according to any kind of subjective or objective criteria is a Likert item. Generally it is a measure of the level of agreement or disagreement on the particular statement. Odd numbers of response categories (five, seven, and nine) are usually used, as well as the least popular even number of responses which are called force-number Likert scale. Four to six numbers of responses are used in this technique. The odd number of three response categories can also be used (Jacoby & Matell 1971). However, often in practice, five ordered response levels are used although many psychometricians suggest using seven or nine levels. The reason for this is based on a recent empirical study by Dawes (2008) that a 5- or 7- point scale may produce slightly higher mean scores relative to the highest possible attainable score, compared to those produced from a 10-point scale, Further, he stressed that this difference was statistically significant. On the other hand, In terms of the other data characteristics, there was very little difference among the scale formats in terms of variation about the mean, skewness or kurtosis. The format of a typical five-level Likert item is: 1. Strongly Disagree, 2. Disagree, 3.Neither Agree nor Disagree (undecided or neutral), 4. Agree, 5. Strongly agree.

Likert scaling is a bipolar scaling method, measuring either positive or negative response to a statement. Likert scales may be subject to distortion from several causes. Respondents may avoid using extreme response categories (central tendency bias); agree with statements as presented (acquiescence bias); or try to portray themselves or their organization in a more favorable light (social desirability bias). Designing a scale with balanced keying (an equal number of positive and negative statements) can obviate the problem of acquiescence bias, since acquiescence on positively keyed items will balance acquiescence on negatively keyed items, but central tendency and social desirability are somewhat more problematic.

In this study, the author's interest is presented in the way the Likert scale is applied to descriptive interpretation after it had underwent several stages of summing up and related procedures of the central tendency measures.

## Scoring and analysis

If a questionnaire is completed, each item may be analyzed separately or in some cases item responses may be summed to create a score for a group of items.

An issue on whether Likert items maybe considered an ordinal level data or an interval level data is a subject of disagreement. In most practice, they are considered ordinal or interval level data, many regard such items as ordinal data because at only five levels, one cannot assume that respondents' perceived all pairs of adjacent levels as equidistant. On the other hand, often the wording of response levels clearly implies symmetry of response levels about a middle category; at the very least, such an item would fall between ordinal- and interval-level measurements; to treat it as merely ordinal would lose information. Further, if the item is accompanied by a visual analog scale, where equal spacing of response levels is clearly indicated, the argument for treating it as interval-level data is even stronger.

If considered as an ordinal data, Likert responses maybe displayed in a graph particularly bar charts. Its center being median or the mode but not the mean. Its spread (variability) uses the range across quartiles but not the standard deviation since mean and standard deviation are inappropriate measures for ordinal data (Jamieson 2004); analysis uses non-parametric tests such as the chi-square test, Mann–Whitney test, Wilcoxon signed-rank test, or Kruskal–Wallis test. If guaranteed by the Central Limit Theorem that ordinary averages of the Likert scale data behave normally, parametric analysis maybe performed; however, some would disagree that ordinary averages should be used instead for Likert scale data.

Responses to several Likert questions may be summed with the assumption that all questions use the same Likert scale and that the scale is a defendable approximation to an interval scale, in which case they may be treated as interval data measuring a latent variable. Hence, parametric statistical tests such as the analysis of variance maybe applied. Note these can be only applied when more than five Likert questions are summed.

**Level of measurement**

The five response categories are often believed to represent an interval level of measurement. But this can only be the case if the intervals between the scale points correspond to empirical observations in a metric sense. We bear in mind that treating ordinal scales as an interval scales has long been controversial; however, in principle it will be used as a basis for obtaining interval level estimates on a continuum by applying item response models. In polytomous Rasch model, data maybe obtained that fits this model. In addition, the polytomous Rasch model permits testing of the hypothesis that the statements reflect increasing levels of an attitude or trait, as intended. For example, application of the model often indicates that the neutral category does not represent a level of attitude or trait between the "Disagree" and "Agree" categories. Again, not every set of Likert scaled items maybe used for Rasch measurement. The data has to be thoroughly checked to fulfill the strict formal axioms of the model (www.rasch-analysis.com/rasch-model-specification.htm).

**Some applications**

When items are Likert scaled and assumed to have interval measurement, often in practice, the information to all the respondents are summarized in the form of weighted mean. It then interpreted using an interval which in turn corresponds to a verbal description. For example, a recent research in the common errors committed by nursing students in the clinical exposure (Jamora et al, Table 1):

Table 1. Perceived responses on the common clinical errors among the nursing students, Jamora et al, 2010.

| Common clinical errors | Weighted mean |
|---|---|
| 1. Wrong dosage | 1.00 |
| 2. Wrong time | 1.75 |
| 3. Failure to do a procedure correctly | 2.25 |
| 4. Failure to check the equipment | 2.00 |
| 5. Able to break a sterile field | 2.75 |

Table 1a used a Likert Scale in the following manner: 1. Never, 2. Rare, 3. Sometimes, 4. Often and 5. Always. Giving a verbal description, one has to create an interval of means in order to give interpretations for the weighted mean. Results of most studies treat the problem in this manner. Some authors construct a legend indicating description that runs like the table below.

| Likert Scale | Interval | Description |
|---|---|---|
| 1 | 1.00-1.50 | Never |
| 2 | 1.51-2.50 | Rare |
| 3 | 2.51-3.50 | sometimes |
| 4 | 3.51-4.50 | often |
| 5 | 4.51-5.00 | always |

The table 1 would be then presented as

Table 1b. Perceived responses on the common clinical errors among the nursing students, Jamora et al, 2010.

| Common clinical errors | Weighted mean | Description |
|---|---|---|
| 1. Wrong dosage | 1.00 | Never |
| 2. Wrong time | 1.75 | Rare |
| 3. Failure to do a procedure correctly | 2.25 | Rare |
| 4. Failure to check the equipment | 2.00 | Rare |
| 5. Able to break a sterile field | 2.75 | Sometimes |

However, the kind of interval given above is biased in the sense that the difference in the upper limit and lower limit of the first and last intervals is much lower as compared to the three middle intervals (as shown in the table below). This observation will result to items having more weighted mean in the middle.

| Likert Scale | Interval | Difference | Description |
|---|---|---|---|
| 1 | 1.00-1.50 | 0.50 | Never |
| 2 | 1.51-2.50 | 0.99 | Rare |
| 3 | 2.51-3.50 | 0.99 | sometimes |
| 4 | 3.51-4.50 | 0.99 | often |
| 5 | 4.51-5.00 | 0.49 | always |

To correct this problem, the author reduced or eliminated the bias, by making the difference in each interval to have a uniform difference; hence, the new interval given below have a constant uniform difference in each interval. That is,

| Likert Scale | Interval | Difference | Description |
|---|---|---|---|
| 1 | 1.00-1.79 | 0.79 | Never |
| 2 | 1.80-2.59 | 0.79 | Rare |
| 3 | 2.60-3.39 | 0.79 | sometimes |
| 4 | 3.40-4.19 | 0.79 | often |
| 5 | 4.20-5.00 | 0.80 | always |

Thus, Table 1b will have new description for item 2 (from rare to never) that is,

Table 1c. Perceived responses on the common clinical errors among the nursing students, Jamora et al, 2010.

| Common clinical errors | Weighted | Description mean |
|---|---|---|
| 1. Wrong dosage | 1.00 | Never |
| 2. Wrong time | 1.75 | Never |
| 3. Failure to do a procedure correctly | 2.25 | Rare |
| 4. Failure to check the equipment | 2.00 | Rare |
| 5. Able to break a sterile field | 2.75 | Sometimes |

The correction of the bias in the interval making the difference uniform changes the descriptive interpretation of the item. Thus, a recommendation for three, four, and seven response categories Likert scale "How good do you think was Macapagal-Arroyo as president?" The following may be studied:

Table 1d. Perceived responses on the common clinical errors among the nursing students, Jamora et al, 2010.

| Likert Scale | Interval | Difference | Description |
|---|---|---|---|
| 1 | 1.00-1.66 | 0.66 | Bad |
| 2 | 1.67-2.33 | 0.66 | Undecided |
| 3 | 2.34-3.00 | 0.66 | Good |
| Likert Scale | Interval | Difference | Description |
| 1 | 1.00-1.75 | 0.75 | Very bad |
| 2 | 1.76-2.51 | 0.75 | Bad |
| 3 | 2.52-3.27 | 0.75 | Good |
| 4 | 3.28-4.00 | 0.72 | Very good |
| Likert Scale | Interval | Difference | Description |
| 1 | 1.00-1.85 | 0.85 | Very bad |
| 2 | 1.86-2.71 | 0.85 | Rather bad |
| 3 | 2.72-3.57 | 0.85 | Bad |
| 4 | 3.58-4.43 | 0.85 | Neither good nor bad |
| 5 | 4.44-5.29 | 0.85 | Good |
| 6 | 5.30-6.15 | 0.85 | Rather good |
| 7 | 6.16-7.00 | 0.84 | Very good |

In this manner, descriptive interpretation of the weighted mean of the item for three, four, and seven response categories will be properly addressed.

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|---|---|
| 1. Wrong dosage | 1.00 |
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| 3. Failure to do a procedure correctly | 2.25 |
| 4. Failure to check the equipment | 2.00 |
| 5. Able to break a sterile field | 2.75 |

Table 1a used a Likert Scale in the following manner: 1. Never, 2. Rare, 3. Sometimes, 4. Often and 5. Always. Giving a verbal description, one has to create an interval of means in order to give interpretations for the weighted mean. Results of most studies treat the problem in this manner. Some authors construct a legend indicating description that runs like the table below.

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| 2 | 1.51-2.50 | Rare |
| 3 | 2.51-3.50 | sometimes |
| 4 | 3.51-4.50 | often |
| 5 | 4.51-5.00 | always |

The table 1 would be then presented as

Table 1b. Perceived responses on the common clinical errors among the nursing students, Jamora et al, 2010.

| Common clinical errors | Weighted mean | Description |
|---|---|---|
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| 2. Wrong time | 1.75 | Rare |
| 3. Failure to do a procedure correctly | 2.25 | Rare |
| 4. Failure to check the equipment | 2.00 | Rare |
| 5. Able to break a sterile field | 2.75 | Sometimes |

However, the kind of interval given above is biased in the sense that the difference in the upper limit and lower limit of the first and last intervals is much lower as compared to the three middle intervals (as shown in the table below). This observation will result to items having more weighted mean in the middle.

| Likert Scale | Interval | Difference | Description |
|---|---|---|---|
| 1 | 1.00-1.50 | 0.50 | Never |
| 2 | 1.51-2.50 | 0.99 | Rare |
| 3 | 2.51-3.50 | 0.99 | sometimes |
| 4 | 3.51-4.50 | 0.99 | often |
| 5 | 4.51-5.00 | 0.49 | always |

To correct this problem, the author reduced or eliminated the bias, by making the difference in each interval to have a uniform difference; hence, the new interval given below have a constant uniform difference in each interval. That is,

| Likert Scale | Interval | Difference | Description |
|---|---|---|---|
| 1 | 1.00-1.79 | 0.79 | Never |
| 2 | 1.80-2.59 | 0.79 | Rare |
| 3 | 2.60-3.39 | 0.79 | sometimes |
| 4 | 3.40-4.19 | 0.79 | often |
| 5 | 4.20-5.00 | 0.80 | always |

Thus, Table 1b will have new description for item 2 (from rare to never) that is,

Table 1c. Perceived responses on the common clinical errors among the nursing students, Jamora et al, 2010.

| Common clinical errors | Weighted | Description mean |
|---|---|---|
| 1. Wrong dosage | 1.00 | Never |
| 2. Wrong time | 1.75 | Never |
| 3. Failure to do a procedure correctly | 2.25 | Rare |
| 4. Failure to check the equipment | 2.00 | Rare |
| 5. Able to break a sterile field | 2.75 | Sometimes |

The correction of the bias in the interval making the difference uniform changes the descriptive interpretation of the item. Thus, a recommendation for three, four, and seven response categories Likert scale "How good do you think was Macapagal-Arroyo as president?" The following may be studied:

Table 1d. Perceived responses on the common clinical errors among the nursing students, Jamora et al, 2010.

| Likert Scale | Interval | Difference | Description |
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| 2 | 1.67-2.33 | 0.66 | Undecided |
| 3 | 2.34-3.00 | 0.66 | Good |
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| Likert Scale | Interval | Difference | Description |
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| 2 | 1.86-2.71 | 0.85 | Rather bad |
| 3 | 2.72-3.57 | 0.85 | Bad |
| 4 | 3.58-4.43 | 0.85 | Neither good nor bad |
| 5 | 4.44-5.29 | 0.85 | Good |
| 6 | 5.30-6.15 | 0.85 | Rather good |
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In this manner, descriptive interpretation of the weighted mean of the item for three, four, and seven response categories will be properly addressed.

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# An Investigation on the Effect of the Difficulty of an Item in A Multiple Choice Examination When Item Choices are Rearrange

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University of Southern Mindanao, Kabacan, Cotabato, Philippines

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Ascend E-Commerce Phils., Inc. Pasig City, Philippines

**ABSTRACT**

**A study was conducted to know if rearranging item choices in an item of a multiple choice test affects the behavior of the examinees the way they look at the item difficulty of the given items. Two sets of test instruments (pretest and modified posttest) containing fifteen items were made with the same items but item choices for the modified posttest instrument were rearranged. Among the 205 examinees who took the test during a two-week time interval, their responses were modeled using the Rasch model. Results show that the estimates of the item difficulties for the majority of the fifteen items between the two tests were different. Majority of the items given in the exam showed an increase in the difficulty level as viewed by the examinees. The effect on the difficulty maybe due to the time interval the two sets of test were administered, that is first, students forget what they learned and see the items as difficult (time factor) and second, the rearrangement of the choices in each items in the post test affected the student's way they see in dealing the items of the test which partly contributed to the increase of the level of difficulty of majority of the items in the test.**

**Keywords:** Difficulty, Item Response Theory, Item Choices, Rasch Model, Rearrange.

## INTRODUCTION

In educational measurement, conducting experiments whether small or big in which the tester records the responses of the examinee is a very useful way to view a test for example in many academic institutions such as Science, Liberal Arts or any Universities which requires oral or written examination as an assessment for their students to be awarded degrees. These responses of items in these examinations were not direct measurements, but supplies the information from which we deduce the measurements. In most experiments, the issue on problems for method in controlling the responses for the error experiment holds for the tests. In reality, independent variables play a role as well as other variables were operative in the experiment, in general we call the nuisance, background, or error variables, and these perhaps gave an influence concerning the responses in which is where the experimental error with the tests become apparent.

In an established field such as in agriculture, for example, if the amount of application of fertilizers were familiar to be a worrying variables that fluctuate across experimental

conditions and an up to date theory is accessible to forecast how these variables affect the explanatory or response variable such as plant growth, then experimental results can be modified to an ordinary levels of fertilizers after a period of time. [1] mentioned that if there is no real theory is available, it is possible to propose probable equations and to evaluate their parameters from the data on the error and explanatory variables.

A collaborative study mostly connected to Psychology, Education and Statistics known as Psychometrics in practice has been utilized in calculating academic achievement and also been used among health related studies and exhibited variety of applications in the field of Science, Agriculture and Social Sciences particularly in assessment and testing. Psychometric methods have several orientation and have two commonly used and established methods, the first one is the classical test theory (CTT) and a second, the item response theory (IRT) which is a contemporary alternative [2].

CTT can be marked as a theory of measurable errors. Some key concepts involve reliability and validity which are both assessed mathematically. On the other hand, IRT is characterized as one method in the theory of measurement error which is a likelihood or stochastic models for responses of examinees to items of test which assume to depend on unobserved latent variable (ability) and on the characteristics of item. The connection between the responses to the items (which can be generally dichotomous or polytomous ordinal variables) and the latent trait is not linear, and the logistic function is often used as this connection function (linkage).

Item response theory models that consider a unidimensional latent trait, that is the answers to items are impacted by a single dimensional variable which characterized the individuals. Mainstream statistics which have computer software packages like the following: Stata, R, or SAS, allows parameters estimation under IRT model frameworks particularly in the scope of generalized liner mixed models [3, 4, 5, 6].

One best known IRT model, considered to be the simplest IRT model is the Rasch model [7, 8, 9]. The Rasch model (RM), a likelihood based model follows this typical framework, say an examinee or person's answering to a binary or dichotomous item is decided by the examinee ability level and the difficulty of the item. One way of expressing the Rasch models is in terms of the chances that an individual with a particular ability level will correctly answer an item that has a particular difficulty, for example in a multiple choice test. Three assumptions were considered when using the Rasch model. The single dimensionality, item discriminations are all equal for all items and the absence of error due to guessing. Although stated as assumptions, however Rasch theorist often viewed it as a requirement of the rigorous fundamental measurement. The Rasch Model merely provides a mathematical formulation of these fundamental properties that can be used to evaluate a dataset of responses of person taking an item.

IRT models have been extensively applied over the last half century. The propagation of these models has certainly been encouraged as beneficial and advantageous over CTT. These benefits have been realized with its improved estimation techniques and software development. Using IRT methodologies, this paper would like to answer the question: Does rearranging the choices in an item of a multiple choice test will change the level of difficulty of the items when a person answers to an item? The conduct of this study is only limited on the concepts of IRT using the

Rasch model as applied to the obtained data conducted by the researchers which are the student's response to the test items and been modeled, evaluated and analyzed using the said model.

## METHODOLOGY

### Item Response Theory (IRT)

Item response theory is a class of likelihood or stochastic models for two-way data, the responses of persons or individuals to test items. This is so far the latest method in the measurement theory in dealing with item and examinees parameter calculation of estimates as compared to the classical test theory. The description of the model is best described in the work of [2] for its features and applications. Under the single dimensional IRT models for dichotomous or binary items, the probability of a correct response depends on the examinee's single dimensional ability, say $\theta$, and the parameters that identified for the item. A variety of in demand models for items with dichotomous responses were mentioned and describe in [7, 10, 11, 12, 13]. There are also available IRT models that cater multiple dimensional cases described in [14, 15]. Further, IRT models are also accessible for non-monotone items. IRT provides practicality of solving extensive measurement problem cases ranging from item calibration, constructions of high stake test instruments, test scores reporting and many other applications. See [2] and [16] for further discussions.

The initial step that item response theory can be applied to test data is that of calculating the estimates of the parameters that identified the selected model for item response. In fact, the usual application of IRT relies on the acceptable procedure's accessibility for calculating the estimates of the parameters of the model. The steps for the calculations of examinee's ability and item parameter estimates applying the models are available in two methods namely the frequentist likelihood based method and the Bayesian methods. On this study we only limit in the use Likelihood based methods. Examples of some frequentist likelihood based methods are the joint maximum likelihood estimation (JML), conditional maximum likelihood estimation (CML) and marginal maximum likelihood estimation (MML) that employ inferences. See [2] for further discussion. These frequentist methods were utilized in software packages such as in BILOG-MG [17], MULTILOG, TESTFACT [18], MPLUS [19], OPLM [20] and ConQuest [21]. The R package eRm [22] and R Package ltm [6].

### Information Characteristics Curve (ICC)

A curve call the Information Characteristics Curve (ICC) is a representation of the function for item response (IRF) of the examinees to the items, a likelihood of obtaining a positive response to each item which is represented as a function of the ability or latent trait $\theta$. One can represent in the same graph the observed and the anticipated ICCs to evaluate the fit of each item.

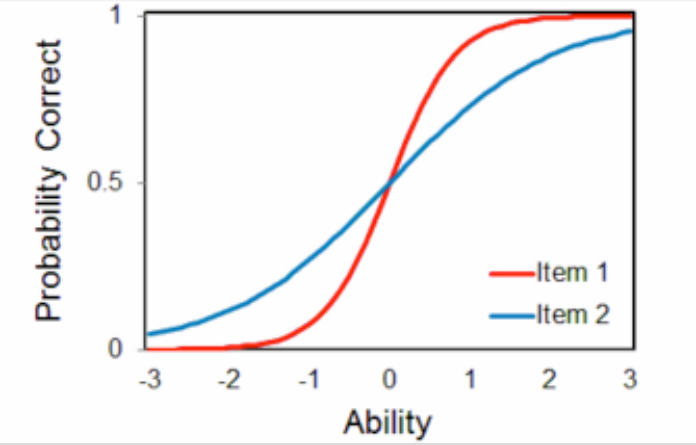



**Figure 1. The ICC, Information Characteristics Curves**

Figure 1 (Reference: Columbia University Mailman School of Public Health) is an example of information characteristic curve for a number of items. ICCs are colored to underline the variation in the probability of a lucky response for a person with ability location at the vertical or upright line. The person is probably to answer correctly to the effortless items (with locations to the left and higher curves) and unlikely to respond correctly to difficult items (locations to the right and lower curves) that is, the x-axis is the conceptual theta (theoretical proficiency or ability) level, the values of theta usually in practice ranged from -3 to +3. The graph of ICC only speaks for the theoretical modeling rather than for the empirical data. To be specific, there may not be examinees that can reach a proficiency level of +3 or fail so miserably as to be in the -3 group. Anyhow, to analyze the "achievement " of an item, we would like to know that if given a person has a $\theta$ of +3, The question is what do you think is his chances of him getting a correct answer to that particular item. The ICC indicates that when $\theta$ is null (0), which in practice considered an average ability, the likelihood of getting correct to the item is almost 0.5 or 50%. When $\theta$ is -3, the likelihood is almost null (0). When $\theta$ is +3, the probability increases to 0.99.

## The Dataset

This research study utilized 205 empirical student's responses to a 15 items multiple choice of a basic statistics examination conducted among first year undergraduate students at MSU-Iligan Institute of Technology, Philippines. See [23] for the data description. Two sets of test instruments (pretest and modified posttest) containing fifteen items were formulated with same items but choices for the other test instrument were rearranged in which the correct answer in the choices in the pretest was move either one move to the right if the correct choice in the item of the pretest is in the left most position or one move to the left. The tests were conducted for the same group of students during a two-week time interval. The posttest was given without the knowledge of the students that they will be taking another set of examination same as their preliminary examination. The respondents who took only the pretest were omitted in the analysis. A total of 205 respondents were used in the study.

An illustration of the entry matrix of the responses of the N students to a particular J items are given below:

| Student | Test Items | | | |
|---|---|---|---|---|
| examinees | Item1 | Item2 | … | Item J |
| 1 | 1 | 1 | … | 0 |
| 2 | 0 | 1 | … | 1 |
| . | 0 | 1 | … | 1 |
| . | 0 | 1 | … | 0 |
| | 1 | 0 | … | 0 |
| N | 1 | 0 | … | 1 |

where entry 1 means the student got the correct answer for the item while zero (0) means that the student has an incorrect answer to a particular item.

**IRT Measurement Model for Dichotomous Response**

For the viewpoint of IRT, the components that affects the probability of an examinee answering a specific way to a specific item can be specified. The mathematical links between an outcome (e.g., an examinee's score on a specific item) and the components that affect the outcome (e.g., qualities of the examinee and/or qualities of the item) can be expressed using a measurement model. In this research, we will apply the Rasch model (RM) so we can get the estimates of the item difficulty estimates. The RM is given by,

$$P\left(X_{nj}=1\middle|\theta_n,\beta_j\right)=\frac{e^{(\theta_n-\beta_j)}}{1+e^{(\theta_n-\beta_j)}}$$

where: $X_{nj}$ represents the response made by examinee $n$ to item$j$.

$\theta_n$ represents to the latent trait level or ability of examinee $n$; and $\beta_j$ represents to the difficulty of item$j$.

So, $P\left(X_{nj}=1\middle|\theta_n,\beta_j\right)$ represents to the likelihood that examinee $n$ responded to item $j$ correctly. The likelihood that the examinee correctly responded to the item will depend on (i.e., conditional) the examinee's ability level $(\theta_n)$ and the item's difficulty $(\beta_j)$. In an IRT framework, ability or trait levels and item difficulties are standardized scaled metric, that is their means are zero (0) and has one (1) standard deviations. RM expressed an examinee's response to a binary or dichotomous item (i.e., correct or incorrect) and is determined by the examinee ability or trait level and the difficulty of the item. RM employed assumptions for generalization of results. Namely, single dimensionality of ability, raw score is sufficient, present of local independence and parallel item characteristic curves (ICCs). Further discussions can be obtained in [24] and corresponding mathematical derivations and proofs for the model can be found in [25].

**Item Difficulty Estimation with RM using Conditional Maximum Likelihood (CML)**

The software package Latent Trait Models under IRT (ltm) by [6] rooted on the flat-form of the software R which adapted the conditional maximum likelihood (CML) and marginal maximum likelihood (MML) estimation methods was used to estimate item parameters. In our study we

only employed the CML. For more details, please refer to [26], [27, pp.219-224], [28, pp.136-144] and [29, pp.138-141].

## RESULTS AND DISCUSSIONS

The item difficulty of the different items of both test was fitted and estimated using the Rasch model. The package ltm [6] in the software R was used to do the calculations of the item difficulty estimates.

The parameter $\beta_i$ represents the item location which, in the case of attainment testing is referred as the item difficulty and can be categorized into different levels such as very easy, easy, average, difficult and very difficult. This location index is the point on $\theta$ where the item response function has its maximum slope and where the value is half-way between the minimum value of the guessing parameter and the maximum value of 1. The higher the difficulty parameter then there is also a need for higher ability level for the examinee to be in order to get a correct answer to the item. In case $\beta_i = 0$ the item is in an average difficulty. For $\beta_i > 0$, the item ranges from difficult to very difficult while for $\beta_i < 0$, the item difficulty ranges from easy to very easy. In practice, the value of $\beta_i$ ranges between -3 to to 3. Results of the analysis using our data, the estimate of the item difficulty using the Rasch Model is presented in Table1.

**Table 1 Item Difficulty of the 15 Item Test Using the RM**

| Items | Pretest | | Modified Posttest | | Changes in the Difficulty |
|---|---|---|---|---|---|
| | $\beta$ | Difficulty Description | $\beta$ | Difficulty Description | |
| 1 | 0.44 | D | 0.04 | A | Decrease |
| 2 | 0.21 | D | 0.12 | A | Decrease |
| 3 | -1.01 | VE | -0.77 | E | Increase |
| 4 | -0.01 | A | -0.23 | D | Decrease |
| 5 | 0.23 | D | 0.24 | D | Same |
| 6 | -0.59 | E | -1.0 | VE | Decrease |
| 7 | 0.21 | D | 0.77 | VD | Increase |
| 8 | 1.23 | VD | 1.49 | VD | Increase |
| 9 | 0.03 | A | 0.08 | A | Increase |
| 10 | -0.10 | E | -0.03 | A | Increase |
| 11 | 0.75 | D | 0.56 | D | Decrease |
| 12 | 1.91 | VD | 1.59 | VD | Decrease |
| 13 | -1.09 | VE | -0.77 | E | Increase |
| 14 | -1.20 | VE | -0.39 | E | Increase |
| 15 | -0.96 | VE | -0.55 | E | Increase |

**Legend: VE = very easy, E = easy, A = average, D = difficult, VD = very difficult**

$$\boldsymbol{\beta = item\ difficulty\ Index/parameter}$$

Note that under the RM, items not fitting the model were removed [30]. As a consequence, RM does not permit abilities to be estimated for outlying items and persons in which information are limited.

Showing the results of the estimated item difficulty for all the fifteen items included in the test for both tests (pretest and posttest), The estimated level of item difficulty showed that for most items in the pretest in particular items 1, 2, 5, 7, and 11 were difficult or demanding items while items 8 and 12 were considered very difficult (very demanding) items. Further, items 4 and 9 were items on an average difficulty (par). Items 3, 13, 14, and 15 on the other hand, were the simplest (very easy) items which were easily answered by the examinees and while items 6 and 10 were considered easy items respectively. For the modified posttest, the result showed that items 6 was a very easy (obvious) item. Items 3, 13, 14, and 15 were easy items while terms 1, 2, 9, and 10 were average items. Further items 4, 5, and 11 were difficult items while items 7, 8, and 12 were very difficult items.

As we observed in table 1, most item difficulties of the different items from the pretest to the modified posttest when compared change from an average, easy or very easy items from pretest to become average, difficult to very difficult in the modified posttest or vice versa. Other items' difficulty did not change, an example is the items 5, 8, 9, 11, and 12. One aspect of question needed to be answered to this change is whether the rearrangement of the item choices or the two-week time interval were the reason for such changes in the item difficulties of the different items. Particular items namely items 3, 7, 8, 9, 10, 13, 14 and 15 in the pretest to modified posttest show an increase in values of the difficulty. While items 1, 2, 4, 6, 11, and 12 showed a decreased in the value of its difficulty. Further, only item 5 shows same level of difficulty for both pretest and modified posttest. In some items, the difficulty estimates ranges from difficult in pretest to average in posttest, this phenomenon was observed in items 1 and 2. Others from very easy to easy is shown in items 3, 13, 14 and 15. On the other hand, item 4 gone from an average in pretest to become difficult in the modified posttest. One item, the item 6 shows an easy item in the pretest to become a very easy item in the modified posttest. Another item from difficult to very difficult is shown in item 7. Lastly, item 10 has an easy item in pretest and becomes average item in the modified posttest.

Further, the item response functions among the different items mentioned above were transformed to an Item Characteristic Curves (ICC) which are graphical functions representing the examinee's ability as a function of the likelihood of getting correctly the item. The different item ICCs for both the pretest and modified posttest are shown in figures 2 and 3.

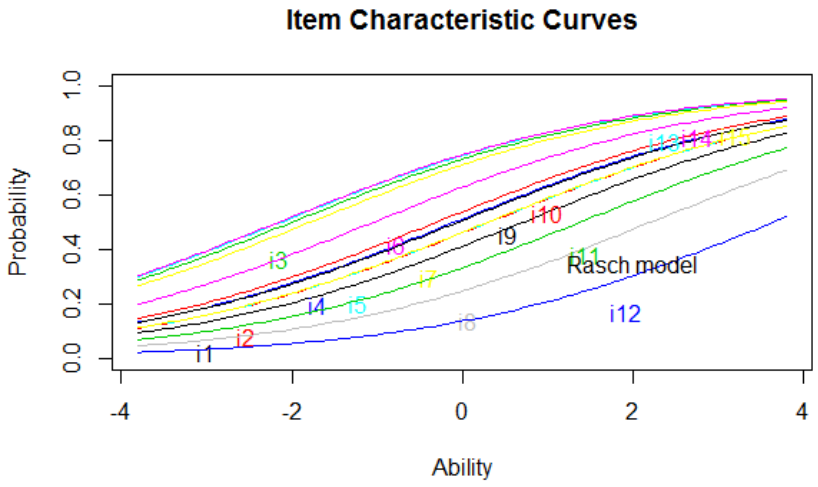



**Figure 2. ICCs under Rasch Model (Pretest)**

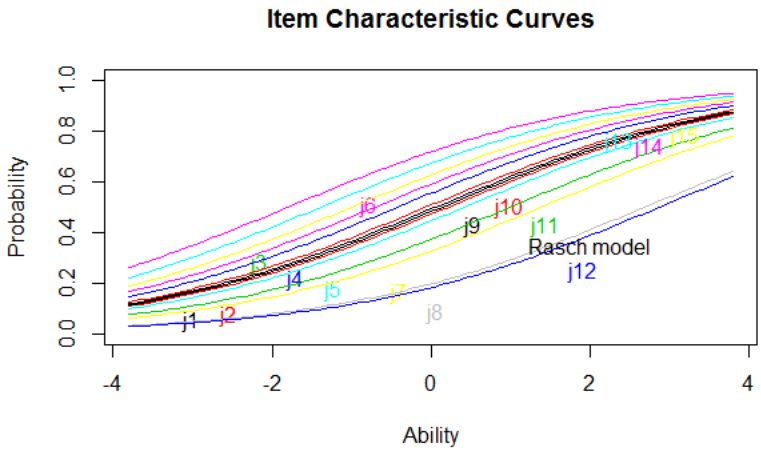



**Figure 3. ICCs under Rasch Model(Modified Posttest)**

In Figures 1 and 2, when the data were fitted with the RM, the item difficulty in the pretest changed from its level when evaluated in the modified posttest. The ICC have shown that when the item was difficult the higher was the ability parameter $\theta$ of the examinee needed to get 50% of getting a correct answer to an item. In the Figure 1, which were the items in the pretest, we have seen that most of the items were difficult. Hence, higher abilities from the examinees were needed to get higher probabilities of getting correctly the answer. In Figure 2, the items in the modified posttest, we observed that the item difficulties of the items shifted to further more difficulties. Thus, much higher abilities were needed to obtained higher probability of getting a correctly the answer. In terms of the model fit of the Rasch model, the results showed that some items were "misfit", a terminology in which items did not fit in the RM. As we discussed previously, items that did not fitted the RM will be removed Table 2 shows items that did not fit the RM.

**Table 2 Fitting the items under RM**

| Items | Pretest | | Modified Posttest | |
|---|---|---|---|---|
| | $X^2$ | $p$-value | $X^2$ | $p$-value |
| 1 | 18.49 | 0.01* | 9.26 | 0.23 |
| 2 | 5.66 | 0.46 | 8.19 | 0.32 |
| 3 | 11.51 | 0.07 | 21.75 | <0.01* |
| 4 | 14.41 | 0.03* | 19.41 | 0.01* |
| 5 | 22.23 | <0.01* | 12.91 | 0.07 |
| 6 | 24.78 | <0.01* | 17.66 | 0.01* |
| 7 | 9.97 | 0.13 | 6.51 | 0.48 |
| 8 | 23.08 | <0.01* | 14.01 | 0.05* |
| 9 | 15.16 | 0.02* | 11.09 | 0.13 |
| 10 | 11.84 | 0.07 | 14.43 | 0.04* |
| 11 | 7.70 | 0.26 | 15.87 | 0.03* |
| 12 | 7.49 | 0.28 | 6.82 | 0.45 |
| 13 | 14.01 | 0.03* | 17.67 | 0.01* |
| 14 | 34.71 | <0.01* | 25.85 | <0.01* |
| 15 | 22.62 | <0.01* | 27.45 | <0.01* |

**Legend: * significant at 5% (using Chi-square test)-model fit**

The following items were, in particular items 2, 3, 7, 10, 11, and 12 in both pretest and in modified posttest items 1, 2, 5, 7, 9, and 12 fitted to RM. While items 1, 4, 5, 6, 8, 9, 13, 14, and 15 in the pretest and items 3, 4, 6, 8, 10, 11, 13, 14, and 15 in modified posttest did not fitted to RM. Further observations, the results showed that items 2, 7 and 12 fitted in RM for both in the pretest and also in modified posttest. Items that did not fitted the RM means that RM is not a good model for these items. In practice, researchers look for an appropriate IRT models for the items to be fitted which is usually a recommended.

## CONCLUSION

In this study, majority of all the items that were given in the exam showed an increase in the difficulty level. Firstly, this increase may be due to forgetfulness of the examinees of what they learned in the class. The time interval in the administration of the two sets of test on which students forget that they have seen the items as difficult which can be due to time factor in which memory retention was not there. This is consistent to the study of [31] that maturity in mathematics can be obtained for longer period of time. Secondly perhaps the rearrangement of the choices in each items in the post test really affected the student's way and behavior to see in dealing the items of the test. This explained partly why an increase of the level of difficulty of majority of the items in the test appeared. We recommend a further study in which the different methods of rearranging the choices of the items in the test using different IRT models may be considered and result may be compared to the results of this paper. Further we suggest that test developers whether in classroom examinations or in high stake examinations might consider adopting this method for quality assessment and selection.

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# Caught in the crossfire: biodiversity conservation paradox of sociopolitical conflict

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The current state of global biodiversity is confronted with escalating threats arising from human-induced environmental changes and a growing array of unpredictable challenges. However, effective conservation efforts are often hindered by limited knowledge, especially in developing economies such as the Philippines. The limitations imposed by these shortfalls in biodiversity knowledge hamper the capacity to protect biodiversity in light of the continuing extinction crisis. Our study revealed that areas with higher conflict levels exhibited lower species richness, fewer occurrence records, and reduced forest cover. This finding provides initial evidence for the relationship between sociopolitical conflict and biodiversity in the Philippines. We posit that the security risks caused by sociopolitical conflicts could have a negative impact on conservation efforts, particularly in terms of monitoring and implementing measures to protect natural resources. The links that bind armed conflict and biodiversity conservation are multifaceted and complex issues that warrant greater scientific and political attention. Finally, we identified 10 meaningful approaches to address shortfalls in biodiversity knowledge in conflicted areas, particularly incorporating conflict-sensitive approaches, considering the geopolitical context and conflict dynamics to adapt and align their strategies with local realities for more effective conservation efforts.

Reducing the rate of global biodiversity loss, halting extinction risks, and preserving intact ecosystems are central to conservation biology[1]. The latest Kunming-Montreal Global Biodiversity Framework aims to safeguard large proportions of species and habitats[2], and conservation biologists and ecologists rely on accurate and robust biodiversity data to effectively develop conservation priorities[3,4]. Despite the progress made to catalogue biodiversity on Earth[5], there are persistent gaps in biodiversity knowledge across various areas[6,7]. Consolidating biodiversity information is often challenged by insufficient funding[8], limited capacity to implement conservation on the ground, and issues related to national security[9].

Armed conflicts and violent extremism have long been recognised as significant threats to national security, altering social stability[9,10]. The impacts of armed and sociopolitical conflicts on the environment and biodiversity have also gained considerable attention in recent years[10,11]. For instance, the world witnessed the destruction of forests during the Vietnam War[9,12], the draining of Mesopotamian marshes during the Gulf War[13], and a decline in wildlife during civil wars in the Democratic Republic of the Congo[14]. At the same time, the 21st-century wars in Afghanistan, Syria, and Ukraine continue to affect biodiversity at an alarming rate[15–18]. Undeniably, armed conflicts and violent extremism can lead to extensive habitat destruction, altering patterns of biodiversity. In fact, of the major armed conflicts between 1950 and 2000, over 90% occurred in countries with biodiversity hotspots, and 80% occurred directly within hotspot areas[10]. The environmental footprints of military activities, explosives, and landmines cause long-lasting damage to ecosystems, leading to the loss of critical habitats for diverse flora and fauna[19]. These activities significantly worsen

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the problem of biodiversity depletion by promoting unsustainable practices, which disturb delicate ecological equilibriums such as overharvesting species, habitat destruction, and deforestation[20–22]. Moreover, armed conflicts tend to undermine the effectiveness of environmental governance systems, enforce environmental regulations, and safeguard vulnerable ecosystems.

These warfare-driven threats to biodiversity prompted the United Nations (UN) General Assembly in 2001 to declare every 6th of November as the *International Day for Preventing the Exploitation of the Environment in War and Armed Conflict*[23]. Twenty-three years later, many gaps in policy and research still need to be addressed, especially in developing economies, where biodiversity capacity building remains insufficient. Biodiversity hotspots, protected areas, and indigenous territories lack protection under international humanitarian law during armed and sociopolitical conflicts[22]. Previous analyses have demonstrated how sociopolitical conflicts could negatively impact the documentation of biodiversity[10,19], which consequently limits effective conservation. Yet, there is a lack of well-researched case studies[24], particularly in the Philippines, a megadiverse country.

Mindanao is the second largest group of islands in the Philippines, consisting of 27 provinces and 33 cities within six administrative regions with an estimated population of 26,252,442 (24% of the country's population), and its mainland is the seventh most populous island in the world[25]. Its large fertile landmass makes it a major raw material producer in the Philippines, producing around 40% of the country's agricultural produce and 60% of agricultural exports[26]. In addition, owing to its unique biogeographical history and position, Mindanao is a biodiversity hotspot for diverse flora and fauna[27,28]. Mindanao has over 30 Key Biodiversity Areas (KBAs) that are globally significant sites for biodiversity conservation because of the high concentration of endemic and threatened species, including the globally threatened Philippine Eagle (*Pithecophaga jefferyi*), and other keystone species[28–31].

Whilst Mindanao is known for its rich and diverse wildlife and valuable natural resources, the region confronts a disconcerting reality marked by a confluence of sociopolitical adversities encompassing armed conflicts, religious tensions, feuds among clans, abductions, and other incidents of violence that paint a grim picture of the region[32]. The history of war and armed conflict in Mindanao is complex[32,33], which can be traced back to the colonial era when the Philippines was under Spanish rule during the 16th century. Spanish colonisers encountered resistance from Muslim communities in Mindanao, who fought against their conversion to Christianity and the imposition of colonial rule[34]. In the 21st century, the Philippine government faced multiple parallel domestic armed conflicts and violence[35]. Data from the United Nations Office for the Coordination of Humanitarian Affairs (OCHA) have estimated that Mindanao has experienced more than half (53%) of the sociopolitical conflicts in the Philippines from 1989 to the present[36] (Fig. 1).

Contemporary conflicts spanning 60 years in Mindanao might lead to considerable ecological and environmental degradation. Moreover, the collection and analysis of biodiversity information may have been significantly impeded in areas facing sociopolitical and armed conflicts. Apart from direct environmental impacts, sociopolitical conflicts can disrupt research activities, hinder access to remote or affected areas, and create risks for scientists and conservation biologists[20,21,37]. This, in turn, limits our ability to gather accurate data on local flora and fauna, which is essential for making informed decisions about biodiversity conservation and management. Recent Philippine-wide assessments have highlighted differences in survey efforts, notably the low number of studies and recorded species in Mindanao (for example, in bats[38] and primates[39]). However, the lack of formal studies linking armed conflict to biodiversity knowledge shortfalls in the Philippines is a significant gap.

Addressing the biodiversity knowledge shortfall is crucial for understanding species distribution, the extent of the environmental impacts of conflict, formulation of effective policies and strategies to mitigate these effects, and promoting sustainable development and conservation in the region. Here, we present a perspective highlighting the link between sociopolitical conflicts and biodiversity knowledge shortfalls[6], specifically in the context of the Southern Philippines. To gain insight into the influence of

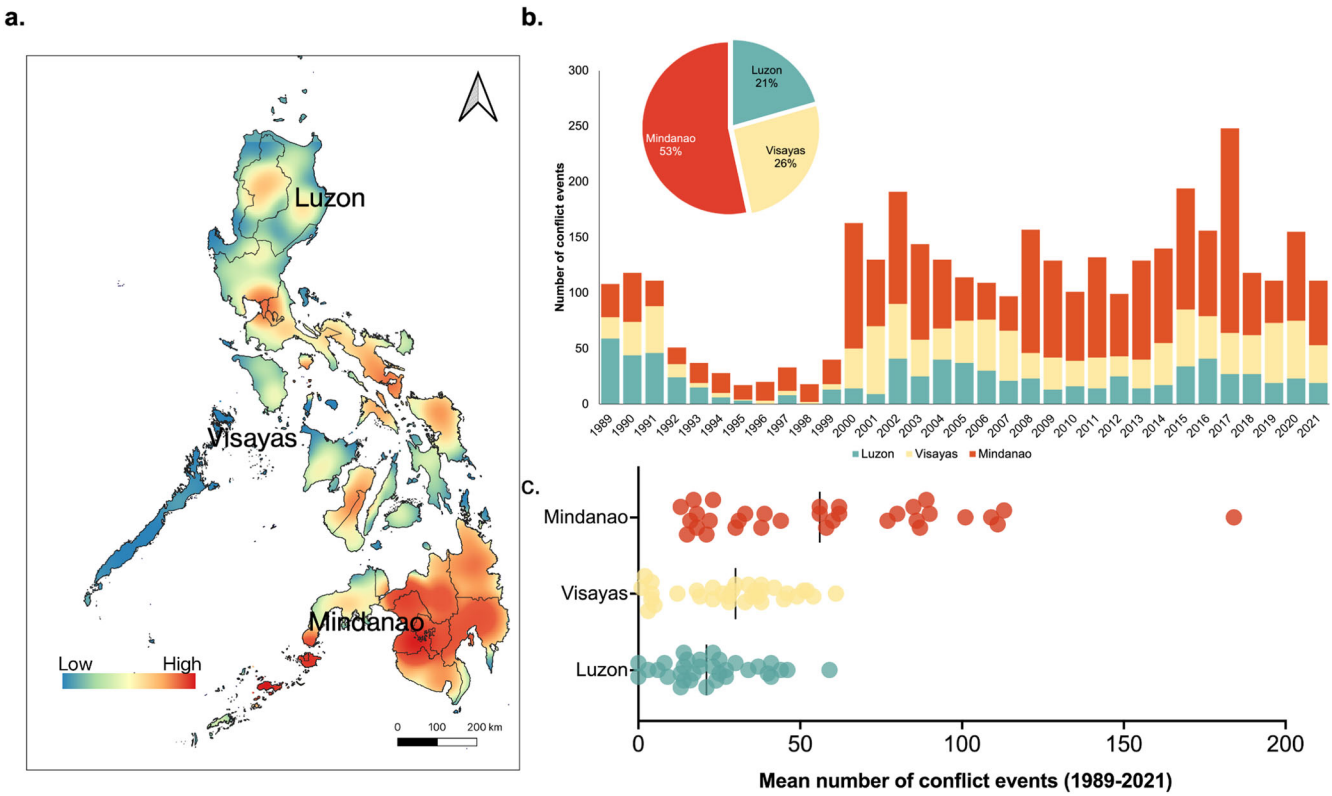



**Fig. 1 | Distribution of war and conflict in the Philippines. a** Density occurrence, **b** temporal patterns, and **c** average number of conflict events in the Philippines from 1989 to 2021 based on the UN OCHA database[33].

sociopolitical conflicts on species richness and occurrence records in Mindanao, we analysed the spatial distribution pattern of biodiversity data and remote sensing variables related to habitat transformation (namely tree cover and density, and forest height) within Mindanao and their possible association with sociopolitical conflict events between 2000 and 2021. Finally, we identified meaningful approaches to address biodiversity shortfalls in conflicted areas.

## Results

### Patterns of biodiversity knowledge shortfalls

Our analysis revealed how sociopolitical conflict promotes gaps in biodiversity knowledge within a biodiverse island in the Philippines. First, a total of 2174 conflicts (103.52 conflicts/year) were recorded in Mindanao from 2000 to 2021, with the highest levels of violence recorded in Sulu (mean = 82 annually; 18%) and Maguindanao (mean = 329 annually; 15%) provinces (Fig. 2). This has an impact on observed biodiversity. We found a significant difference in observed species richness between high- (mean = 0.50 ± 1.60) and low-conflict (mean = 1.27 ± 2.37) areas (Mann–Whitney $U$ test = 1340, $p = 0.0027$) (Fig. 3).

We then modelled the link between species occurrence records and conflict events in Mindanao at the provincial level. The best model for predicting spatial variation in species occurrence records (species record ~1 + conflict events + average distance from conflict events + taxonomic groups, AIC = 7864.53) showed that an increase in the number of conflict events was associated with lower recorded species richness ($\beta = -0.002$, $p < 0.0001$) (Fig. 4a). Conversely, we showed that the spatial distribution of species richness was higher in areas farther away from conflict events ($\beta = 0.003$, $p < 0.0001$) (Fig. 4b), particularly for insects ($\beta = 2.067$, $p < 0.0001$) and birds ($\beta = 1.545$, $p < 0.0001$).

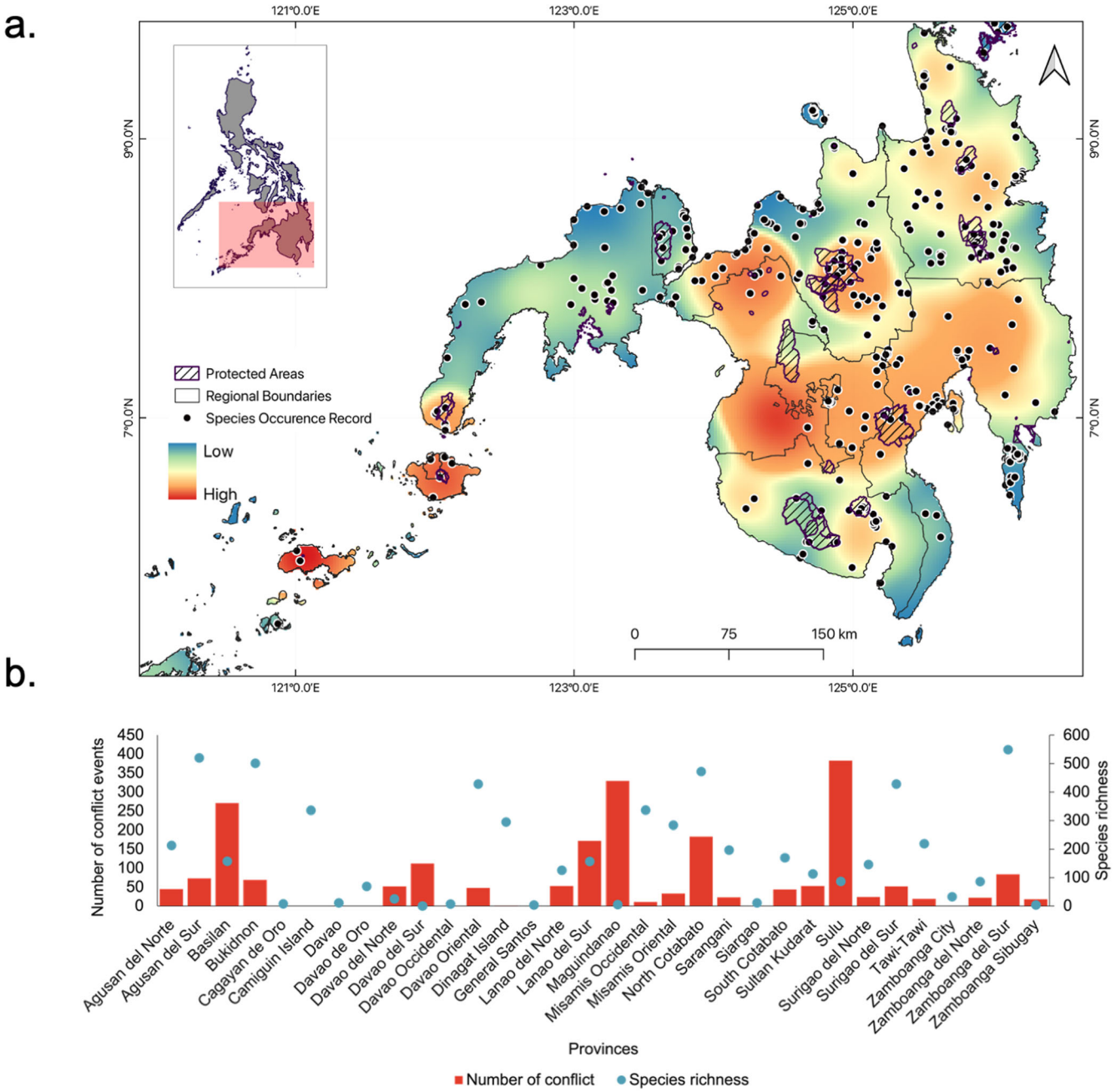



**Fig. 2 | Distribution of war and conflict in Mindanao relative to its provinces. a** Spatial distribution of species occurrence records (dots) and density of sociopolitical conflict events in Mindanao from 2000 to 2021 and **b** comparison of conflict events and recorded species richness at the provincial level.

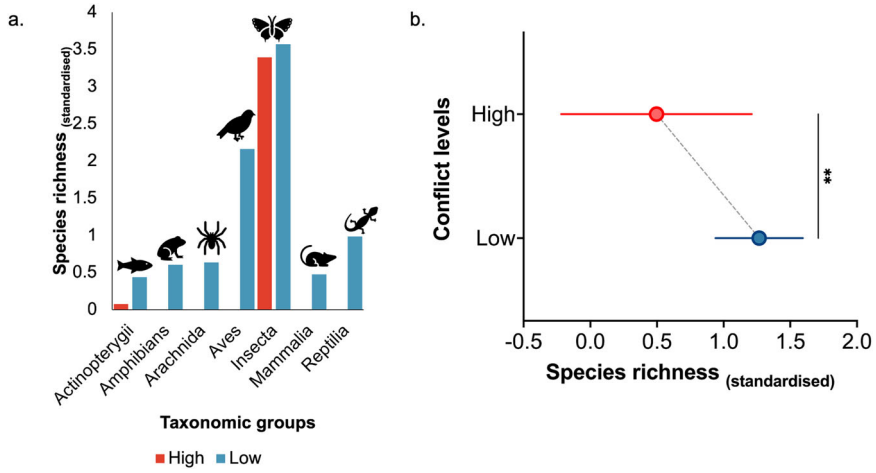



**Fig. 3 | Differences in species richness between low- and high-conflict areas. a** Comparison of normalised species richness among taxonomic groups and **b** overall group comparison between low- and high-conflict areas. Note: ** indicates significance at $p < 0.001$; whiskers represent 95% CI intervals.

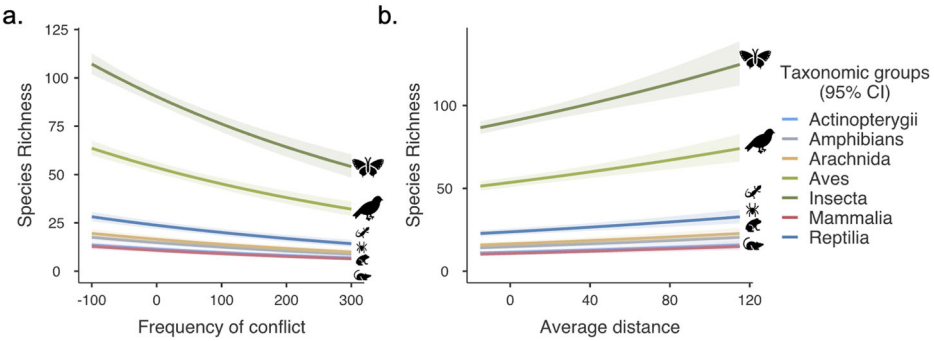



**Fig. 4 | Relationship between conflict and distance from species occurrence.** Visualised results of the generalised linear model (Poisson GLM) showing the association between species richness, **a** frequency of events, and **b** average distance at the provincial level. Note: Shading represents the 95% CI. Graphics were from Microsoft Office.

### Conflict in forests and protected areas

The spatial distribution of conflict events varied across habitat types ($\chi^2 = 716$, df = 6, $p < 0.0001$). The majority of the conflict events fell within open habitats, such as agricultural areas (61%) and grasslands (19%), with only 6% of the recorded conflict events from 2000 to 2021 within protected areas. Moreover, we found a significant negative correlation between a number of fatalities with tree density (Pearson's $r = -0.05$, $p = 0.013$) and tree cover (%) (Pearson's $r = -0.06$, $p = 0.002$) but not with canopy height (Pearson's $r = -0.04$, $p = 0.054$).

## Discussion

Our findings provide valuable insights into the relationship between sociopolitical conflict and shortfalls of biodiversity knowledge in the Philippines. First, our analysis showed that species richness and occurrence decreased in areas with higher numbers and distances to conflict but were taxonomically dependent. Birds and insects exhibited a more robust response to the dependent variables than other taxonomic groups. This can be attributed to their greater detectability, making them easier to sample and collect than other groups like mammals and herptiles[28,31,40]. Unlike these groups, birds and insects are often more conspicuous and exhibit behaviours and characteristics that facilitate easier observation and data collection. This makes them useful indicators of changes in species turnover in conflict zones[24]. Here, we posit that in high-conflict areas, the number of species or their populations have likely declined, potentially resulting in observed biodiversity that falls well below the expected levels due to conflict-related pressures. Another potential reason could be the difficulty in recording and inventorying biodiversity within highly conflicted areas. These areas often require staying in the field, which can be challenging and may deter biologists from making efforts to study biodiversity owing to security risks. Scientists often face challenges when conducting fieldwork in regions that experience ongoing disputes or unstable political situations. These range from personal safety concerns to logistical difficulties in accessing remote and unstable areas. Consequently, it may be impractical to conduct extensive biodiversity assessments in areas marked by high levels of conflict.

Although most areas in Mindanao are now relatively accessible, such as the Ligawasan Marsh in the BARMM region[30], conducting biodiversity research remains a challenge because of the fear brought about by past conflict events and the disruption of local peace and order in some areas. Several non-state armed groups operate in Mindanao, particularly in Maguindanao, Lanao Del Sur, Basilan, Sulu, and Tawi-Tawi[41]. Other violent tensions, such as clan wars or *'rido'* and political disputes, brought brutal incidents, such as the Maguindanao massacre in 2011[42]. Another example was the kidnapping of bird watchers in Tawi-Tawi by the *Abu Sayyaf* group in 2012[43], which prompted foreign and local authorities to advise their citizens, including biodiversity researchers, to refrain from travelling or visiting Mindanao. In regions where security risks impede the ability of scientists to conduct biodiversity assessments, documented biodiversity may underrepresent the actual diversity of species. This disparity can have profound implications for conservation efforts and for our understanding of the true biodiversity status of conflict-stricken regions. For example, heavily conflicted areas such as Basilan, Zamboanga del Norte, Tawi-Tawi, Zamboanga Sibugay, Sulu, and Isabela remain lacking biodiversity information in the past two decades[40].

While it is clear that war and conflict impede filling in existing knowledge gaps, the complex interplay of conflict, governmental policies, and their impact on biodiversity conservation in conflict areas constitutes a nuanced and multifaceted subject[44]. Although sociopolitical conflict can be acknowledged as offering transitory advantages to biodiversity conservation by creating no-go zones and improving vegetation recovery[45], our evidence from the Philippines tends to be negative. Our study revealed that areas with high conflict levels, as indicated by high fatality rates, had lower tree cover and forest density. We argue that a greater variety of plants and animals may thrive in low-conflict areas, which are typically characterised by more intact ecosystems than in high-conflict areas[46–48]. Moreover, our current finding

aligns with many previous studies suggesting that conflict zones often experience increased habitat destruction, ecosystem disruption, and wildlife population reduction due to the lack of statutory regulations and challenging implementation of environmental policies in high-conflict areas[10,11,49,50]. However, our results need careful interpretation, and it is crucial to explore whether the observed relationship is causal or merely correlational, considering factors like human displacement and changes in land use during conflicts[24,49,51]. If armed conflict indeed proves to be a significant driver of biodiversity loss in Mindanao, it has profound implications for conservation efforts in conflict-affected regions, necessitating collaborative strategies among policymakers, conservationists, and humanitarian organisations working in the region.

Discussions on the impact of armed conflict and violent extremism *vis-à-vis* biodiversity remain the elephant in the room, which is often neglected in biodiversity prioritisation efforts[10], especially in the Philippines[52]. Specific connections between armed conflict and biodiversity conservation have garnered limited attention[53]. Schulte to Bühne et al. [54] stated that the current legal and policy frameworks regulating global biodiversity conservation do not address the challenges of conducting activities in areas affected by conflicts. They added that to incorporate conflict-sensitive protection into international policymaking, peace and scientific organisations should openly address the consequences of armed conflicts on biodiversity. Hulme[55] and Hemptinne[56] suggested that international environmental laws (IEL) should be reinforced and incorporate the principles of international humanitarian laws (IHL) as a tool and guide to protect the environment during and post-conflict regimes.

Another significant challenge in effectively implementing conservation initiatives in conflicted areas in the environment is the absence of baseline evidence for biodiversity status[57]. The absence of comparative data on pre- and post-conflict conditions adds complexity to our understanding of the impacts of conflict on biodiversity. Moreover, implementing biodiversity regulations and policies is particularly problematic in regions affected by conflict where maintaining law and order is difficult. Resource allocation, a key governmental responsibility, is vital for conservation initiatives; however, in conflict zones, financial resources may be redirected to address immediate security concerns, potentially affecting biodiversity conservation efforts[10,53]. Moreover, the direct and indirect impacts of sociopolitical conflicts on biodiversity, including habitat destruction and community displacement, highlight the need for mainstream post-conflict initiatives to rehabilitate and restore ecosystems[37].

A concerted effort must be made to thoroughly document and monitor regions with notable shortfalls in biodiversity, to identify areas where gaps exist in biodiversity data, and to establish comparative monitoring assessments that employ standardised, transparent, accessible, and reproducible methods to accurately document unrecorded biodiversity. This is crucial for ensuring a comprehensive understanding of the state of biodiversity and for making informed decisions regarding conservation and management efforts[28,40]. Additionally, conservation biologists can work with local communities in conflict-affected areas to establish community-centric conservation projects. Strengthening the engagement of local communities to build awareness ensures sustainable resource management and provides economic alternatives to destructive activities. However, specific actions are needed to effectively address biodiversity shortfalls in conflict areas. Conservation biologists must integrate conflict-sensitive approaches into the planning processes. This involves considering the geopolitical context, understanding the conflict dynamics, and adapting conservation strategies accordingly. By aligning conservation efforts with local realities, we can better navigate the challenges posed by conflicts. Here, we recommend meaningful actions and considerations to bolster biodiversity conservation efforts in areas affected by sociopolitical conflict during the post-conflict period (Box 1).

In conclusion, our research sheds light on the complex and multifaceted relationship between sociopolitical conflict and biodiversity knowledge shortfalls, which restricts our understanding of biodiversity patterns in areas affected by war and conflict. Additionally, conflict zones are difficult to explore, leading to a lack of dependable biodiversity information, such as current threats and their influence on species and ecosystems. Moreover, our proposed actions extend beyond Mindanao and offer applicability to other regions that face comparable challenges. We emphasise that there is no single silver bullet that resolves the challenges brought about by sociopolitical conflicts with biodiversity. Conservation efforts in conflict zones require a multidimensional approach that addresses both ecological and sociopolitical aspects of the situation. Governmental efforts should focus on conflict prevention, post-conflict environmental restoration, initiatives to strengthen environmental governance, and engaging local communities to ensure the sustainable management of natural resources. Appropriate policies must be implemented to create a supportive environment for long-term biodiversity conservation efforts. Advocating policies and frameworks, such as the recent Kunming-Montreal Global Biodiversity Framework[2], to bolster biodiversity conservation in conflict areas is crucial in addressing the inadequacies in these regions while simultaneously ensuring balanced benefits from nature.

Ensuring the security of conservation biologists and personnel, including park rangers, is paramount for implementing policies. Therefore, it is necessary to integrate conservation efforts into broader national security strategies[10]. Future studies should examine the extent of conflict that is identified as off-limits for researchers and conservationists. Additionally, post-conflict reconstruction and peacebuilding initiatives can offer opportunities to incorporate sustainable development and conservation measures, facilitating the restoration and protection of biodiversity in regions affected by conflict[37,53], especially in countries with developing economies, where the overlap between conflict and biodiversity is particularly high.

## Methods

### Comparing species richness and occurrence in conflict areas

We compared and determined the link between sociopolitical conflict and species occurrence in Mindanao. We first obtained biodiversity data from the MOBIOS$^{+}$ database[40] and conflict-related information for the Philippines from the UN-OCHA[36] from 2000 to 2021. For biodiversity data, we filtered the dataset and only included biodiversity data records for insects, arachnids, fishes, amphibians, reptiles, birds, and mammals. We standardised the species occurrence data by dividing the values by the duration of the dataset and subsequently analysed the differences in species occurrence by comparing the average species richness per year between provinces with low (<10 conflicts per year) and high (>10 conflicts per year) levels of conflict using the Mann–Whitney *U* test.

We then assessed the association between the conflict events and species occurrence. To do this, we measured the distance (in m) of species occurrence records to the nearest conflict events using the 'join attributes by nearest' in QGIS[58]. Employing a Poisson generalised linear model, we utilised the Gamlj module within the open-source software JAMOVI 2.3.19 to predict the impacts of taxonomic groups, distance from conflict areas, and frequency of conflict events on species richness at the provincial level[59,60]. We built two GLM candidates and evaluated the best model based on the lowest values of the corrected Akaike information criterion (AICc) and Akaike weight (wAICc)[61].

### Mapping conflicts events distribution

Assessing the impact of war and conflict on the environment is difficult in the absence of pre-war conflict data, which serves as a baseline. To provide insight into the relationship between conflict and the environment, we initially mapped and sampled conflict events to determine whether they were covered within the protected zones. We then correlated tree cover (%)[62], tree density[47], and forest canopy height[63] with the number of fatalities using Pearson's correlation coefficient (*r*). In our analysis, we used the number of fatalities per conflict event as an arbitrary measure, assuming that

## Box 1 | Recommended conservation strategies in conflict areas

1. *Establishment of a regional biodiversity database*: While large-scale biodiversity databases, such as the Global Biodiversity Information Facility (GBIF), are available and accessible, it is important to recognise that collecting data in the Philippines and in areas near conflict zones remains challenging. Additionally, valuable local studies may not be adequately organised and may not be readily available in biodiversity repositories (for example[40,64]). A collaborative and Findable, Accessible, Interoperable and Reproducible (FAIR) biodiversity database would be a valuable tool for harmonised conservation planning and assessment of biodiversity status on the ground[30].
2. *Crisis mapping and data analysis*: The use of crisis mapping and data analysis tools helps scientists assess the immediate and long-term impacts of conflicts on biodiversity. This data-driven approach, based on harmonised data (see #1), aids in identifying priority areas for intervention and enables rapid response to emerging threats.
3. *Use of eDNA and barcoding technologies to survey biodiversity*: In conflict-prone areas, where it may be risky to directly observe wildlife, environmental DNA (eDNA) and DNA barcoding offer a non-intrusive way to study and monitor biodiversity[65], even in challenging and high-conflict areas. These novel techniques can provide valuable data with or without less direct contact with the species or disturbing the environment for an extended period, which reduces exposure to threats in high-conflict areas.
4. *Biodiversity monitoring using remote-sensing technologies*: Conservation biologists can use satellite technology to monitor changes in land use and vegetation cover in conflict zones[66] (as demonstrated in this study). This allows for the assessment of the impact of war and conflict on biodiversity, identifying areas of concern, and developing targeted spatial conservation strategies[67].
5. *Applications of conservation drones, bioacoustics, and camera traps for surveillance*: Novel technologies, such as unmanned vehicles (i.e. drones) equipped with cameras and sensors, remote bioacoustics, and camera traps, can monitor wildlife populations and detect illegal activities in conflict zones, such as poaching and habitat destruction, in conflict zones[68]. This technology enables real-time or consolidated data collection without putting conservationists at risk (see the sections "Discussion" and "Methods"). However, the application of these technologies requires careful consideration before implementation in conflicted areas[69].
6. *Establishment of wildlife corridors*: Conservation biologists can establish wildlife corridors connecting fragmented habitats in conflict-affected regions during the postwar period. These corridors could help maintain genetic diversity and enable species to migrate, adapt, and survive despite conflicting challenges and environmental vulnerabilities.
7. *Creation of 'Peace Parks'*: Peace parks are transboundary conservation areas that span regions or territories affected by conflict[70]. These initiatives involve cooperation between neighbouring territories to protect shared biodiversity, promote regional stability, and encourage collaboration among communities rather than conflict.
8. *Mainstream adaptive strategies for conflict context*. Recognising the unique challenges and risks associated with conducting biodiversity studies within a conflict zone. Adapt conservation strategies that account for security considerations, access limitations, and flexibility. Develop contingency plans and implement risk management strategies to ensure the safety and well-being of personnel and local communities involved in conservation efforts.
9. *Partnership with the military for management and enforcement*: Conservation organisations and academic institutions may collaborate with governments, military forces, and international agencies to enhance the management and enforcement of protected areas in conflict zones. This involves deploying trained and experienced personnel to train the military, employing technology for biodiversity surveillance, and implementing strategies to prevent illegal activities (see #5). Additionally, military activity may be beneficial under certain conditions, such as when an exclusion zone is created, which facilitates the recovery of terrestrial and aquatic habitats[19]. This inadvertently safeguards wildlife and creates makeshift protected areas. However, reflecting on militarised actions and interventions in conservation zones is of utmost importance. Neglecting the balance between conservation and security may significantly increase the inclination to resort to violence, resulting in counterproductive and unjust consequences for both people and wildlife[21].
10. *Promoting citizen science in post-conflict management*: Citizen science engages the public, including non-professional scientists, in biodiversity research. In areas vulnerable to war and conflict, civilians can be crucial in gathering information about their local biodiversity in the post-conflict period. By involving residents as citizen scientists and creating accessible platforms (e.g. smartphone-based biodiversity reporting[71]), valuable knowledge and access to hard-to-reach areas can be obtained remotely.

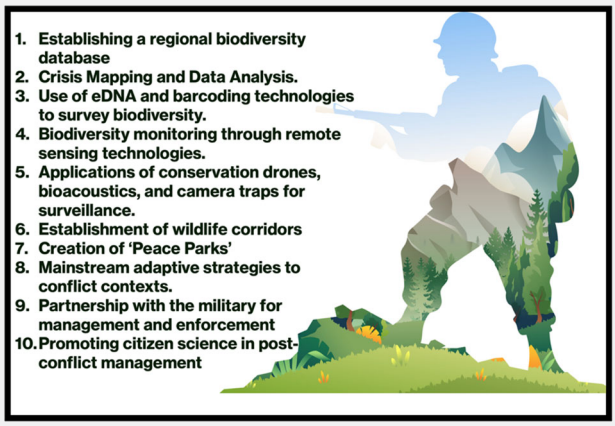



**Figure Box**. Recommended strategies to address biodiversity shortfalls within conflicted areas. Note: the image used from https://www.vecteezy.com/ under the Creative Commons Attribution-NonCommercial (BY-NC) License.

a higher fatality count corresponds to an increased level of conflict within a specific area.

## Data availability

All the data used in this study are publicly available online. For consolidated Mindanao biodiversity data, the Darwin Core Formatted dataset can be accessed here https://doi.org/10.15468/rtedgk. Conflict records can be accessed here https://data.humdata.org/dataset/philippines-acled-conflict-data.

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## Author contributions

B.A.H.H., K.C.T., and A.R.A. conceived the idea; K.C.T., K.C.D., S.S.A., and J.M.V.R. compiled and organised the biodiversity data; K.C.D., A.K.L, and J.M.V.R. organised the war and conflict data; K.C.T. led the spatial and statistical analyses; B.A.H.H, K.C.T., and A.R.A. wrote the main manuscript text; K.C.T. and K.C.D. visualised the data and prepared figures. All authors reviewed, discussed, and approved the manuscript.

## Competing interests

The authors declare no competing interests.

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# Caught on the Crossfire: Biodiversity Conservation Paradox of Sociopolitical Conflict

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# Abstract

Biodiversity loss is a growing concern globally, but there's a lack of knowledge on where to focus conservation efforts. It is therefore essential to determine priorities and barriers to biodiversity knowledge generation. In this context, we present evidence from the Philippines on how sociopolitical instability drives biodiversity shortfall. Our study showed that conflict leads to fewer biodiversity records, with low-conflict areas having higher turnover by more than 50%. Further, tree cover loss is lower in high-conflict areas. Security risks due to sociopolitical conflicts can significantly affect biodiversity recording efforts, potentially leading to underestimating species diversity in these regions. This study highlights the link between conflicts and biodiversity shortfalls, and the negative impact on biodiversity documentation efforts. While rare positive consequences exist, they are incidental and overall negative. Addressing the convergence of conflict, extremism, and biodiversity conservation is crucial for safeguarding ecosystems and human and nature's well-being.


# Introduction

Armed conflicts and violent extremism have long been recognized as significant threats to human security and stability. Armed conflict is a state of organized, violent confrontation involving weapons and military force between two or more parties, including governments, non-state actors, or a combination of both[1]. While sociopolitical conflict is a type of conflict or discord that arises from differing social and political beliefs, values, interests, or ideologies within a society[2,3]. It typically involves disputes, disagreements, or tensions between different groups or individuals with distinct social, cultural, or political backgrounds and perspectives[4]. However, the impacts of these conflicts on the environment and biodiversity have gained considerable attention in recent years[5,6]. For instance, the world witnessed the destruction of forests during the Vietnam War[7,8], the draining of Mesopotamian marshes during the Gulf War[9], and a decline in wildlife during civil wars in the Democratic Republic of the Congo[10]. At the same time, post-millennium wars in Ukraine, Afghanistan, Syria, and some parts of Africa continue to devastate biodiversity at an alarming rate[11–14]. Undeniably, armed conflicts and violent extremism can lead to extensive habitat destruction. In fact, of the major armed conflicts between 1950 and 2000 within countries, over 90% were within biodiversity hotspots, and 80% occurred directly within hotspot areas[6]. The environmental footprint of military activities, explosives, and landmines causes lasting damage to ecosystems, leading to the loss of critical habitats for diverse plant and animal species[4]. In conflict zones, a pivotal concern is their transformation into channels and central hubs for illicit wildlife trafficking and unregulated exploitation of natural resources. These actions significantly worsen the problem of biodiversity depletion by involving unsustainable practices, such as overharvesting species, habitat destruction, and the disturbance of delicate ecological equilibrium[15,16]. Additionally, armed conflicts tend to undermine the effectiveness of environmental governance systems, rendering enforcing environmental regulations and safeguarding vulnerable ecosystems formidable. This, in turn, amplifies issues such as poaching, deforestation, and the unlawful extraction of resources, thereby posing even greater threats to biodiversity[17].

These anthropogenic-driven threats to biodiversity prompted the United Nations (UN) General Assembly in 2001 to declare every 6th of November the *International Day for Preventing the Exploitation of the Environment in War and Armed Conflict*[18]. Twenty-two years later, many gaps still need to be addressed, especially in the global south, where biodiversity capacity building is lacking. Biodiversity hotspots, protected areas, and indigenous territories lack protection under international humanitarian law during armed and sociopolitical conflicts[17]. Previous analyses have demonstrated how warfare and conflict directly impact biodiversity[4,6], yet our understanding of how they drive biodiversity knowledge turnover is not well discussed in the current literature.

This perspective paper primarily aims to present empirical evidence drawn from the specific context of the Southern Philippines. We highlight the critical role that sociopolitical and armed conflicts play as limiting factors in generating comprehensive biodiversity information[19]. However, in regions affected by sociopolitical and armed conflicts for decades, the collection and analysis of this information can be impeded significantly. Conflicts can disrupt research activities, hinder access to remote or affected areas, and create safety concerns for scientists and conservationists. This, in turn, limits our ability to gather accurate data about local flora and fauna, which is essential for making informed decisions about biodiversity conservation and management. Therefore, understanding the impact of such conflicts on biodiversity information generation is crucial to developing effective conservation strategies in conflict-prone regions, such as the Philippines.

## War and armed conflict in the Philippines: Why is Mindanao a ‘hotspot’?

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Mindanao is the second-largest group of islands in the Philippines, consisting of 27 provinces and 33 cities with six administrative regions with an estimated population of 26,252,442 (24% of the country’s population), hailing it as the seventh-most populated area in the world[20]. Its large fertile landmass makes it a major breadbasket of the Philippines, producing around 40% of the country’s agricultural produce and 60% of agricultural exports[21]. In addition, owing to its unique biogeographical history and location, Mindanao is renowned for its vast flora and fauna, making it a biodiversity hotspot[22,23]. Mindanao has over 30 Key Biodiversity Areas (KBAs) that are globally significant sites for biodiversity conservation because of the high concentration of endemic and threatened species, including the globally threatened Philippine Eagle (*Pithecophaga jefferyi*) and other keystone species[23–26].

Whilst Mindanao is known for its rich and diverse wildlife and valuable natural resources, the region confronts a disconcerting reality marked by a confluence of sociopolitical adversities encompassing armed conflicts, religious tensions, feuds among clans, incidents of abduction, and other incidents of violence that paint a grim picture of the region[27]. The UN Office for the Coordination of Humanitarian Affairs (OCHA) has estimated that Mindanao shared around 53% of sociopolitical conflicts in the

Philippines from 1989 to the present[28] (Fig. 1). The history of war and armed conflict in Mindanao is complex, spanning several decades [27,29]. Conflict in Mindanao can be traced back to the colonial era, when the Philippines was under Spanish rule, which started during the 16th century. The Spanish colonizers encountered resistance from the Muslim communities in Mindanao, who fought against their conversion to Christianity and the imposition of colonial rule[30]. Today, the Philippine government is involved in multiple parallel, non-international armed conflicts and violence[2].

Continuous conflict spanning 60 years has probably led to considerable ecological and environmental degradation in the Philippines, especially in Mindanao. This damage could manifest in various forms, including deforestation, habitat destruction, pollution, and overexploitation of natural resources[31]. As armed conflicts often involve the displacement of communities, pressure on local ecosystems may increase as displaced populations resort to unsustainable practices for their livelihoods. Furthermore, armed groups and the instability associated with conflicts can hinder effective environmental conservation efforts, exacerbating the environmental toll. In addition, socioeconomic challenges can also indirectly impact the environment, as people may resort to resource-intensive activities, such as illegal logging or mining, to cope with the economic hardships caused by the conflict.

Despite these apparent potential links between armed conflict and environmental and socioeconomic consequences, there remains a significant absence of empirical evidence explicitly exploring the connection between war and biodiversity shortfalls in the Mindanao region. While some Philippine-wide studies have shown apparent differences in biodiversity in Mindanao (for example, in bats[32] and primates[33]) in recent years, no clear evidence has shown the relationship between sociopolitical conflict and the generation of biodiversity information in the region. Addressing this knowledge gap is crucial for understanding the full extent of the environmental impacts of conflict, formulating effective policies and strategies to mitigate these effects, and promoting sustainable development and conservation in the region.

To gain insight into the influence of socio-political conflicts on biodiversity generation in Mindanao, we compared the information from the distribution of biodiversity data with conflict-related information for the Philippines between 2000 and 2021. We then investigated the differences between low (< 10 conflicts/year) and high (> 10 conflicts/year) conflict areas in both biodiversity turnover (i.e., species records) and tree cover loss (2000–2022), as a proxy to determine whether conflict events affect environmental disturbance. Finally, we also assessed the effects of taxonomic groups, conflict distance, and number of conflict events on species turnover.

# Results

Our analysis reveals how sociopolitical conflict promote biodiversity shortfalls within hotspots. Firstly, a total of 2174 (103.52 conflict/year) was recorded in the entire Mindanao region from 2000 to 2021, with the highest percent records in Sulu (n = 382 per year, 18%) and Maguindanao province (n = 329 per year, 15%) (Fig. 2a). We found that there is a significant difference in the biodiversity record turnover between

high (mean = 0.50 ± 1.60) and low (mean = 1.27 ± 2.37) conflict areas (Mann-Whitney U test = 1340, $p$ = 0.0027) (Fig. 2a-b).

Secondly, the best model that predicts biodiversity turnover (species record turnover ~ 1 + conflict events + average distance from conflict events + taxonomic groups, AIC = 7864.53) showed that an increase in the number of conflict events is associated with lower species records (β = − 0.002, $p$ < 0.0001). Conversely, as conflict events moved farther away, our model showed an increasing turnover of species records (β = 0.003, $p$ < 0.0001). This association was particularly strong among insects (β = 2.067, $p$ < 0.0001) and birds (β = 1.545, $p$ < 0.0001) (Fig. 2c).

Contrary to our expectation, in terms of tree cover loss, we found a significantly lower tree cover loss within high-conflict areas (mean = 9262 ha) than in lower-conflict areas (mean = 3738 ha) in Mindanao (Mann-Whitney U test = 735, $p$ < 0.0001) (Fig. 3), with highest tree cover loss in Agusan del Sur province (22%).

# Discussion

Our findings provide valuable insights into the link between sociopolitical conflicts and biodiversity shortfalls within a biodiverse region of the Philippines. Notably, a significant difference in biodiversity record turnover was observed between the high- and low-conflict areas within these regions. High-conflict areas exhibited a lower biodiversity record turnover (more than 50% difference). These differences and evident biodiversity shortfalls within highly conflicted areas may indicate that conflict events notably impact the stability of species populations in these areas[4]. In high-conflict areas, the number of species or their populations has likely declined, resulting in an observed biodiversity that falls well below the expected levels due to conflict-related pressures, such as habitat destruction and overexploitation. In addition, recording and inventories of biodiversity within highly conflicted areas are likely to hinder biologists due to security risks brought about by sociopolitical conflicts. Our analysis supports the notion that species turnover increases, especially among insects and birds, as the number of conflict events moves farther away from a conflict area.

These findings emphasize the detrimental impact of conflict events on biodiversity within biodiversity hotspots, and how conflict limits our understanding of biodiversity in biodiverse areas. Understanding these dynamics is crucial for conservation efforts in conflict-affected areas, where targeted strategies are needed to mitigate the consequences of conflicts on biodiversity, restore habitats, and promote sustainable recovery. Moreover, these insights may inform policy decisions to minimize the environmental and ecological consequences of armed conflicts, thereby reducing biodiversity shortfalls in these regions.

Our analysis sheds light on the complex and multifaceted interplay between sociopolitical conflicts and biodiversity. The negative correlation between the frequency of conflict events and species richness underscores the vulnerability of biodiversity in regions marked by unrest, particularly in terms of limiting

the generation of biodiversity information. Thus, we highlight the urgent need for conservation efforts and protective measures in conflict-affected areas. Conversely, the positive relationship between distance from conflicts and biodiversity records indicates the importance of the absence of war for biodiversity. Additionally, regions less affected by human disturbance act as refuges for various species, contributing to the overall conservation of biodiversity. This highlights the importance of creating wider safe zones. The positive relationship of species record turnover with distance to conflict areas suggests that some taxa thrive in environments with low conflict events.

Although most areas in Mindanao are now relatively accessible, conducting biodiversity research is still challenging due to fear brought about by the breakdown of local peace and order in some areas, such as the Liguasan Marsh[25]. One of the important factors that greatly affects biodiversity shortfalls within conflicted areas is the threat to security associated with sociopolitical conflicts. Biologists and conservationists often face challenges when conducting fieldwork in regions with ongoing disputes or unstable political situations. These challenges range from personal safety concerns to logistical difficulties in accessing remote and unstable areas. As a result, comprehensive biodiversity inventories might be limited or even impossible to carry out in such high-conflict regions. Several non-state armed groups operate in Mindanao, particularly in Maguindanao, Lanao Del Sur, Basilan, Sulu, and Tawi-Tawi[34]. Other violent tensions, such as clan wars or *'rido'* and political disputes, brought brutal incidents, for example, the Maguindanao massacre in 2011[35].

Another example is the kidnapping of bird watchers in Tawi-Tawi by the *Abu Sayyaf* group in 2012[36]. This prompted foreign and local authorities to advise their citizens, including biodiversity researchers, to refrain from travelling or visiting Mindanao. Consequently, conflict areas such as Basilan, Zamboanga del Norte, Tawi-Tawi, Zamboanga Sibugay, Sulu, and Isabela remain lacking in biodiversity information. In regions where security risks impede the ability of scientists to conduct biodiversity assessments, the documented biodiversity may underrepresent the actual diversity of species. This disparity can have profound implications for conservation efforts and our comprehension of the true biodiversity status of conflict-stricken regions. Interestingly, our findings indicate that as conflicts recede, researchers may become more willing to conduct biodiversity assessments in previously conflicted areas.

## Conflict areas: Nature's unintended refuge?

We posit that armed conflict can positively impact biodiversity in certain instances. For example, conflict zones become inaccessible to tourists or dangerous for human activities, reducing human disturbances such as hunting, deforestation, and other destructive activities. This inadvertently safeguards wildlife and creates makeshift protected areas. During periods of armed conflict, local communities, including indigenous communities, may retreat to remote areas, where they rely on traditional knowledge and sustainable resource management. These practices often prioritise protecting and conserving biodiversity and safeguarding valuable ecosystems. In addition, armed conflicts can attract significant media

coverage and international attention, which may result in increased conservation funding, enabling the protection and restoration of ecosystems affected by conflicts, thus benefiting biodiversity.

Additionally, military activity may be beneficial under certain conditions, such as when an exclusion zone is created that generally results in population increases and population recovery in terrestrial and aquatic systems[4]. Although war and armed conflicts can be acknowledged as offering transitory advantages to biodiversity conservation by creating no-go zones that inadvertently safeguard certain species and their fragile habitats, it is essential to understand that these are unintended and incidental and do not justify or outweigh their negative consequences. However, it is crucial to recognize that these areas remain inherently unstable and susceptible to transformation into active conflict zones with unpredictable impacts on biodiversity preservation. It is imperative to recognize that the consequences of war and conflict on socioeconomic stability are far-reaching and have significant long-term effects on the ecosystem, greatly outweighing any potential benefits.

# Addressing biodiversity shortfalls in a heavily conflicted area

Conversation of the impact and recognition of armed conflict and violent extremism vis-à-vis biodiversity remains the elephant in the room, which is often neglected in biodiversity prioritisation[6], especially in Mindanao[31]. Specific connections between armed conflict and biodiversity conservation have garnered limited attention[3]. Schulte to Bühne (2022) state that the current legal and policy frameworks regulating global biodiversity conservation do not address the challenges of conducting activities in areas affected by conflicts[38]. He adds that, to incorporate conflict-sensitive protection into international policymaking, these organizations should openly address the consequences of armed conflicts on biodiversity. Hulme (2022) suggests that international environmental laws (IEL) can be a tool for environmental protection during armed conflicts[39].

Moreover, the interplay among armed conflict, violent extremism, and biodiversity conservation is a multifaceted and complex issue that warrants greater scientific and political attention. Acknowledging the link between conflict and biodiversity is essential for shaping effective national and international policies and legal frameworks that can address the unique challenges posed by conservation efforts in conflict-ridden areas, ultimately contributing to protecting diverse ecosystems and species. To address these biodiversity shortfalls within conflicted areas, we recommend actions and considerations for biodiversity conservation (Fig. 4).

1. **More effort should be spent to document and monitor areas with high biodiversity shortfalls.** It is imperative that a concerted effort be made to thoroughly document and monitor regions with notable deficits in biodiversity, to identify areas where gaps exist in biodiversity data, and to establish comparative monitoring assessments that employ standardized, transparent, accessible, and reproducible methods to accurately record previously unrecorded biodiversity. This is crucial for

ensuring a comprehensive understanding of the state of biodiversity, and for making informed decisions regarding conservation and management efforts[23,40].

2. **Collaborate with local communities to promote biodiversity assessments and inventories.** Initiate meaningful engagement and foster collaboration with local communities residing in conflict-affected zones. Cultivate relationships grounded in trust, respect, and mutual comprehension. Include community members in decision-making processes, prioritizing and incorporating their perspectives and needs, as they are pivotal in safeguarding and overseeing natural resources.
3. **Strengthen capacity building in highly impacted areas.** Education and awareness programs play a significant role in fostering a sense of environmental stewardship and creating a supportive network for conservation efforts[41]. It is imperative to invest in genuine capacity-building initiatives that empower local communities, conservation practitioners, and researchers in conflict zones. Long-term effective training on conservation techniques, sustainable resource management, and conflict-sensitive approaches should be provided to promote the conservation of biodiversity in these regions.
4. **Secure funding and establish equitable partnerships.** Collaborate with international organizations, non-governmental organizations, research institutions, and governmental agencies to harness resources, expertise, and funding for conservation projects in conflict zones, seeking support from donors and foundations that specialize in environmental and humanitarian issues. Strong partnerships and financial stability are crucial for the longevity of conservation efforts.
5. **Mainstream adaptive strategies to conflict contexts.** Recognize the unique challenges and risks associated with conducting biodiversity studies within a conflict zone. Adapt conservation strategies that account for security considerations, access limitations, and flexibility. Develop contingency plans and implement risk management strategies to ensure the safety and well-being of personnel and local communities involved in conservation efforts.
6. **Advocate for policy support towards biodiversity conservation**: It is imperative that appropriate policies are implemented to create a supportive environment for the success of long-term biodiversity conservation efforts. The advocacy of policies that enhance biodiversity conservation in areas of conflict is crucial in addressing the inadequacies in these regions. This involves collaborating with local and national authorities to develop and implement legislation that promotes biodiversity research to provide evidence for the effective protection of natural resources, the establishment of conservation areas, and the promotion of sustainable development practices in highly conflicted regions.

# Moving forward

We presented clear evidence of the impact of sociopolitical conflicts on biodiversity. Conservation efforts in conflict zones require a multidimensional approach that addresses both the ecological and sociopolitical aspects of the situation. This requires resilience, adaptability, and a commitment to building peace and protecting biodiversity, even under challenging circumstances. Recognizing the need to address the intersection of armed conflict, violent extremism, and biodiversity conservation is crucial

for protecting ecosystems, and securing the well-being of humans and the natural world. Governmental efforts should focus on conflict prevention, post-conflict environmental restoration, initiatives to strengthen environmental governance, and engaging local communities to ensure sustainable management of natural resources. Peace, stability, and effective environmental governance are crucial for the long-term protection and conservation of biodiversity. Post-conflict reconstruction and peacebuilding efforts can provide opportunities to integrate sustainable development and conservation practices, promoting the recovery and preservation of biodiversity in conflict-affected regions, especially in the global south, where both conflict and biodiversity are highly concentrated.

# Methods

We determined the association of sociopolitical conflicts on biodiversity turnover in Mindanao. We first obtained biodiversity data from the MOBIOS$^{+}$ database[40] and conflict-related information for the Philippines from UN-OCHA[28] spanning 2000 to 2021. For the biodiversity data, we only included frequency of data records for insects, arachnids, fishes, amphibians, reptiles, birds, and mammals. We normalised species record turnover by dividing the values to 22 years. We then examined the differences in biodiversity turnover by comparing the average species records per year between low (< 10 conflicts/year) and high (> 10 conflicts/year) conflict areas using the Mann-Whitney U test. Using the same analysis, we compared tree cover loss (2000–2022) using Global Forest Watch data[37] as a proxy to determine whether conflict events contribute to environmental disturbance. We measured the distance (in metres) of biodiversity records to nearest conflict events using the 'join attributes by nearest' in QGIS[42]. Furthermore, using a simple Poisson generalised linear model, we determined the effects of taxonomic groups, conflict distance, and number of conflict events on species turnover at the provincial level using *Gamlj* module in the open software JAMOVI 2.3.19.[43,44]. We evaluated the best model based on the lowest values of the corrected Akaike information criterion (AICc) and Akaike weight (wAICc)[45].

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# Figures

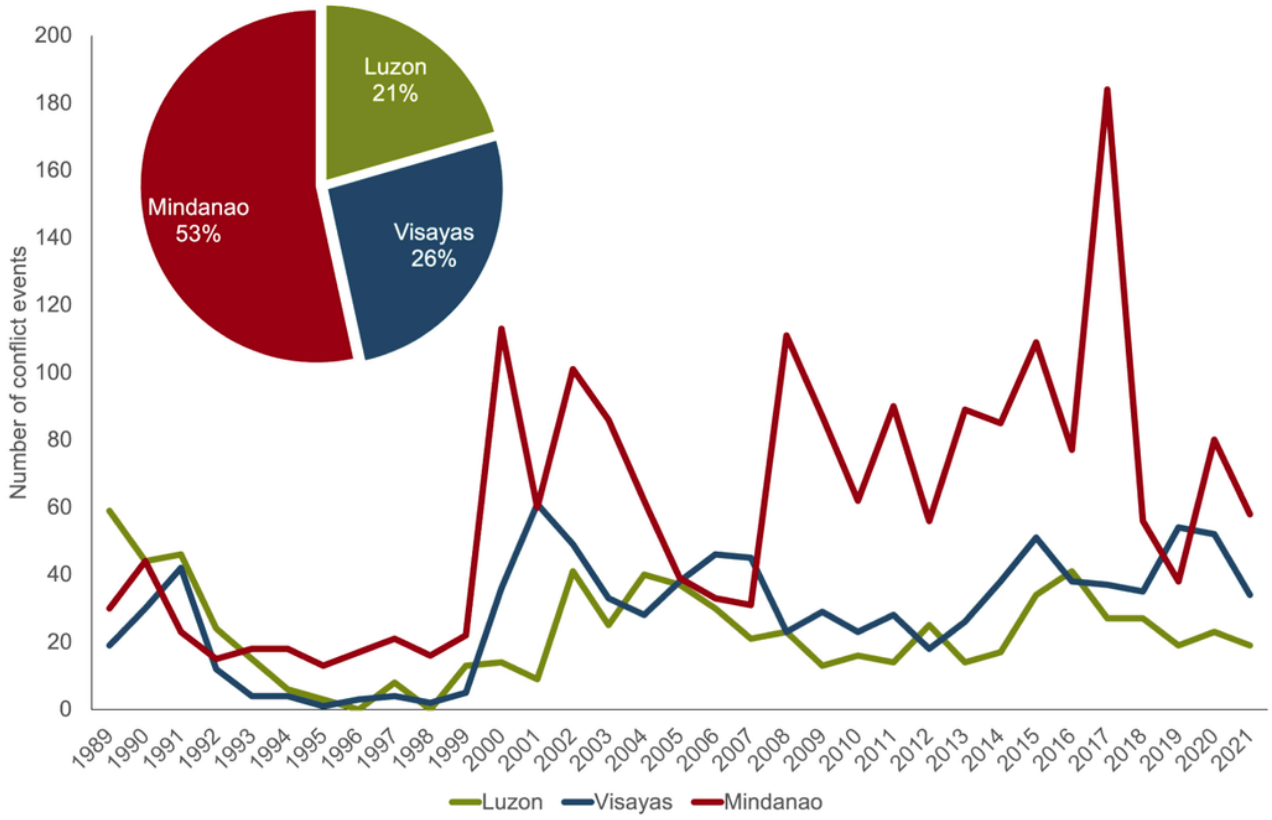
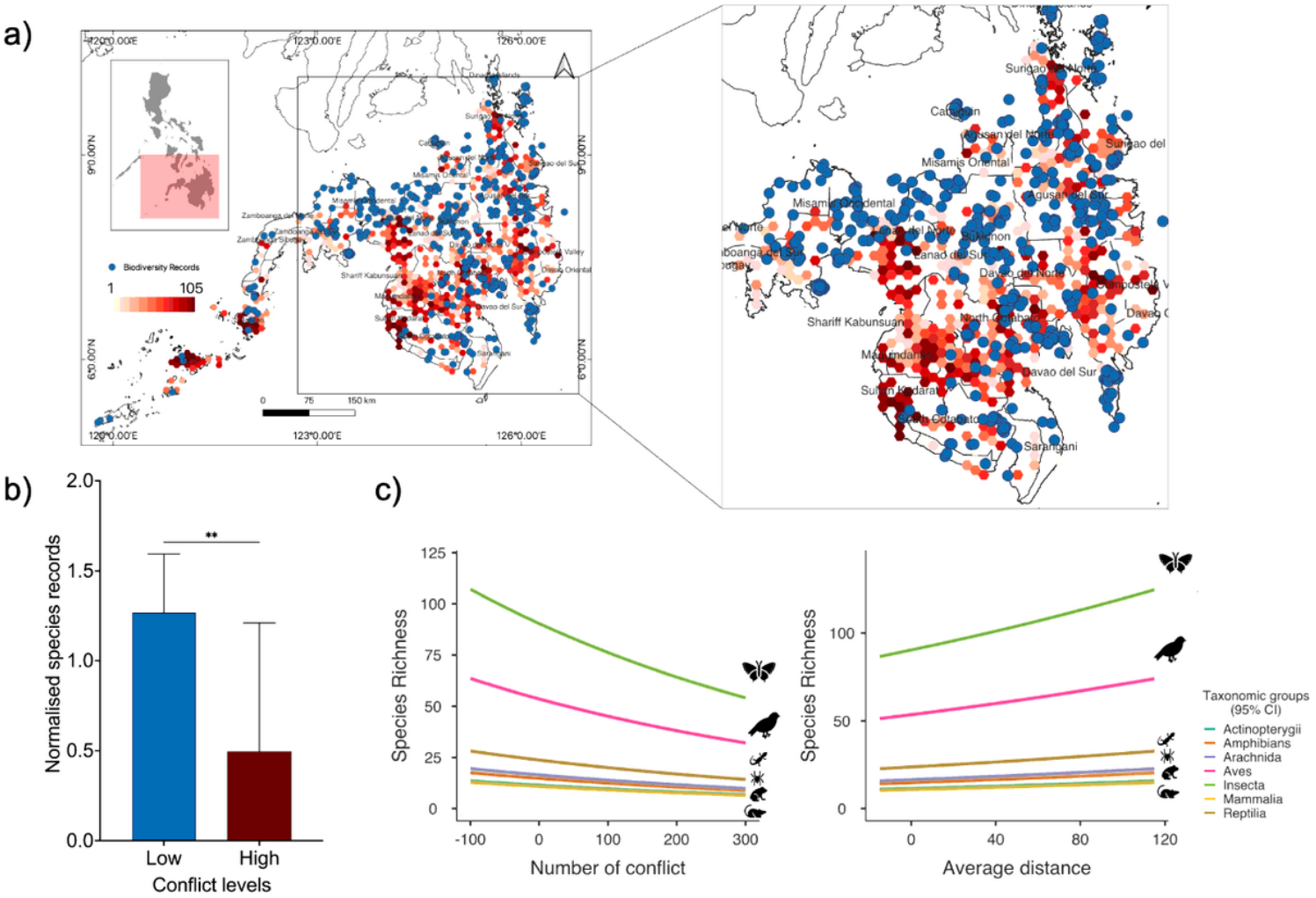



**Figure 1**

Temporal trends in conflict events in the Philippines from 1989 to 2021 based on the UN OCHA[28].




## Figure 2

Relationship between sociopolitical conflict and biodiversity in Mindanao: a) distribution and overlapping of conflict events (red dots) and biodiversity records (blue dots); b) comparison of low- and high-conflict areas, c) visualised results of the generalised linear model (Poisson GLM) showing the association of conflict event and species record turnover. Note: *** indicates significance at $p < 0.0001$; whiskers and shading represent 95% CI.

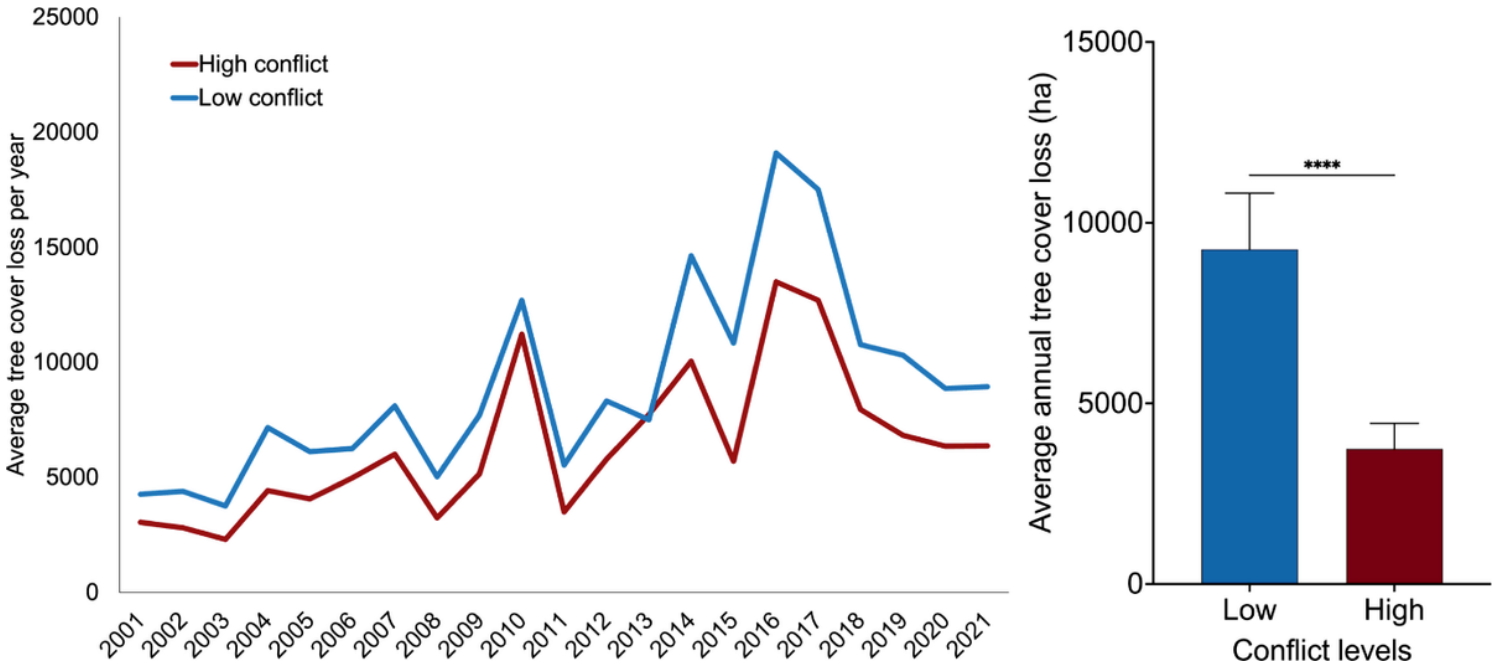



**Figure 3**

Comparison of tree cover loss (in hectares) in Mindanao from to 2001-2021 based on remotely sensed data from Global Forest Watch[37]. The average tree cover loss was significantly different between the low- and high-conflict areas. Note: *** indicates significance at $p < .0001$; whiskers represent 95% CI.

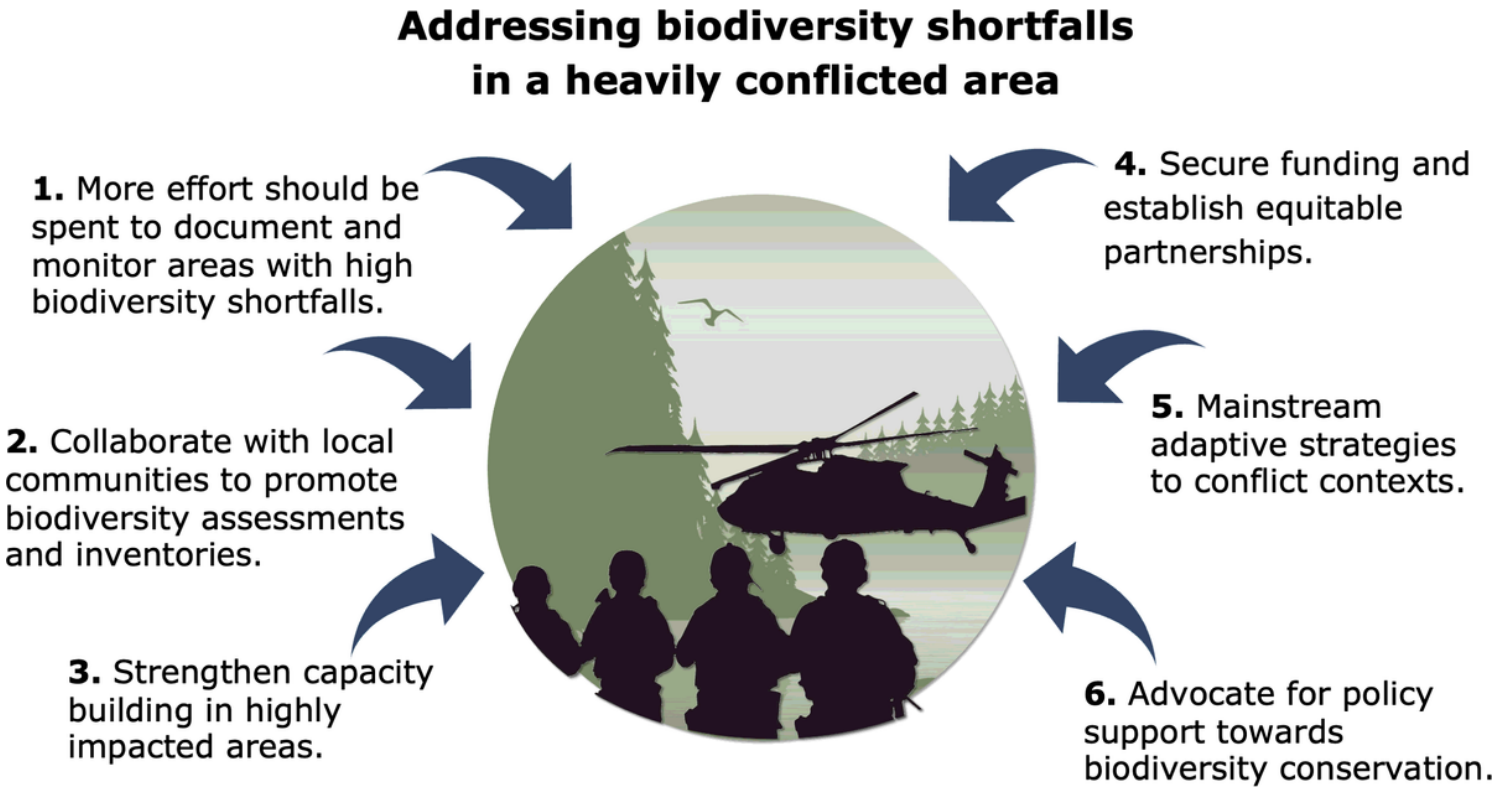



**Figure 4**

Recommended strategies to address biodiversity shortfalls within conflicted areas.

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# Comparison of Item Difficulty Estimates in a Basic Statistics Test using ltm and CTT Software Packages in R

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# Comparison of Item Difficulty Estimates in a Basic Statistics Test using ltm and CTT Software Packages in R

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***Abstract*—Two free computer software packages "ltm" and "CTT" in the R software environment were tested to demonstrate its usefulness in an item test analysis. The calibration of the item difficulty parameters given the binary responses of two hundred five examinees for the fifteen items multiple choice test were analyzed using the Classical Test Theory (CTT) and Item Response Theory (IRT) methodologies. The software latent trait model "ltm" employed the IRT framework while the software classical test theory functions "CTT" operated under CTT. The IRT Rasch model was used to model the responses of the examinees. The conditional maximum likelihood estimation method was used to estimate the item difficulty parameters for all the items. On the other hand, all the item difficulty indices using the "CTT" software were also calculated. Both the statistical analyses of this study were done in the R software. Results showed that among the fifteen items, the estimates of their item difficulty parameters differed mostly on their values between the two methods. In an IRT framework, items showed extreme difficulty or easy cases as compared to CTT. However, when the estimated values were categorized into intervals and labelled according to its verbal difficulty description, both methodologies showed some similarities in their item difficulties.**

***Keywords*—Classical test theory; indices; item calibration; item difficulty; item response theory; R software**

## I. Introduction

In the field of education particularly in test and measurement, it is important that any method that uses technology should be upgraded from time to time. This technology that performs computing and analysis requires speed and precision especially if the data is huge. Hand computation seems to be tedious and possible but it will take a longtime. In the case of a test or item test analyses, it is important that the item calibration for the estimates of its item parameters is accurate, fast and reliable. Statistical software packages that perform these calculations are available, either purchased commercially or as a free software in the internet.

Test item analysis is very important especially in the test construction. First, test can be classified with its degree of difficulty and second, for item banking that is, the calibrated items are stored traditionally in a box or electronically in a database. These items were given labels for its corresponding levels or index of difficulty of which it can be retrieved anytime for test construction. This method is useful for test makers in the composition of test items and the determination of the difficulty or easiness of the test instrument.

The primary objective of this paper is to demonstrate the usefulness of the two computer software programs, the latent trait model "ltm" [1] and the classical test theory functions "CTT" [2] in the R software environment in the calibration of item difficulty parameter estimates/indices for a multiple-choice test. Two methodologies, the item response theory and the classical test theory will be used. Specifically, this study will employ the Rasch model [3], an IRT probabilistic model which is part of the logistic model family, to model the responses of all the examinees for all the items. Estimation for all the item difficulty parameters will be carried using the conditional maximum likelihood estimation [4]. The calculated item difficulty estimates will be compared to the calculated difficulty indices of the same test examination that uses the scores of the examinees under the classical test theory methodology. One point of interest in this study is the comparison of the verbal description of the items in terms of its difficulty labels. Here we will know whether each item estimates are comparable for both methodologies or they both possess extreme differences.

## II. Background of the Study and Related Studies

### A. Item Calibration

In the calibration of item parameters, specifically the difficulty indices $\beta$ of an examination test say in the case of a multiple-choice test in which the resulting data is a matrix of binary responses of the number of examinees who took the examination and the number of items being answered, Two methodologies are available at present in the literatures to handle such calibration. These methods are the Classical Test Theory (CTT) which is based on prediction of outcomes on a test that is, in particular an examinee's observed score which is composed of a true score and an error score and the Item Response Theory (IRT) which is based on a response probabilistic modeling [4]. CTT usually do the estimation of the reliability of a test and the item difficulty indices which comes from the score of the examinees. In practice, these indices are also known as the p-value and is valued from 0.0 to 1.0 for each item and it is based on the proportion of all the examinees who got the correct answers over the total

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examinees. The higher the proportion of getting correct answers, the easier is the item. CTT however, has many limitations as cited by [5]. On the other hand, test makers are also adopting model based IRT because it is powerful and can provide a framework for evaluating how good assessment do its job and how good its item do its job. For calculating item difficulty for example in a multiple-choice test, IRT traditionally applied based on a large number of historical correct or incorrect information gathered from the test [6] and in turn applies probabilistic models as mention by [4]. See also [7], [8], [9] and [10] for more discussion about these item response theory modeling.

### B. Available Statistical Software Programs

Statistical software packages are presently available for calibrating item parameters in which CTT and IRT models are used. These includes powerful commercial software such SAS [11], STATA [12], SPSS [13], M plus [14], the BILOG-MG software [15] and ConQuest software [16] for fitting item response latent regression models and many more. However, there are some commercial software packages that are not easy to learn as well, hence it is must to do an extensive training if you want learn it because some of the software corresponding documentations are difficult to comprehend and sometimes have program failures and limitation which can be frustrating.

There is also a software package developed by The National Institute for Educational Measurement of the Netherlands (CITO) called OPLM [17] which is free and can be obtained by request. Starting in the year 2000, a quite number of new IRT packages uploaded as a library were developed in the open source in R software environment [18]. These includes computer software programs called the latent trait model (ltm) which was intended for unidimensional item response theory [1] as mentioned earlier, the extended Rasch models called eRm [19], the software called mirt which is intended for multidimensional IRT [20], and the software called mlirt which is intended for multilevel and Bayesian estimation [21]. Also, a software in R that uses Bayesian methods is also available called R2WinBuGs [22]. Lastly the software called "CTT" is intended for the estimation of items parameters under the classical test theory methodology [2].

## III. Material and Methods

### A. The Dataset

The data used in this study were the responses of two hundred five (205) students who responded to a fifteen (15) items multiple choice test. The test was just part of the Basic Statistics Preliminary Examination of the Mathematics and Statistics Department of the College of Science and Mathematics, Mindanao State University –Iligan Institute of Technology during the second semester school year 2014-2015 [23]. The test questionnaire was made by the authors and was validated for its content. The responses of these students were tabulated in a 205 by 15 matrix of 1's, when student got correct answer to the given item and 0's, when student got a wrong answer to the given item (see Table I for the illustration).The data then was stored as a text file having file name. The data was processed using a personal computer.

TABLE. I. Illustrating the Data Matrix

| examinee | Item 1 | Item2 | … | Item 15 |
|---|---|---|---|---|
| 1 | 1 | 0 | . | 0 |
| 2 | 0 | 0 | . | 1 |
| . | . | . | . | . |
| . | . | . | . | . |
| 205 | 1 | 0 | . | 1 |

### B. Item Response Theory Models for Binary Response

In an IRT framework, one can specify the components affecting the probability that an examinee will respond in a particular way to a particular test item. We can choose a particular measurement model that will relate the responses of the examinees and the qualities of the items. In this study the Rasch model was the model used to obtain the estimates of the item difficulty applying the conditional maximum likelihood estimation method. This model is one of the simplest item response theory models [24]. In general, the model is characterized as a two parameter models with the ability parameter of the examinee and the other parameter is the characteristics of the item which is the difficulty parameter [25]. The model is given by.

$$P\left(X_{nj}=1\middle|\theta_n,\beta_j\right)=\frac{e^{(\theta_n-\beta_j)}}{1+e^{(\theta_n-\beta_j)}}$$

where $X_{nj}$ refers to a response made by an examinee $n$ to an item j, $\theta_n$ refer to the trait level or ability of an examinee $n$; and $\beta_j$ refers to the difficulty characteristics of the item $j$ and it may take values between -3 (Very easy) to 3 (very difficult). The expression, $P\left(X_{nj}=1\middle|\theta_n,\beta_j\right)$ is the chance or the likelihood that an examinee n will give an answer to an item correctly conditional to his ability $(\theta_n)$ and the difficulty of the item $(\beta_j)$. It is common in IRT that all measurements in the ability and difficulty before being subjected to estimation under the Rasch model are transformed into standard normal so normal measurements will be used. In the Rasch modeling, an examinee's answer in a form of a dichotomous response (that is, in our data, 1 refers to an examinee who got a correct response to an item while the entry 0, means that the examinee got a wrong answer) can be explained by the examinee's ability and the difficulty characteristic of the item. Now, in order for the model to be generalized, assumptions are considered that will make the model hold. Please see [26] and [27] for more explanations. The majority of applications of item response theory models usually to categorical data as known in [3], [7], [8], [9] and [10] but they were also being applied to data where there are continuous responses. These can be seen in the literatures [28] and [29].

### C. Information Characteristics Curve (ICC) under the Item Reponse Theory

The Information Characteristics Curve (ICC) represents the item response function (IRF) which is the likelihood or chance of getting a positive response to each item which is represents the function of the proficiency or ability $\theta$ of the examinees. One can represent in the same graph the observed and the expected ICCs to get the fit of each item [30].

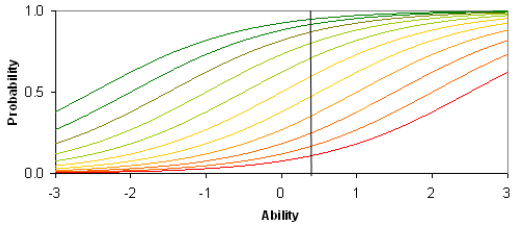



Fig. 1. Information Characteristics Curves (ICCs).

Fig. 1 illustrates ICCs for a number of items. ICCs highlight the change in the chances or likelihood of a successful response for an examinee with its ability location at the vertical line. The examinee will likely respond correctly to the easiest items (with locations to the left and higher curves) and unlikely to respond correctly to difficult items (locations to the right and lower curves) that is, the x-axis is the theoretical ability or proficiency level, ranging from -3 to +3. This graph only represents theoretical modeling rather than empirical data. To be specific, there may not be examinees that can reach a proficiency or ability θ level of +3 or fail so miserably as to be in the -3 group. Nonetheless, to study the characteristics of an item, we are interested in knowing, given a person whose θ is +3, what the probability of giving the rights answer to an item. The ICC indicates that when θ is zero, the examinee is on an average ability or proficiency hence, the chances of the examinee of answering the item correctly is approximately 0.5 or 50%. When the ability level θ is -3, the probability is almost zero to correctly get the item. When θ is +3, the probability to correctly answer the item increases to 0.99 or 99%.

### *D. Maximum Likelihood Estimation for the Item Difficulty with the Rasch Model*

In order to calculate the values of the estimates of the item difficulty parameters, the computer software program "ltm" which means Latent Trait Models under the IRT framework [1] will be used in the calibration of item difficulty estimates and is based on the environment of the software R. Further, the "ltm" adapted both the conditional maximum likelihood (CML) and marginal maximum likelihood (MML) estimation methods that handles the calculation in estimating item parameters and person parameters mathematically. In our study we employed the conditional maximum likelihood to calculate the item difficulty parameter. For more details, please see [6], [27], [31] and [32]. Although, maximum likelihood methods are the common estimation methods for years in the calibration of examinee's proficiency or ability and item parameters particularly the discrimination, difficulty and the guessing parameters another alternative estimation method emerged. The development of the Bayesian framework as an alternative but very powerful sampling-based estimation techniques have encouraged the application of Bayesian methods. The Markov Chain Monte Carlo (MCMC) methods, such as Gibbs sampling and Metropolis-Hastings (M-H), were used to simultaneously estimate all model parameters. An MCMC implementation are introduced for the sampling of all model parameters that combines various advantages of different MCMC schemes for sampling IRT parameters [33]. For example [34] estimated the ability and item parameters of the IRT model for the observed data using MCMC.

The Bayesian inference requires the computation of the posterior distribution for a collection of random variables (parameters or unknown observables). At present, numerous simulation-based methods emerged. On sampling by [35] and [36], Other Bayesian works see [37] and [38]. Statistical software packages like WinBUGS (Bayesian inference Using Gibbs Sampling) [39] is a popular software for analyzing complex statistical models using MCMC methods.

### *E. Classical Test Theory*

The classical test theory (CTT) is a theory of measurement error. The classical test theory has an assumption that each examinee's actual observed score X is the sum of the examinee's true score T and the error score E that is X=T+E. The key concepts of this theory involved the determination of test's reliability and validity for which test can be assessed mathematically [40]. The study and application for the classical test theory has been continuing which can be seen in the literatures [41]. Further, major applications of this theory are also on the test and item analysis and observed score equating. An article published in [42] looks for working on the classical test theory in combination with the concept of the item response theory. Their paper, emphasized that since the classical test theory was built in the assumption of exchangeability and the item response theory was based on conditional independence then they concluded that item response theory can be considered as an extension of the classical test theory where the concepts for both theories are related with each other. What is interesting in their work is the capability of IRT to provide the classical test theory statistical values where it can provide.

In our study, the software package "CTT" will be used to calculate the item difficulty indices of the test which is based on the proportion of the total number of examinees who got a correct answer on the given item and the total number of examinees. The closer the value of the index of difficulty of the given item to 1, the easier is the item and the closer it is to 0 the item will be very difficult. An index of 0.5 means that the item is average in its difficulty. We will also categorize the different intervals so that item difficulty indices can be given a verbal description. Moreover, in this study for reasons of simplicity and completeness of the estimation of item difficulty parameter for each of the 15 items, we assume that the item response theory's Rasch model fits the data, that is in every responses of examinees on each item fits in the model. Although, we will check the goodness of fit of the items to the model by statistical means as done by [43] in the process. For the sake of comparison, we do not discard items in the estimation that do not fit the given item response model, that is we need the complete 15 item difficulty estimates so we can compare it to the values in the classical test theory.

## IV. Results and Discussions

This section will present three results. First is the presentation and discussions of the information characteristic curve (ICCs) of the fifteen items under the item response theory methods. Second and third is the simultaneous tabular

presentation of the calculations and analysis of the results for the estimation of the item difficulty under the item response theory (IRT) and the classical test theory (CTT) methods.

### A. The Information Characteristics Curves of the Items

Fig. 2 are the information characteristic curves of the fifteen items, the ICCs of the fifteen items above can be converted into an Item Characteristic Curves (ICC) which are graphical functions that shows the examinees proficiency or ability as a function of the likelihood or chances of answering correctly the item. We can see through the curve that most of the items (9 out of 15 items) are difficult because higher abilities are needed to get higher probabilities of getting correct answer. We can see in the figure that items 8 and 12 are the very difficult items. To further support these observations, we will calculate mathematically using the Rasch model under the item response theory methods the values of the estimates of the difficulty of the fifteen items. Then we will incorporate the results of fifteen difficulty indices of the items under the classical test theory.

### B. Fit of the Item to the Rasch Model

Checking the fit of the data to the Rasch model, the results show that some items are a "misfit", a terminology in modeling for those items that do not fit the model. As we mentioned above those items that do not fit in the Rasch model are supposedly discarded but for the purpose of comparison with difficulty indices under the classical test theory we will retain it. Table II shows those items that fit and also do not fit the Rasch model. To test the fit of the data responses of the item to the model, we use the Chi-square test. If the p-value of that item is less than 0.05 or 5%, we do not reject the hypothesis that the item fits the model. Based on the results in Table II, the following items fit the model in particular items 2, 3, 7, 10, 11, and 12. On the other hand, items 1, 4, 5, 6, 8, 9, 13, 14, and 15 in the test did not fit the model. Items that misfit the Rasch model means that the Rasch model is not a good model for these items, hence for model fitting purposes, another type of item response theory should be considered as a recommendation.

### C. Comparison of Item Difficulty Estimates Between IRT and CTT

The item difficulty estimates of the data using the IRT and CTT methodology are presented in Table III.

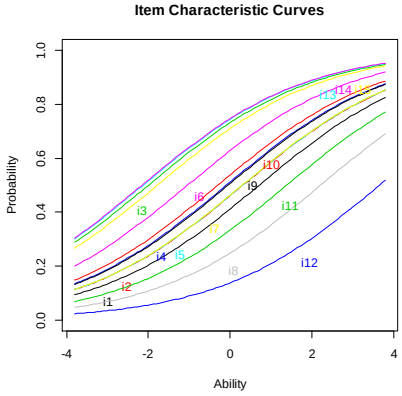



Fig. 2. ICCs of the 15 items using IRT Rasch Model.

TABLE. II. FITTING THE DATA USING RM (MODEL FIT)

| Items | Test | |
|---|---|---|
| | $X^2$ | $p$-value |
| 1 | 18.49 | 0.01* |
| 2 | 5.66 | 0.46 |
| 3 | 11.51 | 0.07 |
| 4 | 14.41 | 0.03* |
| 5 | 22.23 | <0.01* |
| 6 | 24.78 | <0.01* |
| 7 | 9.97 | 0.13 |
| 8 | 23.08 | <0.01* |
| 9 | 15.16 | 0.02* |
| 10 | 11.84 | 0.07 |
| 11 | 7.70 | 0.26 |
| 12 | 7.49 | 0.28 |
| 13 | 14.01 | 0.03* |
| 14 | 34.71 | <0.01* |
| 15 | 22.62 | <0.01* |

Legend: * Significant at 5% (using chi-square test)

TABLE. III. ITEM DIFFICULTY ESTIMATES/ INDICES

| Items | $\beta$ $(IRT)$ | description[a] | $\beta$ $(CTT)$ | description[b] |
|---|---|---|---|---|
| 1 | 0.44 | D | 0.40 | A |
| 2 | 0.21 | D | 0.45 | A |
| 3 | -1.01 | VE | 0.71 | E |
| 4 | 0.01 | A | 0.50 | A |
| 5 | 0.23 | D | 0.45 | A |
| 6 | -0.59 | E | 0.62 | A |
| 7 | 0.21 | D | 0.45 | A |
| 8 | 1.23 | VD | 0.25 | D |
| 9 | 0.03 | A | 0.49 | A |
| 10 | -0.10 | E | 0.52 | A |
| 11 | 0.75 | D | 0.34 | D |
| 12 | 1.91 | VD | 0.16 | D |
| 13 | -1.09 | VE | 0.72 | E |
| 14 | -1.20 | VE | 0.74 | E |
| 15 | -0.96 | VE | 0.70 | E |

Legend: $\beta$=item difficulty values IRT $\in$ [-3,3], CTT $\in$ [0,1]
[a]Description: VE = Very Easy, E = Easy, A = Average, D = Difficult, VD = Very Difficult
[b]Description: VD=0-0.125, D=0.126-0.375, A=0.376-0.625 E=0.626-0.875, VE=0.876-1.0

Includes corresponding verbal descriptions of all the items. Discussing the item difficulty estimates of the fifteen items that was included in the test under the IRT framework that employed the Rasch model, results showed that the level of difficulties, the test in particular items 1, 2, 5, 7, and 11 are difficult items because they are above 0. Note that an item whose difficulty is zero is considered an average item. Items 8

and 12 can be considered very difficult items because they are almost near at the upper right extreme.

Further, items 4 and 9 are items on an average difficulty. On the other hand, items 6 and 10 can be considered easy items while items 3, 13, 14, and 15 are very easy items because they are in extreme left near the value -3 considered the easiest item. In the case with CTT methods, results of the analysis show that items 8,11 and 12 are difficult items and items 1, 2, 4, 5, 6, 9 and 10 are items with average difficulty while the rest of the items, items 3, 13, 14 and 15 are easy items.

For the point of comparisons in accordance to the item difficulty estimates calculated under the two methodologies in particular, the items verbal descriptions, with regards to classical test theory (CTT), results revealed that there were no very difficult items in the test. Examination and further there were also no very easy items. This can be explained maybe due to the choice of the categorized interval from 0 to 1. Three items out of the total fifteen items namely items 4, 9 and 11 showed similar descriptions in their item difficulty for both item response (IRT) and classical test theory (CTT) methodologies. However, there are also some items that do not have the same common description (about 9 out of 15 items or 60% of the items). These items are items 1, 2, 3, 5, 6, 7, 8, 10 and 12. These results are expected since the two methodologies have different assumptions in their formulations.

As we mentioned above, the assignment of the degree of difficulty depends on the kind of interval that was made. As we observed, some intervals are narrow and some are wide. In the case of item 1, it is difficult under the IRT formulation but is an average item in CTT. It is also the same result with items 2, 5, 7 while item 3 is very easy in IRT but is an easy item in CTT. Items 6 and 10 are both easy in IRT but were average items in CTT while items 8 and 12 are very difficult items in IRT but only difficult items in CTT and lastly items 13,14,15 are very easy items in IRT but are easy items in CTT. A study by [44] compared CTT and IRT for the examinee change assessment. According to them a lot of investigators were eager to know of how IRT can be used in greater advantage as compared to CTT in change assessment but available results showed that they did not differ when compared based the examinee change assessment. However, when compared in term of their type 1 errors and detection percentages, their results showed that IRT is better than CTT in the examinee ‘s change detection with the condition that the test must consists twenty (20) items or more. For shorter tests, however they further mentioned that CTT has the advantage of correctly knowing change in the examinees. In our study, however there was also some variations in the results between IRT and CTT among the item difficulties when they were compared but the objective of this study was achieved. The two free computer software programs the “ltm” and “CTT” were very useful in doing the statistical analysis using the R software for the item test calibrations.

## V. Conclusions and Recommendations

This paper demonstrated the usefulness of the free computer software programs, the “ltm” and “CTT” in the R software environment for the calibration of the item difficulty parameter estimates/indices of the multiple-choice test examination using both the item response theory (IRT) and classical test theory (CTT) methodologies. We also demonstrated the usefulness of the Rasch model, an IRT probabilistic logistic model used to estimate the values of the item difficulty parameters of the test examination which were compared to the estimated values under the CTT method. Further, we also demonstrated that it was possible to plot the item characteristics of different items, so the proficiency or ability of the examinee can be estimated so that we will be able to know the higher chance of getting the item correctly. The Item characteristic curves (ICC) also gave us a glimpse of the difficulty characteristic of the item. The study also found some differences and similarities in the interpretation with the labeling of the item difficulty in the form of a verbal description for the items. The study concluded that these differences are due to the assumptions of the different methods in the item analysis.

The study further concluded that for the possibility of convenience for teachers in all levels and test constructors, they can do an item analysis for their test electronically using either ltm or CTT software packages in R for free in which first, they can do item calibration and assigned description for the item level of difficulty or indices and second, for the purpose of item banking especially in the test construction where items are stored in a database and labeled with their corresponding item level difficulty or indices.

This study further recommends that other item characteristics namely, the item discrimination and the item guessing parameters shall also be investigated to complete the test item analysis using both the classical test theory functions and other appropriate item response theory models that involves item calibration for discrimination and guessing.

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# Does Retention Interval Matters in Mathematics Performance?

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**Abstract**

This study describes and investigates if an association exist between the retention intervals of the three-grouped student respondents in high school and their performance in the basic algebra. The respondent's performances were categorized into different proficiency levels namely the far below basic, below basic, basic, above basic and proficient. Results revealed that a highly significant association was found between the retention intervals and the level of performance of the respondents. The level of performance of the respondents significantly increased with varying retention interval. An improved shift in the level of performances were observed from test to retest. The recall of information in the basic algebra was strengthened as retention interval increases particularly to high performing students. Respondents' exposure to related advance academic mathematics learnings, practice as well as maturity may have contributed to the mathematical performance across retention intervals of the respondents.

***Keywords:*** Basic algebra; performance; proficiency; association; retention interval.

## 1. Introduction

In the field of education and psychology many issues particularly on the individual's learning deteriorates over times.

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Is this true or not true? Perhaps it can be true to some specific field of study, however many studies have some conflicting results. Psychological theories were very beneficial for mathematics education since psychology has a strong influence in the field especially in learning numbers. On the other hand, mathematics education theories on the learning of numbers had been strongly influenced by the work of [1] that is for example that the assumption that children's ability to understand numbers is limited by their general cognitive maturation which perhaps involve time has certainly influence shaping educational approaches to teaching of mathematics in the classroom and the way teaching of mathematics particularly numbers was sequenced in the curricula [2]. In the aspect of retention, many researchers discussed many results on the studies on retention interval or sometimes called spacing over mathematics performance. For example, in the study of [3] on improving student's long term retention performance particularly in the personalized retention schedules, they suggested that personalized knowledge retrieval schedules are more effective than fixed schedules that is, in particular the students' long-term performance can be improved by giving them tests that are well spaced out and should be scheduled appropriately before gradually expanding the spacing between these tests. In their method, they were concerned with estimating the effects of personalized expanding intervals on improving students' long-term mastery level of skills. They developed a method called Personalized Adaptive Scheduling System (PASS) in ASSISTments' retention and relearning workflow. After applying the PASS, they conducted an investigation to the impact of personalized scheduling on long-term retention by comparing results from 97 classes in the summer of 2013 and 2014. They observed that students in PASS outperformed significantly students in traditional scheduling systems on long-term retention performance and that in particular, students with medium level of knowledge demonstrated significant reliable improvement with had a moderate effect size. In addition, the data we gathered from this study also helped to expose a few issues we have with the new system. These results suggested that personalized knowledge retrieval schedules were more effective than fixed schedules and suggested that more study should be made on examining approaches to optimize PASS. Reference [4] investigated the effect of distributed practice on the mathematical performance of eighty-one 7th graders. After a random lesson, one group of students worked three sets of exercises massed on one day, while the other group of students worked the same exercises distributed over three days. When they analyzed the data, they found out that performance for two weeks after the last practice revealed no evidence of an effect of practice condition. But, in a test given after six weeks, the result suggested a strong evidence for a positive effect of distributed practice came out. Further analysis indicated particularly for students in the medium performance range benefitted from distributed practice. This distributed practice in mathematics answered the questions of why and under which circumstances distributed practice proves a useful learning strategy in realistic learning contexts, even beyond learning of rather simple verbal content. They argued that while the effect of distributed practice emerges for roughly all learners if simple verbal content or mathematical routines are practiced, the effect of distributed practice on learning of more complex content for example on the acquisition and application of higher learning mathematical procedures is effective for students in the medium performance range. According to the authors, these assumptions were based on exploratory analyses and there is a need for further empirical confirmation. They concluded that that distributed practice remains a promising learning strategy and more studies on a broad range of content and learners could help to deepen the understanding of when and why it works which is quite related to our study. Reference [5] discussed issues regarding increasing retention without increasing study time which is quite interesting on our study with retention intervals. They argue that because people forget much

of what they learned, students could benefit from learning strategies that yield long-lasting knowledge. Yet surprisingly little was known about how long-term retention is most efficiently achieved. In their study, they examined how retention was affected by two variables: the duration of a study session and the temporal distribution of study time across multiple sessions. Their study resulted and suggested that a single session devoted to the study of some material should continue long enough ensuring that achievement of mastery but that immediate further study of the same material is an inefficient use of time. Their data showed that the benefit of distributing a fixed amount of study time across two study sessions called the spacing effect that depends jointly on the interval between study sessions and the interval between study and test. They said their findings showed some practical implications to mathematics learning. References [6,7] made contribution in the studies regarding effects of retention on the performance of Calculus. Reference [6], in their study on retention of concepts and skills in traditional and reformed applied calculus mentioned that a recent calculus reform initiatives have shifted emphasis from rote memorization and symbol manipulation to conceptual understanding and practical application to strive to make calculus more meaningful to students. A question they posed that said, is reform calculus making a difference? Their study compared outcomes of a traditional and reform calculus course in terms of students' retention of basic concepts and skills after the passage of time. Although in their result, traditional and reform students did not significantly differ on the overall performance however reformed students retained better conceptual knowledge and traditional students retained better procedural knowledge. Those reformed calculus students demonstrated that concepts and can be understood before computational competence will be achieved while [7] in their study among engineering students on the long-term retention of basic mathematical knowledge and skills, they focused on the long-term retention of basic mathematical techniques in a first-year calculus course, involving a sample group of engineering students at the University of Pretoria. They investigated which and how much of the basic mathematical knowledge and rote skills acquired in the first year of study were retained after a further two years of study. Their results showed that there was a significant decline in the performance over a two-year period. There were, however, areas in which students still performed reasonably well after the elapsed period or even showed improvement. The research was of diagnostic value since it assisted course designers in determining what basic mathematical skills and knowledge were retained after a period of two years in their teaching approach to and emphasis of different topics. This paper wanted to explore whether an association exist between the retention intervals and the performance of the spacing three retention interval groups of high school respondents particularly in their basic algebra subject. Some studies of similar objectives can be read in the work of [8] which studied relationship of time and learning retention, Reference [9] who investigated the relationship between retention in first grade and performance on high stakes tests in third grade, Reference [10] who were interested in a longitudinal study of mathematics and retention specifically on placement, retention and success and finally [11] who were interested in the length of the retention interval, forgetting and subject similarity. The results of this study would be helpful in providing teachers some perspective on how students learn and retain information about mathematical concepts and procedures in particular give them ideas on appropriate actions to facilitate better retention of information. As many centers and clients of educational endeavors, results can benefit students to have a better understanding on their mental processes and maturation, thereby, giving them ideas to what actions can be done to improve their performance and retain more information. Results also of this study may be used by school administrators in their decision-making concerning the arrangement of subjects in the school curriculum which

should always be designed for the improvement of human learning capacity particularly in mathematics education. This study also provides insights for further research investigations of similar nature and will give a new perspective and trends in educational researches.

### *1.1. Scope and limitation of the Study*

The respondents of this study will only focus on the performance of 295 students comprising grade 8, grade 9, and grade 10 students respectively of the University Laboratory School in the University of Southern Mindanao, Kabacan, Cotabato, Philippines for academic year 2012-2013. These different groups determine the three-year levels which corresponds to their length of retention interval in their high school. The respondent's performance was only based in the result of the summed examination results that covers the first and second grading period in their elementary Algebra which covers topics on the Real Number System, Percentage, Ratio and Proportion, Measurement, Algebraic Expressions, and Polynomials. Other topics on the succeeding grading periods were not included in the study. We further assumed that the respondents received the same coverage and level of instruction in their grade 7, since the respondents were taught by the same teacher for the last three years, Further, we disregard those students who are transferees they will not satisfy the requirements of our respondents do not have data results in their grade 7. For further information about the data please refer to the work of [12].

## 2. Materials and Methods

### *2.1. Experimental Design*

This research employed a Test-Retest Comparative Design where the three year levels represented the different retention intervals. All the three different groups respondents totaling 295 students (118 students for 1-year retention, 90 students for 2 years' retention and 87 students for 3 years' retention groups) were given the same teacher validated test in their grade 7 by the same teacher who taught them the subject. Under the one-year retention interval, the grade 8 students received their test on October 2011. Those treated under two years' retention interval were the grade 9 students and received their test on October 2010 and lastly those treated under three years' retention interval were the grade 10 students and received their test on October 2009. For the retest, the same test instrument was administered to the same three groups on February 2013 by the same teacher who taught them in their grade 7 on their basic algebra. The performance of the respondents was determined by their scores in the first test and their re-test. The examination instrument used to get the performance of the students contained 125 multiple choice items. Only the retest was carried out by the researchers since test data were already available and the students took the test during their grade seven in high school as part of their quarterly examination. However, it is also customary to describe the events that took place between the test and the retest. Normally, in the Philippines, for high school, the academic year starts in June and ends in March in the following year but at present due to the COVID 19 pandemic there was an adjustment made by the department of education to refrain from doing face to face instruction but instead doing online and modular instruction.

## 3. Results and Discussions

In order to determine if an association exist between retention interval and the mathematical performance of the students, the data obtained during the experiment was analyzed using Chi-square test for association (dependence), this test is useful for categorical data in a contingency table. Prior to this, the performance level of students was divided into five categories according to the percentage of correct answers of the students in their 125 item test examination. The Categorization is shown in Table 1 below.

**Table 1:** Categorization of Performances. ULS-USM, Kabacan, Cotabato. A.Y. 2012-2013.

| Performance level | | Description | Percentage of Score Obtained (%) |
|---|---|---|---|
| Numerical Equivalent | Symbol | | |
| 1 | FBB | Far Below Basic | 0-34 |
| 2 | BB | Below Basic | 35-48 |
| 3 | B | Basic | 49-64 |
| 4 | AB | Above Basic | 65-83 |
| 5 | Prof. | Proficient | 84-100 |

Remark: Shaded cells represent merged categories on the following analyses

Adapted from Mathematics Performance Bands based on 2009 CST Results

However, after running the preliminary analysis of the data, Results revealed that we need to divide the student's performance into three groups where the Far Below Basic group is merge with the Below Basic group and the Proficient Group merged with the Above Basic group. These merging is necessary to satisfy the assumption of the Chi-square test, that is each cell in the table should have at least five frequency counts. Frequencies of students who belong to different categories after the test and retest were tallied in Tables 2 and 3.

### *3.1. Description of the Performance level of each Retention group in their Grade 7*

Variations in the frequency in the different retention interval groups can be noted from Figure 1.

Apparently, all retention groups in their grade 7 test after they were given instruction were found to have more students who belonged to the basic group and below basic group and in the order above basic, proficient and the far below basic group. In details, 1 out of 118 students or 0.8 % from the 1 year retention interval group; 5 out of 90 students or 5.6% from the 2 years retention interval group and 1 out of 87 students or 0.8 % from the 3 years retention interval group belong to far below basic while 49 out of 118 students or 41 % from the 1 year retention interval group, 30 out of 90 students or 33.3% of the 2 years retention interval group and 25 out of 87 students 28.7 % from the 3 years retention interval group below basic category. The basic category, however, were composed of 55 out of 118 students or 46.6% from the 1-year retention interval group, 41out of 90 students or 45.6% from the 2 years' retention interval group and 47 out of 87 students or 54 % from the 3 years' retention interval group. The above basic category was composed of 9 out of 118 students or 7.6 % from the 1-

year retention interval group, 8 out of 90 students or 8.9 % from the 2 years' retention interval group and 10 out of 87 students or 11.5 % from the 3 years' retention interval group. Moreover, there were only 4 out of 118 students or 3.4 % from 1-year retention interval group, 6 out of 90 students or 6.7% from the 2 years' retention interval group and 4 out of 87 students or 4.6% from the 3 years' retention interval group who belonged to the above basic to Proficient category.

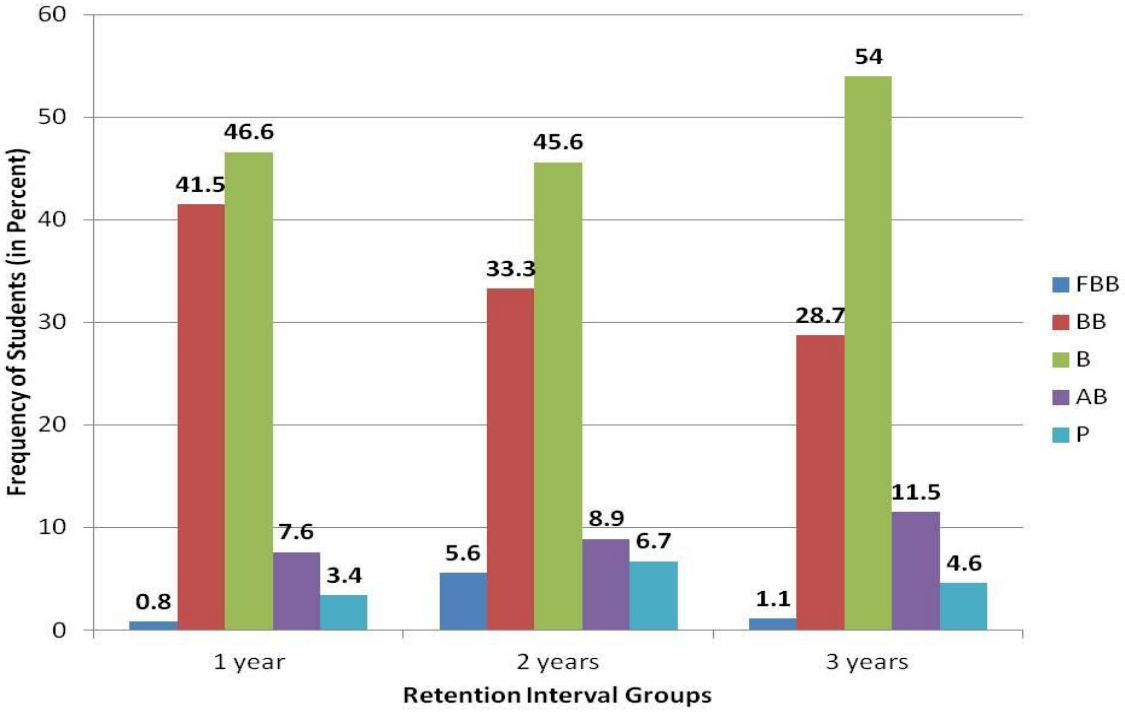



**Figure 1:** Frequency of Students (in percent) in Different Categories (Test). ULS-USM, Kabacan, Cotabato. A.Y. 2012-2013.

Generally, there were 7 out of 295 students or 2.4% under far below basic category, 104 out of 295 students or 35.3% under below basic category, 143 out of 295 student or 48.5 % fell under basic category, 27 out of 295 students or 9.2 % fell under above basic category, and only 14 out of 295 students or 4.7 % of all students reached the proficient level.

### *3.2. Analysis on the Association Between Performance and Retention Interval (Test)*

The analysis of the categorical data in table 2 revealed that there was no significant association between the retention interval and the level of performance of the students ($\chi^2 = 4.111$, $p > 0.05$). This means that we do not have sufficient evidence to conclude that a significant association exist after the learning instruction between with retention interval and the level of performance of the students. This held true since all the subjects of the experiment were tested when they were all first year student and no amount of retention interval were given to each group and their mathematical abilities perhaps were found to be equivalent and comparable with results from the literatures. Further, their exposure to learning is uniformly delivered since they have the same teacher who thought them during the period so retention is not a big issue yet.

**Table 2:** Test of Significant Association between Retention interval and Performance level (Test) ULS-USM, Kabacan, Cotabato. A.Y. 2012-2013.

| Retention Interval Group | Performance Level | | | Total |
|---|---|---|---|---|
| | FBB – BB (1) | B (2) | AB - Prof. (3) | |
| 1 year | 50 | 55 | 13 | 118 |
| 2 years | 35 | 41 | 14 | 90 |
| 3 years | 26 | 47 | 14 | 87 |
| Total | 111 | 143 | 41 | 295 |

$X^2$= 4.111, *df*= 4, *p-value* = 0.391$^{ns}$, ns=not significant at 5% level of significance

### *3.3. Description of the Performance level of each Retention group during Retest*

In contrast to the data presented in figure 1, figure 2, however presents a different pattern. After treating each group with the specified retention interval, it was found out that after a year, the frequency of students under below basic and basic categories dropped substantially which contributed to the large increase in the frequency of students who fell under above basic and proficient categories. Similar conclusion can also be deduced from the group treated with 2 and 3 years' retention interval. The frequency of students under far below basic fell down to none after 2 and 3 years' retention interval. Categorical analysis on each group revealed that after 1-year retention interval, far below basic category increased to 2 out of 118 students or 1.7%, below basic category decreased to 28.8 % or 34 out 118 students, basic category decreased to 29 out of 118 students or 24.6%, above basic category increased to 24.6 % or 29 out of 118 students, and the proficient category increased to 20.3 % or 24 out of 118 students.

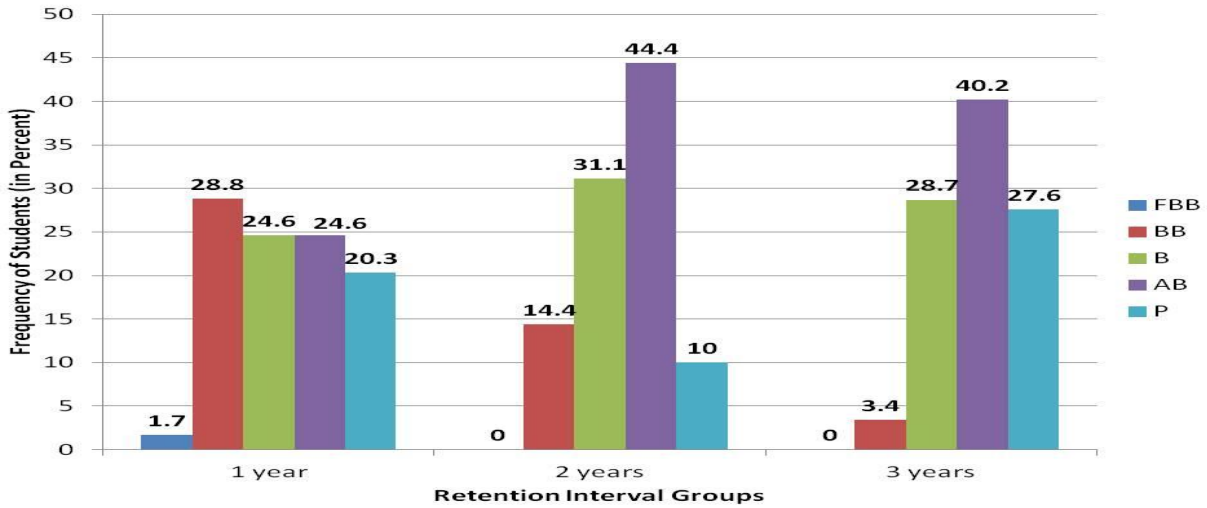



**Figure 2:** Frequency of Students (in percent) in Different Categories (Retest) ULS-USM, Kabacan, Cotabato. A.Y. 2012-2013.

However, after 2 years' retention interval, the group was 0 % or 0 out 90 students for far below basic performance level and 14.4 % or 13 out of 90 students from below basic performance level, 31.1 % or 28 out of 90 students from basic students and 14.4 % (40 out of 90 students) and 10 % or 9 out of 90 students from above basic and proficient performance levels respectively. On the other hand, after 3 years' retention interval, the group has 0 % or none of 87 students from far below basic performance level and 3.4 % or 3 out of 87 students from below basic, 28.7 % or 25 out of 87 students from the basic performance level and 40.2 % or 35 out of 87 students for above basic and 27.6 % or 24 out of 87 students from proficient performance level. Overall for all students regardless of the student's retention interval groups, there was 2 out of 295 student or, 0.7 % from far below basic, 150 out of 295 students or 6.9 % of students fell under below basic, 82 out of 295 students or 27.8% of students fell under basic category, 104 out of 295 students or 35.3 % fell under above basic category and 57 out of 295 students or 19.3% fell under proficient category with the treatment of retention intervals.

### *3.4. Analysis on the Association Between Performance and Retention Interval (Retest)*

**Table 3:** Test of Significant Association between Retention interval and Performance (Retest) ULS-USM, Kabacan, Cotabato. A.Y. 2012-2013.

| Retention Interval Group | Performance level | | | Total |
|---|---|---|---|---|
| | FBB – BB | B | AB - Prof. | |
| | (1) | (2) | (3) | |
| 1 year | 36 | 29 | 53 | 118 |
| 2 years | 13 | 28 | 49 | 90 |
| 3 years | 3 | 25 | 59 | 87 |
| Total | 52 | 82 | 161 | 295 |

$X^2$= 27.184, df= 4, p-value =0.000**,** highly significant at 0.01 level of significance

Using Chi-square test for independence or association on the preceding data as shown in Table 3, results revealed that there was a highly significant association between the retention intervals and the performance levels of the students ($X^2$= 27.184, p-value < 0.01). We observed that there was a shifting of frequencies of students across each different category. One can see from the comparison of the categories in Figure 2 that any increase in retention interval causes a decline in the frequency of students who were under far below basic up to basic category. Even though there was no definite pattern, relative frequency of students in the above basic and proficient category generally continues to push upward as retention interval increases. It should be noted that far below basic category fell down to zero frequency when retention interval was increased. An important revelation that substantiated the claim that the properties of retention interval strengthens the students' prior mathematical knowledge. Thus, it would only mean that increasing retention interval tends to enhance students' expertise in mathematics as they are exposed to numerous academic trials and learning that provided avenues for practice of old and new knowledge as well as elaboration of newly learned materials with the already learned materials [12]. Further, [13] mentioned in their experiment that students assigned in the distributed rereading condition showed no forgetting from the short to the long retention interval and have beneficial effects on

learning. In relation to our study, more importantly in the retest, it was being observed that the frequency of students shifted towards higher level of achievement as a result of increasing retention interval and its properties like frequency of practice, exposure to related academic trials, and maturity entwined with time are possibilities to explain this phenomenon however our result is in contrast to the result of [11]. They argued that a long retention interval tends to result in the poor retention known as forgetting. A high subjective similarity between stimuli frequently produces their poor retention. Further, they said that a high subjective similarity between stimuli frequently produces their poor retention hence, a long retention interval may increase the subjective similarity between stimuli and this increase may produce forgetting and therefore may produce overall poor performance. Further, in the business field, Reference [8] in his focus of examining the importance of course sequencing in a curriculum, explored the degree to which students' understanding, or knowledge, may deteriorate over time. They measured the student performance in the quantitative business courses and found that over time, a correlation existed between students' performance in those courses but it declined significantly and these decline was nonlinear. Our study resulted in a direct association, where there was an increase in the overall performance as retention intervals increased. One explanation is due perhaps to maturation and exposure of the student respondents to more advanced mathematical concepts especially for those have more retention years. This is expected usually in mathematics education, where the curricula were arranged in such a manner that a student exposed first with basic course in mathematics then in an increasingly manner they were exposed to more advanced mathematics as retention interval increases as mentioned by [2] and their mathematical basic concept were retained [6]. The student as being taught and exposed gradually, leading to a more polished skills and expectedly and efficiently able to recall their basic mathematics because the procedural process which requires skill to solve advanced learning in mathematics is necessarily needed therefore there is a higher tendency basic mathematics were retained specifically in a particular subject like in our study the basic algebra as mentioned by [7].

## 4. Conclusions

As we observed in this study, the level of performance of the student respondents significantly increased with their varying retention interval. An improved shift in the level of performances among the three groups of students were observed from test to retest and a significant association existed between these performances and the retention years in the retest. Thus, with respect to this experiment, the recall of information in the content of the basic algebra that was in consideration in the test examination resulted to a better overall performance of the students. Perhaps this is strengthened due to the maturation and exposure of the students to more advance mathematics learning and practice in between the test and retest period in which greater advantage been seen on the overall performance to students with more retention years.

## 5. Recommendations

This study only considered in particular basic mathematics content in the experiment, the researchers recommend that this study might be replicated using other fields like in natural sciences or in languages, maybe a different scenario in turn may occur.

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# Fish catch assessment of the nine fish species at Ligawasan marsh, North Cotabato, Philippines

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Full Text Article

# Fish catch assessment of the nine fish species at Ligawasan marsh, North Cotabato, Philippines

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## Abstract

Farmers' nine important fishes were assessed to determine relative abundance from data of fish catch landings and catch per unit effort (CPUE) in Ligawasan marsh from January to March 2022. The study gathered data from total fish catch landings, gear selectivity, catch per unit effort (CPUE) and length-weight relationship from fish catch landings. Results of the study showed that mudfish (*Channa striata*), tilapia (*Oreochromis niloticus*), and common carp (*Cyprinus carpio*) were the abundant species in the marsh. Gear selectivity was observed in some species. Length-weight relationship was compared to a similar wetland data. Shorter range for length were found in Ligawasan marsh fish species except for gourami (*Trichopterus trichogaster*) compared in the report from the study in Agusan marsh. The present study can provide baseline data on relative abundance of fish resources present in the marsh which can be used as reference to develop policy on sustainable management based in Ligawasan marsh, North Cotabato, Philippines.

## Introduction

Ligawasan marsh is a wetland located in Mindanao which surpasses three provinces-South Cotabato, Maguindanao, and North Cotabato, Philippines. This wetland is included in the list of protected areas under the 1992 National Integrated Protected Area System (NIPAS) Act or Republic Act 7586. With this law, Ligawasan marsh has been declared as game reserve and bird sanctuary. However, other aspects pertaining to fishery resources management have not yet been fully maximized. In contrast to marine scenario wherein Marine Protected Area (MPA) system was already established to provide measures and systematic approach in coastal resource management (Alcala, 1988), the conservation system in freshwater environment has not yet been fully elucidated. There is a need to substantiate the ecological and social scheme in the area to develop an effective conservation approach. Hence, assessment of the fishery resources in the area was conducted in this study.

The previous study conducted by the DENR (2004) showed that the majority of settlers in Ligawasan marsh depended on farming, livestock or poultry production, and fishing as major livelihood sources. Fishing serves as primary source of income and sustenance. It becomes an alternative livelihood source when farming is not feasible when water levels rise, and the marshland communities are submerged. This also holds the integral role of fishing in the community as a common means in providing source of livelihood for each family. Moreover, DENR identified 33 species of freshwater fishes from Ligawasan marsh (DENR, 2004). However, fish stock assessments in the area was not explored and studies

have been limited to ecological descriptions and sociodemographic factors. Therefore, there is a need for stock assessment to provide quantitative data since fish stocks in this wetland are vulnerable to exploitation pressure due to the open access system. This assessment updates the local government units (LGU) and other agencies on the fishery resources in the area and helps explore ecologically-sound policies addressing the modern-day needs of the marshland communities. In this way, the data from this study can be an indicator if the existing municipal policies have been effectively implemented.

To evaluate the relative abundance of the species present in the marsh, fish stock assessment was conducted using catch per unit effort (CPUE). The most common index of relative abundance was calculated through CPUE data from fish stock samples (Fabrizio and Richards, 1996; Hubert 1996; Maunder et al., 2006; Ney, 1999). CPUE measures the proportion of the population that is vulnerable to fishery, considering gear selectivity, size, age of fish, horizontal and vertical distribution of fish, and fishing practice. For this study, factors considered were the types of fishing gear used, number of hours spent fishing as part of the fishermen's practice, and weight and length of the fish.

The goal of the present study is to assess fish stock on the relative abundance of nine important fishes caught at Ligawasan marsh based on fish catch data. Four of the species are native to Ligawasan marsh included mudfish (*Channa striata*), climbing perch (*Anabas testudineus*), native catfish (*Clarias macrocephalus*) and giant mottled eel (*Anguilla marmorata*). The cyprinid species-common carp (*Cyprinus carpio*), rohu carp, (*Labeo rohita*), silver carp (*Hypothalmichthys molitrix*), and other fish such as snakeskin gourami (*Trichogaster pectoralis*) and tilapia (*Tilapia niloticus*) are all introduced to Ligawasan marsh. Fish stock assessment can provide valuable information for fishery resource management. In the future, the results serve as baseline for the development of policies preventing the risk of overexploitation of resources. The data can be used by fishery managers to estimate fish population in a certain fishing ground. The information from fish stock assessment can be used as basis for maximum sustainable yield which determines regulatory actions such as closed season or other measures compliant to RA 8550 (Fisheries Code of the Philippines) and RA 10654 (Law on Illegal, Unreported and Unregulated fishing activities in the Philippines).

## Methodology

*Study area*

The fish landing site was situated at Bagoinged, Pikit in the province of North Cotabato, Philippines (Figure 1) at 6'60'' North, 124'42" East. There are five prominent fish landing sites in North Cotabato that obtain fish from Ligawasan marsh. The fish landing site in Bagoinged, Pikit has the highest percentage of fish landed in the area, thus was selected the site for this study.

Using the Ramsar Convention definition of wetlands as a basis, the Ligawasan marsh is classified into three major wetland habitats: the marsh habitats, artificial habitats, and open water habitats. In the case of Ligawasan marsh, specifically the Bagoinged, Pikit, the habitat is characterized by running open water habitat. The still open water habitat is also present where floaters such as *Pistia stratioides* (water lettuce) and *Eichhornia crassipes* (common water hyacinth) are found. These areas are used by local human communities for fishing and transportation.




Figure 1. The location of sampling site at Bagoinged, Pikit which covers an area of Ligawasan marsh.

*Preliminary survey*

A preliminary survey was conducted last December 2021 prior to the fish stock assessment to gather information on the species most commonly caught in the area and to identify the landing site with the highest fish catch. The activity included an ocular inspection of the landing sites. This information served as a basis for selecting species to be assessed during the nine-week period.

Data on the socio demographic profile of the fisherfolk community at Bagoinged, Pikit were recorded. A sample size of 80 respondents were selected. The survey questionnaires covered socio-demographic information such as family size, number of years in fishing, and age at which respondents started fishing. Descriptive statistics such as counts, averages percentages, tables and graphs were used to describe and summarize the results of the survey.

*Selection of Species for Data Collection*

The data gathering was limited to the nine most abundant fish species caught and landed at the fish landing site. These species include: (1) mudfish (*Channa striata*), (2) gourami (*Trichopodus tricopetrus*), (3) climbing perch (*Anabas testudineus*), (4) native catfish (*Clarias macrocephalus*), (5) giant mottled eel (*Anguilla marmorata*), (6) rohu carp (*Labeo rohita*), (7) silver carp (*Hypophthalmichthys molitrix*), (8) common carp (*Cyprinus carpio*), and, (9) tilapia (*Oreochromis niloticus*).

*Sampling method*

The collection of data was conducted from January to March 2022, corresponding to the first quarter of fishing activities in the Ligawasan marsh area. Sampling was done weekly, except when weather conditions are unfavorable or during periods of fishing restrictions. Data gathering took place during high and low water levels depending on the week covered.

Ten random fish samples per species were selected for measurement each week. Length was measured by a meterstick, and weight was recorded by weighing scale. Fishing and related activities from the time of arrival to the fishing ground to the actual catching

of fish, hauling, dispersal in landing sites and other related activities for 8-12 hours daily were recorded. Total catch was estimated by volume of catch (weight) and recorded according to respective fishing gears used. Small fish and other by-catch not included in the study were discarded. Only the nine fish species identified were included in the inventory. Fish catch landing was presented as relative abundance of fishes caught in the study whereas CPUE is the efficiency of the gears used in fishing by fisherfolks in the marsh. The following are the three fishing gears used by fishermen in Bagoinged, Pikit.

*A. Gill net*

The gill net or *pukot* (Figure 2A-B) is named after its catching principle, where fish are usually caught by "gilling", or when fish are caught in the mesh of the net by their gills. Fish captured by gill nets are most likely to catch fish during feeding or migratory movements. Fish may avoid the gill net if they notice the gear, so catches are normally best in areas with turbid water. The gill net in Ligawasan marsh is commonly constructed at 240 x 182 cm with 8 cm mesh size. Gill nets that are observed in Ligawasan are made from nylon (polyamide) and typically operated as a stationary gear anchored to the bottom. Gillnets are operated from smallest non-mechanized fishing boats to motorized fishing boats.

*B. Improvised Fish Trap*

The traps used by fishermen in Ligawasan have the same features described by FAO-technical paper (Bjordal et al., 2020) however it is constructed at bigger sizes to capture more fish (Figure 3A-B). The traps are normally not baited, instead it catches the fish by leading them into the trap device, and eventually into the fish compartment designed to hold the trapped fish and preventing it to escape. The fish traps are constructed with inner side walls made of nets assembled into mesh sizes forming V-shaped that entrap fish. The fish trap is constructed at 200 cm x 80 cm (length x width), the opening of which is measured at 15 cm and the mesh sizes are ideally at 3 cm. The improvised fish trap or *bubo* is constructed with bamboo to hold the nets together and the nets are made of synthetic twines (polyvinyl). These are submerged in designated areas in the marsh.

*C. Fish pot*

The improvised fish trap and fish pot are similarly called as "*bubo*" or "*buo*". The fish pot in this study (Figure 4A-B) represents a smaller version of the improvised fish trap described previously. The design of fish trap in this area is constructed using bamboo and sticks at 120 cm x 76 cm (length x width). The body is wrapped with synthetic nets with mesh size measuring 6 cm. The design makes use of a V-shaped funnel opening which leads the fishes into the inner compartment and trapped therein.

Of these species, seven species are native to Ligawasan marsh including mudfish, gourami, climbing perch, native catfish, rohu carp and silver carp. Two species are introduced, namely common carp and tilapia. Common carp is also considered as invasive (Pinto et al., 2005; Maiztegui et al., 2019).

*Analysis of Data*

In this study, CPUE is presented as number of fish per amount of fishing time. A separate CPUE is computed according to fishing gear used. Catch was recorded by weight (kg) in terms of total gross catch per species and per species according to type of fishing gear used. The study used nominal fishing effort as elucidated in the study of Ogle (2018) with modifications, wherein CPUE was calculated by dividing the catch of fishing trip by fishing effort (number of fishing hours multiplied to number of fishing boat during the fishing trip). The number of fishing hours corresponds to the whole duration of fishing including the entrapment time for both fish trap and fish pot.

$$\text{Catch per unit effort} = \frac{\text{mass of fish caught (kg)}}{\text{fishing effort}}$$

Length and weight were also recorded. These are then compared to another study on marsh fishery resources particularly in Agusan marsh (Jumawan and Seronay, 2017).

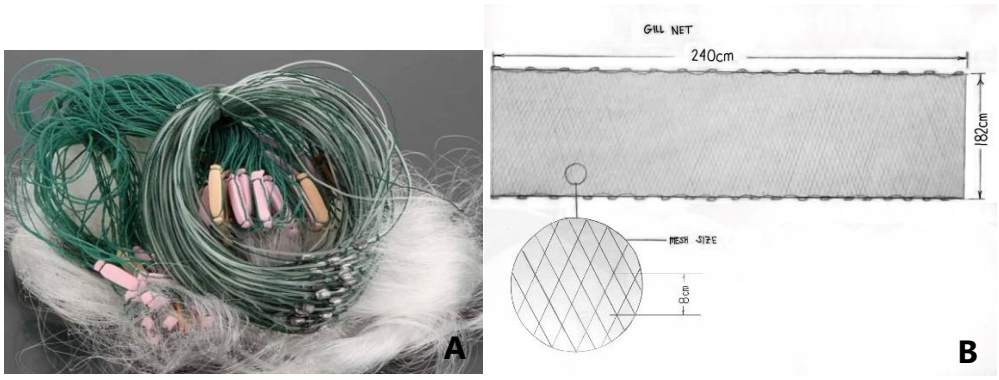



Figure 2A-B. The gill net used by fishermen at Bagoinged, Pikit.

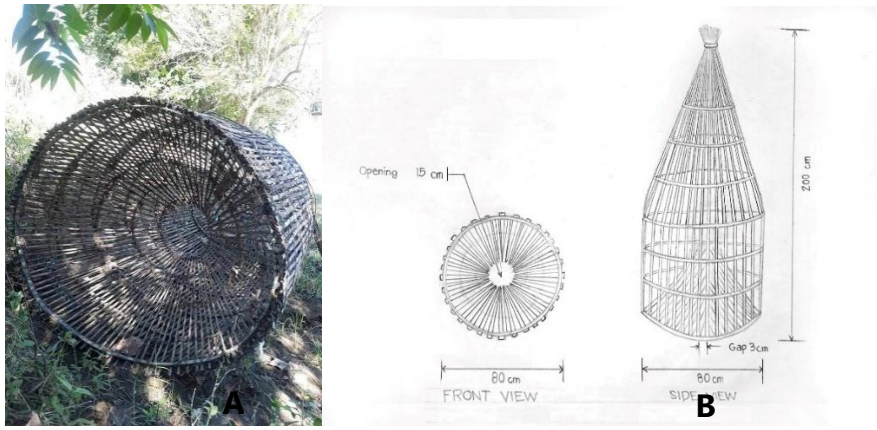



Figure 3A- B. The fish improvesied fish trap used in fishing at Bagoinged, Pikit.

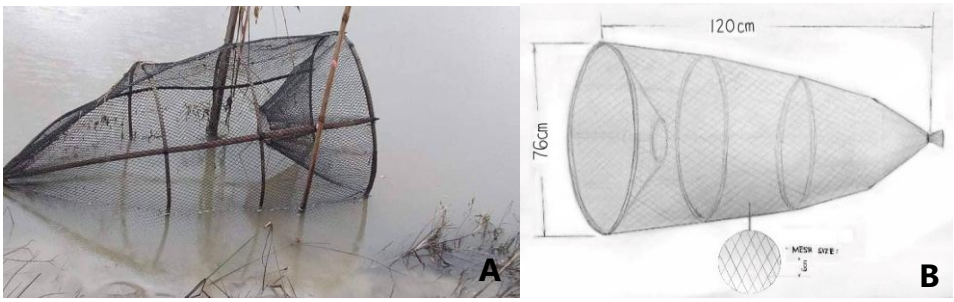



Figure 4A- B. The fish pot used by fishermen at Bagoinged, Pikit.

## Results

The study gathered data on fish catch landing for a nine-week observation. Species observed included mudfish, tilapia, rohu carp, common carp, giant mottled eel, climbing perch, silver carp, native catfish, and snakeskin gourami. The sociodemographic aspect was also investigated to provide valuable inputs in the resource management and sustainable development of Ligawasan marsh communities.

*Sociodemographic Profile*

Data from sociodemographic survey indicated that majority of fishermen (51.25%) belong to ages above 46 years old. Majority of fishermen (78%) had been fishing for 6-10 years. In addition, married status made up the majority (91%) of fishermen in the area. These individuals mostly engaged in fishing belong to families with more than 7 members. The size of family members of fishermen could be one factor that led to high frequency of fishing activities in this wetland community since fish becomes the primary source of food in the area.

In the previous study conducted by DENR last 2004, the households engaged in fishing comprised of 44.5%. A slightly higher percentage were found to rely on farming (46.2%) for subsistence. Fishing became the second major livelihood source, since farming is not feasible when water levels rise, and marshland communities are submerged. The study of Bautista et al. (1999) showed that 81.5% considered fishing as major source of livelihood in Ligawasan marsh. However, the number of fishermen declined from 1999 to 2022. Men are predominantly involved in fishing, while women are actively involved in post-harvest activities. Majority of post-harvest products include dried and smoked fish.

Fishing constitutes the source of living and sustenance of families at Bagoinged, Pikit. It has become a way of life for majority (80%) of the respondents. The community has also adapted fishing strategies to cope with heavy rainfall. The use of fish traps has advantages during rainy weather, as more fish are likely to be trapped in the device.

*Fish Catch Landings*

The highest percentage of catch (22%) was observed in mudfish followed by Nile tilapia (20%), common carp (19%) and rohu carp (14%) as seen in Table 1. Other fish species were recorded at less than 10%. The snakeskin gourami has the least catch recorded.

***Table 1.*** Fish catch landing (kg) at Bagoinged, Pikit, North Cotabato at 9-week survey.

| Fish | Number of weeks | | | | | | | | | Total | % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | | |
| Mudfish | 396.0 | 383.0 | 163.0 | 144.0 | 155.0 | 147.0 | 173.0 | 177.0 | 132.0 | 1,868.7 | 22 |
| Nile Tilapia | 250.5 | 293.3 | 146.6 | 91.4 | 192.7 | 245.0 | 258.0 | 164.0 | 93.7 | 1,735.3 | 20 |
| Common carp | 195.0 | 185.0 | 160.0 | 143.0 | 188.0 | 190.0 | 210.0 | 198.0 | 192.0 | 1,661.0 | 19 |
| Rohu carp | 149.5 | 139.5 | 142.1 | 106.9 | 1,18.0 | 131.0 | 131.0 | 131.0 | 140.0 | 1,188.0 | 14 |
| Climbing perch | 77.7 | 66.8 | 107.1 | 94.7 | 126.4 | 88.6 | 97.7 | 36.1 | 43.2 | 738.3 | 9 |
| Native catfish | 43.1 | 45.7 | 50.3 | 53.2 | 64.7 | 40.1 | 38.3 | 43.9 | 52.0 | 431.3 | 5 |
| Silver carp | 67.4 | 40.3 | 45.7 | 27.8 | 33.0 | 39.8 | 52.8 | 29.6 | 13.4 | 349.8 | 4 |
| Giant mottled eel | 44.7 | 61.1 | 47.6 | 34.9 | 33.4 | 37.6 | 19.5 | 47.2 | 34.7 | 360.7 | 4 |
| Snakeskin gourami | 25.6 | 23.3 | 20.6 | 18.1 | 19.3 | 24.7 | 27.4 | 35.7 | 33.4 | 228.1 | 3 |
| Total | 1224 | 1,215 | 862 | 696 | 911 | 919 | 979 | 826 | 702 | 8,333.1 | |

Mudfish presents the highest number of fish landings during the first two-weeks of observation, but the catch slightly declined in the succeeding 3-9 weeks. Consistent catch was observed for carp species throughout the observation period. Native catfish and snakeskin gourami showed consistently low catch rates. Climbing perch showed declining catch during weeks 8-9.

*Gear Selectivity*

One of the factors affecting CPUE is the type of fishing gear used by fishermen. Gear selectivity was notably observed from the three fishing gears used by fishermen in Bago-inged, Pikit. Thompson and Ben-Yami (1984) considered selectivity as the capacity of any method of gear type to capture certain fractions or sections of the fish population whether grouped by species, age, size or behavior and to exclude others. The type of fishing gear affected both the target species and quantity of catch, with species caught varying according to the type of fishing gear used.

*A. Gill net*

Gill nets are primarily used to target a wide range of species such as mudfish, climbing perch, rohu carp, native catfish, snakeskin gourami, Nile tilapia and silver carp (Figure 5). The range of catch per week was recorded in native catfish (40- 70 kg), snakeskin gourami (18.1-35.7 kg), silver carp (13.4- 67.4 kg), and climbing perch (6.3- 36.1 kg). Highest catch was observed in tilapia ranging from 91.4 to 293.3 kg. It was also noted as one of the most abundant species caught at the site followed by rohu carp (120- 160 kg) using the gill net. In terms of total catch per species over the 9-week fish landing data, gillnet can catch as much as 1,735 kg of mudfish, 1,265 kg rohu carp, 654.7 kg mudfish, 470 kg native catfish, 349.8 kg silver carp, 234.5 kg climbing perch, and 228.1 kg snakeskin gourami.

*B. Improvised Fish Trap*

On the other hand, improvised fish traps captured mudfish, native catfish, and common carp (Figure 6). Data showed that this design of fish trap was effective for catching common carp. This is the only fishing gear capable of capturing this species, and common carp was recorded as one of the highest catch landings during the 9-week observation. In contrast, both fish pot and gill net were effective in catching mudfish and native catfish in the area. In terms of total fish catch, the improvised fish trap accumulated 1,659 kg of common carp, 1,214 kg of mudfish, and 431.3 kg of native catfish. Improvised fish trap is expected to catch more common carp than native catfish and mudfish.

*C. Fish pot*

The fish pot is used for catching giant mottled eel, rohu carp, and native catfish (Figure 7). Both the fish trap and gill net are used in catching native catfish, and rohu carp. Giant mottled eel caught using fish trap was recorded at 33.4 kg- 47.6 kg, rohu carp at 106 kg-149.5 kg and native catfish at 40.1 kg- 64.7 kg in the 9-week fish catch observation. In terms of total catch, rohu carp accumulated 1,188 kg, 431.3 kg native catfish, and 360.7 kg giant mottled eel. If a fish trap was used, the highest number of fish caught was recorded for rohu carp, and the least was for giant mottled eel.

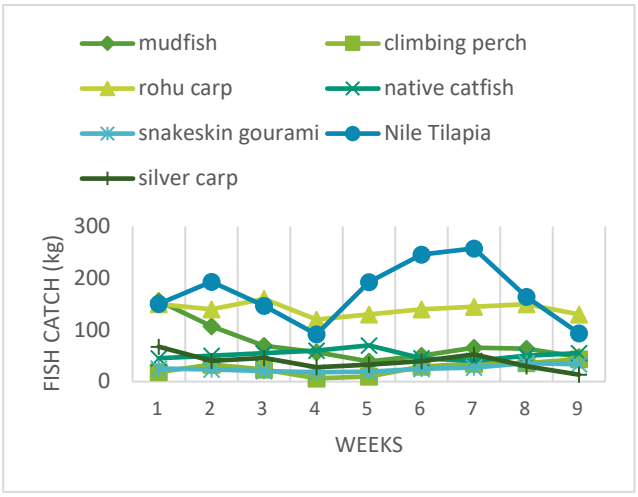



Figure 5. Fish catch landing of fishes caught in Ligawasan marsh using gill net.

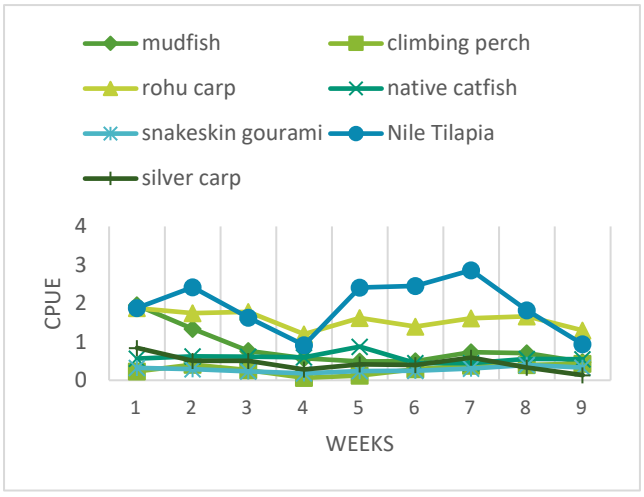



Figure 8. CPUE of fishes caught using gill net.

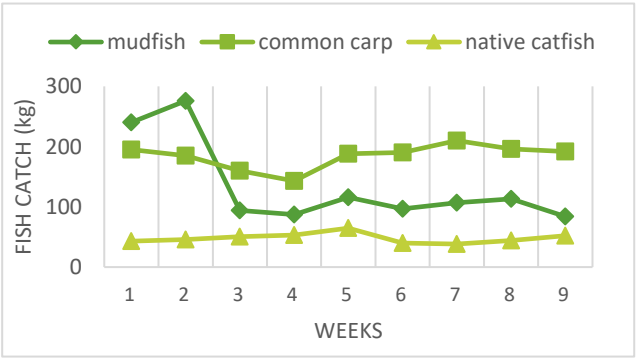



Figure 6. Fish catch landing of fishes caught in Ligawasan Marsh using improvised fish trap.

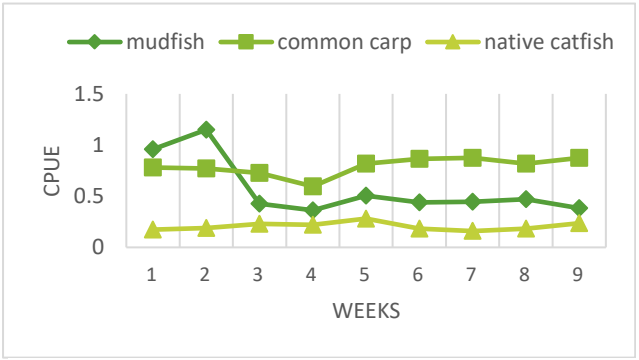



Figure 9. CPUE of fishes caught using improvised fish trap.

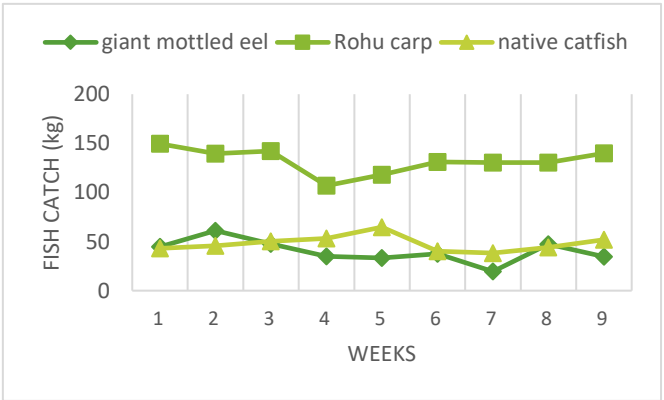



Figure 7. Fish catch landing of fishes caught in Ligawasan Marsh using fish pot.

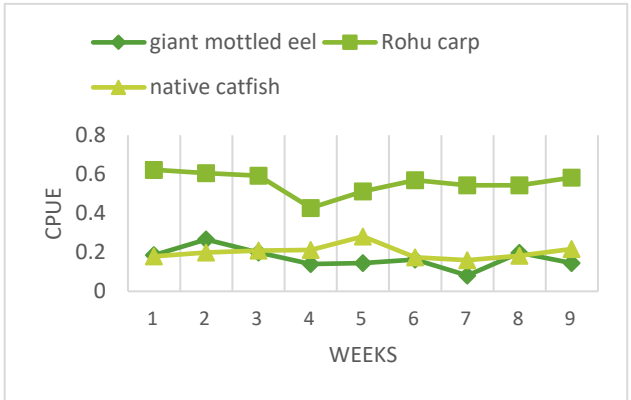



Figure 10. CPUE of fishes caught using fish pot.

*Catch Per Unit Effort (CPUE)*

In this study, CPUE was calculated from the catch according to each type of fishing gear used (Figure 8-10). Since type of fishing gears influenced the catchability of fishes in Ligawasan, gear selectivity was considered as a factor in CPUE. The number of fishing hours varies for each week using gill net on daily basis, fishermen spent around 6-10 hours fishing, from traveling to the fishing grounds to disposal of catch at fish landing sites. By practice, the fishermen in Ligawasan allow 24 hour-entrapment time before harvesting.

*A. Gill net*

Comparing Figure 5 and Figure 8, CPUE and total catch using gill nets showed a similar trend. It means that regardless of fishing effort, relative abundance of fish caught in Ligawasan was already evident in the fish catch landing data. It can be implied that the CPUE of gill nets and the total fish catch by gill nets were directly proportional. CPUE of fishes caught using gill net recorded values for Nile tilapia at 0.91-2.86, rohu carp at 1.2-1.87, mudfish at 0.47- 1.95, native catfish at 0.44-0.87, silver carp at 0.13- 0.84, climbing perch at 0.06-0.43, and snakeskin gourami at 0.18-0.39. The highest CPUE average using gill net was recorded in Nile tilapia and least with snakeskin gourami.

*B. Improvised Fish Trap*

The improvised fish trap CPUE recorded lower values for native catfish (0.16- 0.28) and higher values for mudfish (0.36- 1.15) and common carp (0.59- 0.87). The average CPUE using improvised fish trap was highest for common carp (0.79) and least for mudfish (0.57). Comparing Figure 6 and Figure 9, it showed that similar trends could be detected in mudfish, native catfish and common carp. Fishing effort influenced abundance of fish catch.

*C. Fish pot*

Both fish catch trends (Figure 7) and the trend observed in CPUE of fishes caught using fish pot (Figure 10) showed similar trends. Longer trapping time could have influenced higher probability of catching more fishes into the fishing gear. Figure 10 showed fish pot had lower CPUE data for giant mottled eel (0.08-0.27), native catfish (0.16-0.28), but higher CPUE for rohu carp (0.43-0.62). Average CPUE using fish pot was highest for rohu carp (0.55) and least for giant mottled eel (0.17).

*Fish Length-Weight*

Length of fish samples, including the minimum and maximum ranges from the catch, as well as average length were presented in Table 2, Figure 11. For this study, total length was measured which correspond to the length from the tip of the snout to the tip of the caudal fin.

No evident increase in size was recorded as the sampling progressed. Ranges of fish length were recorded for each species as shown in Table 2. For instance, the total length of giant mottled eel ranged from 89 to 92 cm, rohu carp ranged from 37.5 to 42 cm, the native catfish length ranged from 35.5 to 39.5 cm, common carp ranged from 30.5 to 36.5 cm, mudfish ranged from 26 to 37.5 cm, silver carp ranged from 20 to 22.5 cm, Nile tilapia ranged from 17 to 21.5cm, snakeskin gourami fish ranged from 15 to 18 cm, and climbing perch length ranged from 12.5 to 17cm.

***Table 2.*** Length (cm) of fishes caught in Ligawasan marsh (n=10).

| Week | Giant mottled eel | Rohu carp | Native catfish | Common carp | Mudfish | Silver carp | Nile tilapia | Snakeskin gourami | Climbing perch |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 90.5 | 38.5 | 35.5 | 32.0 | 30.5 | 20.0 | 17.0 | 16.0 | 15.0 |
| 2 | 89.0 | 42.0 | 37.5 | 30.5 | 33.0 | 21.0 | 17.0 | 15.0 | 14.5 |
| 3 | 92.0 | 41.0 | 38.5 | 32.0 | 26.5 | 20.0 | 17.0 | 15.0 | 14.5 |
| 4 | 89.0 | 40.0 | 38.5 | 31.5 | 26.0 | 22.0 | 20.0 | 15.5 | 12.5 |
| 5 | 90.0 | 39.5 | 36.5 | 34.0 | 27.0 | 22.0 | 21.5 | 15.5 | 14.0 |
| 6 | 92.0 | 38.5 | 39.0 | 36.0 | 32.0 | 22.5 | 21.0 | 15.5 | 15.0 |
| 7 | 92.0 | 38.0 | 39.5 | 36.5 | 30.0 | 23.0 | 20.5 | 15.5 | 17.0 |
| 8 | 92.0 | 39.5 | 38.5 | 33.5 | 35.5 | 22.0 | 19.5 | 18,0 | 14.0 |
| 9 | 91.0 | 37.5 | 39.0 | 33.4 | 37.5 | 22.5 | 18.0 | 18.0 | 14.0 |
| Min | 89.0 | 37.5 | 35.5 | 30.5 | 26.0 | 20.0 | 17.0 | 15.0 | 12.5 |
| Max | 92.0 | 42.0 | 39.0 | 36.5 | 37.5 | 22.5 | 21.5 | 18.0 | 17.0 |
| Ave | 90.8 | 39.4 | 38.1 | 33.3 | 30.9 | 21.7 | 19.1 | 16.0 | 14.5 |

## Discussion

The study assessed the relative abundance in terms of fish catch data and CPUE of the nine commercial fishes found in Ligawasan marsh observed from fish landing sites at Bago-inged, Pikit. Wilberg et al. (2009) stated that catchability is an important parameter in many stock assessment models because it relates the index of abundance to stock size. The study also provided sociodemographic profile of the community to reflect the importance of fish-ery resources to wetland community thus sustainable management of resources should be implemented.

*Socio-demographic Profile*

The socio-demographic profile of the community showed that the local population depended on fishing for sustenance and livelihood. Fishing is artisanal in nature. The popu-lation of fishermen is considered as aging, with the highest percentage of fishermen in ages 46 years old and above. This could explain the manner of fishing in the area that is considered traditional, using passive fishing gears such as "bubo", such fishing gears been used for dec-ades. Although, these types of fishing gears are considered as ecologically friendly, which can be related to the fishermen's role to conserve wetland resources against exploitation. However, mesh sizes of the fishing gears are not compliant with the standard mesh size stated in the Fisheries Code. The standard mesh size according to the Fisheries Code is not to exceed 3 cm, as compared to 1.5 cm mesh size observed in the nettings of fishing gears used by the fishermen in this study. This can be attributed to the higher percentage of mar-ried fishermen with large family size. This policy might have been overlooked, as the pressure to catch fish for daily subsistence becomes imminent.

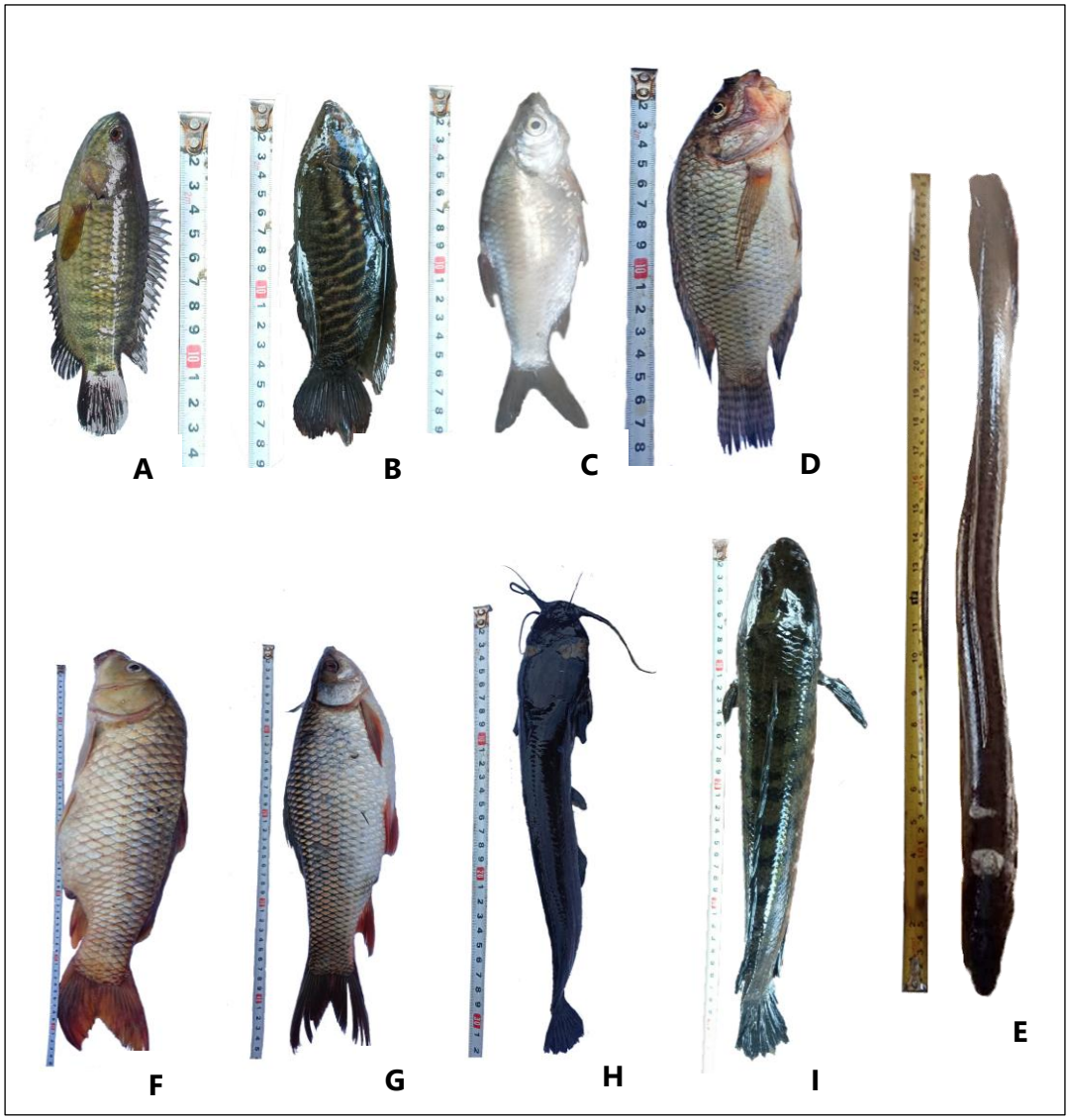



Figure 11. Fishes caught in Ligawasan marsh from fish catch landing site at Bagoinged, Pikit: (A) climbing perch; (B) snakeskin gourami; (C) silver carp; (D) Nile tilapia; (E) giant mottled eel; (F) common carp; (G) rohu carp; (H) native catfish; (I) mudfish.

*Fish Catch Landings*

In terms of relative abundance, the results showed that cyprinids are highly abundant in Ligawasan Marsh. A baseline study in Ligawasan marsh has recorded at least 33 species of freshwater fishes (DENR, 2004). The fishes recorded in this report include: bigeye trevally, broadband sleeper, catfish, climbing perch, common carp, dusky sleeper, giant gourami, giant mottled eel, glass perchlet, guppy, half-beak, Indo- pacific tarpon, java carp, Mozambique tilapia, mudfish, mudskipper, mullet, native catfish, olive flathead, Otomebora mullet, rohu carp, silver carp, snakeskin gourami, spotted barb, silver carp, tank goby, tapiroid grunter, three-spotted gourami, and walking catfish. DENR (2004) recorded the cyprinids as the dominant group which also agrees with the findings of the present study. There was no prior fish stock assessment conducted last 2004 from the DENR study and was limited only to qualitative description of the ecological and socioeconomic data. Since then, the most important fishery species from the marshes are dominated by introduced cyprinids, gouramys, cichlids, and the introduced catfish species. Cyprinids include common carp, rohu carp, and silver carp. The same species were found to be abundant in the present study. The native

fishes are brought to the fish landing sites for sale. In the case of the present study, native catfish which is native to Ligawasan marsh was also found being sold at the landing site.

The Fisheries Situationer (2022) reported inland municipal fisheries production at 205.54 thousand metric tons. In this context, production in Ligawasan marsh can be considered low, despite the abundant catch observed. However, a previous study by Small Grant Program (2012) estimated daily catch at approximately 10-30 tons. The present study estimates daily catch at 0.1-0.17 tons which is evidently lower compared to the data gathered ten years ago.

*Gear Selectivity*

Passive gears are in general commonly practiced in Ligawasan marsh fishing grounds, particularly "*bubo*" and "*pukot*". Gill nets are categorized under passive gears because these are stationary when used. These types of gears are commonly observed in small scale-fishing and are employed for artisanal fisheries because they require less effort and lower cost in the construction. Fishermen in Bagoinged, Pikit are engaged artisanal fishing.

From the data gathered, it can be stated that gear selectivity influenced the fish catch profile at Ligawasan marsh, particularly on fish catch landed at Bagoinged, Pikit. For instance, tilapia, climbing perch, silver carp, and snakehead gourami were only caught using gill net. Fish pot is the only fishing gear used to catch common carp, and fish trap is exclusively used to catch giant mottled eel. But this is not true for all cases of wetlands. In North and South Dakota, both gill nets and fish traps were used to catch common carp as observed in the study of Clark et al. (1991). Overall, from the data gathered, it can be stated that tilapia and common carp, despite its high gear selectivity, have high catchability compared to other fish caught in Ligawasan marsh.

The high relative abundance of tilapia in the area or its dominance can be accounted for its tolerance for a wide range of salinity, temperature and critical conditions. They are 'hardy' species with a high resistance to harsh environmental conditions, thus, they are considered basic food source in rural communities adjacent to freshwater sources. Given its characteristics, high turn-out is somewhat expected for tilapia catching rate however, it is unlikely for common carp.

Considering catch as a common index of relative abundance, it can be noted that common carp is relatively abundant in Ligawasan marsh, with a catch percentage similar with tilapia but with a large difference compared to other fishes such as native catfish, eel, gourami and other cyprinid species. But then, it can be noted that common carp is regarded as invasive species in wetlands (Pinto et al., 2005; Maiztegui et al., 2019). If the population of the common carp continues to grow at this rate, an intervention will be necessary to increase the abundance of other species. Conallin et al. (2012) cited that environmental water affluent can be managed to lessen population dynamics of common carp, to allow native fishes to thrive in the wetland. This may be challenging in the case of Ligawasan due to the regular occurrence of flooding, thus other interventions should be explored.

Low gear selectivity was observed in species such as mudfish, rohu carp and native catfish. According to the practice of fishermen in Ligawasan, rohu carp are caught using gill nets and fish traps while mudfish are caught using gill nets and fish pots. The use of two different fishing gears for these species could account for the high catching rates observed for rohu carp and mudfish species in the area. In addition, fish pot can catch more mudfish than gill net. Fish traps tend to catch more rohu carp than gill nets. A strikingly high catching rate was observed in the first weeks of observation in mudfish species, but this declined in

the following weeks. As compared to rohu carp, which was observed to have consistent high catching rate for the whole 9-week observation period.

Gill nets, fish pots and fish traps can be used to catch native catfish. Despite the use of these three fishing gears, fish catch for native catfish still exhibited incremental percentage compared to other fish species. This suggests that even though all three types of fishing gears were already used to catch this species, it still accounted for only 5% of the total catch, indicating low abundance of this fish in the area. Further assumptions may indicate that low abundance of native catfish was affected by exploitative competition with other fish in Ligawasan, or that its stocks are declining due to overfishing. From this study, it is implied that higher gear selectivity does not always reflect lesser catchability of a particular species. Also, low gear selectivity does not necessarily indicate high relative abundance.

*Catch per Unit Effort*

From the data gathered, respondents indicated that fishing effort was affected by water levels and flooding in Ligawasan marsh. Generally, the fishing effort is affected by flooding thus less catch was observed at some weeks wherein flooding or water level rise was encountered. The highest CPUE was observed in common carp (13-21.8), while lowest CPUE was observed in snakeskin gourami (1.6 -3.97).

Furthermore, higher CPUEs were observed in mudfish, common carp, rohu carp and tilapia as opposed to native catfish, gourami, silver carp, climbing perch and giant mottled eel. The variation incurred in fish pot CPUE can imply that even though fishing effort was increased, fish catch remains low. Hence, there is an indication that relative abundance has evidently declined for giant mottled eel and native catfish, except for rohu carp wherein high CPUE was observed.

*Length- Weight Relationship*

FishBase website recorded the average size of giant mottled eel at 26.3 cm with a maximum length for male species at 70 cm and female species at 200 cm. Comparing the data from the study, giant mottled eel (89-92 cm) from Ligawasan marsh exceeds the average and maximum length for male species. However, the study was not able to observe the reproductive organ of the species which is a limitation of the study.

He et al., (2021) recorded rohu as a fast-growing species reaching a length of 35-45 cm. However, the length recorded in the present study was shorter compared in the study of Sarkar et al. (2017) wherein the size of *Labeo rohita* ranged from 18-24 cm in Kulia beel, a wetland adjacent to the river of West Bengal, India. In another study, Kaur et al. (2018) recorded rohu carp length at 67.6 cm–51.3 cm at Harike wetland, Punjab, India.

The length of native catfish in this study were recorded at 35.5- 39.0 cm which was shorter compared to the maximum length recorded by Fishbase.org at 120 cm. On the other hand, the length of native catfish in Agusan Marsh was comparable at 25.5-41.0 cm (Jumawan and Seronay, 2017). It can be noted that both wetlands recorded almost similar fish length for native catfish thus the wetlans structure and can be a factor for this size limitation.

Common carp can grow up to 93 cm as observed in the study of Abassi et al. (2017) at Zayandehrud dam, Isfahan, Iran. The study of Jumawan and Seronay (2017) recorded a length up to 51 cm for common carp. Fishbase.org reported common carp length at 34.8 cm at maturity, with a common length at 31 cm, but can grow up to 120 cm. This could mean that common carps found in Ligawasan may not have reached its maturity length but not yet its

maximum length. There is also a possibility that common carp caught in Ligawasan have not yet reached its maximum size at this time of the year.

According to Fishbase.org, the maximum length for mudfish is recorded at 100 cm with common length of 61 cm. The length recorded in this study (25-37.5 cm) were shorter and also shorter compared to the range recorded in Agusan Marsh (23-61 cm) in the study of Jumawan and Seronay (2017). A substantial study on the nutrient composition at Ligawasan Marsh could also be considered to further explain these findings as well as the migratory behavior of fishes in the area.

Fishbase recorded the maturity length of silver carp at 51.7 cm with a maximum length at 120 cm. The common length of silver carp was recorded at 18 cm (Abassi et al., 2017). The data from the present study showed a shorter length for silver carp at 23 cm, but longer than the common length mentioned. This suggests that the silver carp in Ligawasan marsh have not yet reached their maximum sizes.

Nile tilapia length in this study are comparable with common sizes observed at harvestable sizes in ponds, although it is slightly increased above average as observation progressed in the latter weeks. The length of tilapia in wetland have not been widely discussed in literatures, which mostly focuses on their invasive nature, thus explaining their proliferation in almost all bodies of water.

According to Fishbase.org, snakeskin gourami can grow to a maximum size of 25 cm, but is commonly observed at 15 cm. The same length was observed in this study (15-18 cm). Welcomme (1988) reported several countries with adverse ecological impact caused by the introduction of this species. The species is naturally occurring in Mekong basin (Kottelat, 2001) and introduced widely in the southeast Asian region (Paepke, 2009).

Climbing perch length were recorded at 12.5–17 cm which is slightly longer compared to the study of Kumary and Raj (2016), where length registered a minimum of 7-10 cm at Kuttanad wetland in Kerala state, India.

This study compares the results with the study of Jumawan and Seronay (2017) relating to the length-weight relationship of fishery resource in Agusan marsh. Although these studies are not interconnected, both are located in Mindanao, making this a relevant comparison of wetlands situated near the Ligawasan. A comparison of the length, weight, linear regression and status of similar species can be shown in the two studies (Table 3).

From the data gathered, comparing two wetland ecosystems, Ligawasan marsh has a lower length-weight range for most species compared to that in Agusan marsh, except for snakeskin gourami (*Trichogaster trichopterus*). It can be noted that minimum lengths recorded in Ligawasan was higher compared to Agusan marsh, but the maximum lengths were greater in Agusan marsh. This difference may be due to the wider variety of fishing gears used by fishermen in Agusan Marsh, which could have affected the catchability rate in the wetland. Fishermen in Agusan Marsh tends to use more types of fishing gears such as cast nets, scissor nets, fish trap, fyke net, and multiple hook and lines (Jumawan and Seronay, 2017). Given that in Agusan marsh, different types of fishing gears were used to catch similar fishes and recorded higher length and weight, there is a possibility that extractive factors in Ligawasan marsh could be limiting the growth of these species to reach its maximum size. This is highly evident in species like mudfish, tilapia, and common carp species. However, considering that fishermen in Ligawasan only used passive fishing gears, another factor may be influencing the growth of fish to a certain length, in this area. It is possible that fish pots

and fish traps are located near the breeding grounds blocking the migration routes obstructing the growth of adult fishes that have not yet reached the maximum size. Otherwise, there is a nutritional factor in Ligawasan marsh that does not permit growth beyond the cited range.

The status of species is similar in two wetland sites except for mudfish that is native to Ligawasan but an introduced species in Agusan Marsh. The dominance of introduced species was noted to pose many dangers to the aquatic habitat. Compared to native fishes, introduced species tend to have higher reproductive rates (Townsend and Pettigrew, 1996; Sakai et al., 2001; Mills et al. 2004) and one reason could be early maturity at a shorter size range. Tilapia for instance can reproduce as early as three month-time.

***Table 3.*** A comparison between Agusan Marsh (Jumawan and Seronay, 2017) and Ligawasan Marsh (this study) fishery resources on total weight and body weights.

| Family and Species | | Total length (cm) | Body weight (g) | Status |
|---|---|---|---|---|
| *Anabantidae* | | | | |
| *Anabas testudineus* | | | | |
| | Ligawasan | 12.5- 15 | 36.1- 77.7 | Native |
| | Agusan | 9.6- 17 | 15.50- 77.6 | Native |
| *Channidae* | | | | |
| *Channa striata* | | | | |
| | Ligawasan | 26- 37.5 | 131.5- 396 | Native |
| | Agusan | 23- 61 | 50- 1500 | Introduced |
| *Cyprinidae* | | | | |
| *Clarias macrocephalus* | | | | |
| | Ligawasan | 35.5- 39.5 | 40.1- 52 | Native |
| | Agusan | 25.5- 41 | 115- 500 | Native |
| *Cichlidae* | | | | |
| *Oreochromis niloticus* | | | | |
| | Ligawasan | 17- 21.5 | 93.7- 293.3 | Introduced |
| | Agusan | 11.5- 47 | 25- 2000 | Introduced |
| *Osphronemidae* | | | | |
| *Trichogaster trichopterus* | | | | |
| | Ligawasan | 15.5- 18.0 | 18.1- 35.7 | Introduced |
| | Agusan | 4.9- 11.5 | 1.7- 21.5 | Introduced |

## Conclusion

From this study it can be inferred that fishery resources in Ligawasan Marsh were considered abundant. In terms of total fish catch in 9- week observation period, fish landing in Bagoinged, Pikit recorded approximately 8 tons of fish catch from 9 fish species. Ligawasan Marsh resources can produce up to 8 metric tons of fish in 9 weeks period. However, data on length-weight relationship revealed that smaller fish are being caught compared to maximum sizes of these species. Although, a comparison of similar study of a wetland in close proximity, such as Agusan marsh, showed higher length-weight range than Ligawasan marsh

except for snakeskin gourami. Gear selectivity was also evident in Ligawasan marsh. Giant mottled eel was only caught using fish pot, while common carp was only caught using improvised fish trap. Climbing persh, snakeskin gourami, Nile tilapia and silver carp were only caught by gill net. Both gill nets and improvised fish traps were effective in catching mudfish in the area, while both gill nets and fish pots were used to catch rohu carp. The low gear selectivity of mudfish and rohu carp may have contributed to its relative abundance in fish catch. However, all three fishing gears were able to catch native catfish and yet low fish catch data were still recorded. Thus, it can imply low relative abundance of native catfish. In contrast to Nile tilapia which is caught only by using gillnet and common carp caught only by using improvised fish trap yet high fish catch data were recorded. This also implies that these areas of Ligawasan marsh are most abundant in Nile tilapia and common carp. Further research is needed to determine the rate of recruitment in Ligawasan marsh to compare the rate of reproduction. If both recruitment and reproduction are determined, then the rate of dwindling stock can be assessed. With this data, the information on how to regulate fishing, or on how to implement closed season to allow time of propagation of a particular species.

## Recommendation

There is a need to determine the sustainable level of fish catch specifically the native fishes. The land use policy of the municipalities bordering the wetland has significant influence on the fishing resources. Fish policy development is needed in Ligawasan Marsh. Although there are existing policies prohibiting catch of young freshwater fish, these are not properly enforced as evidenced by the data gathered in fish stock assessment. If fish caught contains smaller sizes than those commonly observed in wetlands in the Philippines (i.e. Agusan Marsh), then a more specific municipal ordinance should be carried out in the community. The low abundance of native catfish in the area, despite low gear selectivity, suggests a need for proper management of fishery resources. The socio-political structure in Ligawasan Marsh must have a concrete system in place on how policies can be implemented in the community. LGUs play a crucial component in promoting or spearheading policy development in the community, and a strong linkage to the LGU unit of the municipality should be established to facilitate the implementation of new policies in the Ligawasan community.

The study focused on the fish catch landing in the data collection excluding data from breeding grounds. Although it is highly recommended to identify breeding grounds in the marsh in the future studies. For this study, results suggest that the species most likely to be captured depends on gear selectivity. Results showed giant mottled eel tend to be caught only using fish pot. Snakeskin gourami, tilapia and silver carp are caught using gill net. Interestingly, native catfish is the easiest to catch regardless of the different gears used.

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No potential conflict of interest was declared by the authors.

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## The Crocodile Specialist Group

The Crocodile Specialist Group (CSG) is a worldwide network of biologists, wildlife managers, Government officials, independent researchers, non-government organization representatives, farmers, traders, tanners, manufacturers and private companies actively involved in the conservation of crocodilians (crocodiles, alligators, caimans and gharials). The group operates under the auspices of the Species Survival Comission (SSC) of the IUCN. The CSG provides a network of experts to assess conservation priorities, develop plans for research and conservation, conduct surveys, estimate populations, provide technical information and training, and to draft conservation programs and policy. CSG also assists monitoring of international trade and identifying products. Working meetings of the group are held every two years.

## Foreword

The 19th working meeting of the IUCN-SSC Crocodile Specialist Group (CSG) was held in Santa Cruz, Bolivia, on 3-7 June 2008. The meeting was preceded by the CSG Steering Committee meeting, open to all members, which addressed a wide range of current CSG priorities. These included various aspects of crocodilian conservation and management in Madagascar, Colombia, Ecuador, Paraguay and South East Asia generally. They also included the proposed West African CSG sub-regional meeting, to be held in Burkina Faso.

The CSG is extremely grateful to the Beni Department Prefecture which hosted the meeting. The Organizing Committee (Karina Sauma, Alfonso Llobet, Silvia Ovando, Alvaro Velasco, Alejandro Franulic, Ana Karina Bello, Francisco Aguilera, Omar Rocha, Aleida Justiniano) and their support staff did a marvelous job in preparing and running what all considered to be a wonderful meeting. Their efforts make a very significant contribution to the CSG, and through them, to the SSC and IUCN.

None of this would have been possible without the generous financial support provided by the major sponsors of the meeting, whom I thank here on behalf of all CSG members: Programa Nacional de Biocomercio Sostenible (PNBS), Fundacion Amigos de Naturaleza (FAN), Prefectura del Departamento del Beni, Prefectura del Departamento de Santa Cruz, CIENSA, Curtiembre Moxos, Bolivian Leathers and Food, and Bolivian Croco. Additional financial and in-kind support was provided by the Buganvillas Hotel and ICEA.

For the many people who work on crocodilians around the world, the biennial working meetings of the CSG are an important event. Working with crocodilians requires a special effort by special people. Crocodilians live in remote and inhospitable places, where access is difficult. Because they range in weight from less than 50 g to over 500 kg, catching and handling is always a challenge, not to mention the personal risks involved. In the eyes of the general public, it is often a thankless task, because crocodiles are truly viewed as being "wicked" by most people. Not so amongst CSG members.

CSG working meetings, bring together an exceptional array of talented people, from all around the world. For most of them, the time and travel involved is a significant personal cost. The major reward is the ability to share one week with like-minded people, equally passionate about crocodilians. It recharges often tired batteries, stimulates interest, fosters camaraderie, creates new friendships, puts new faces to names, provides a genuinely sympathetic ears for discussion of problems, and most important, provides an opportunity to pass on new results and findings.

The core business of CSG is to help the IUCN and SSC achieve their conservation missions with crocodilians. This involves a raft of different CSG initiatives and activities in different countries, some simple others immensely complex. They are all addressed openly within the Working Meetings. As the complexity of the world expands, so the "biopolitics" of crocodilian conservation becomes more challenging. But the CSG adapts well. We do an exceptional job, quickly, honestly, transparently and usually by consensus. That we do it largely as volunteers, with very few paid staff, is remarkable in its own right.

An important key to the success of the CSG is that its membership includes representation from a great diversity of different stakeholders. We can look at the same problem through many different eyes. Particularly important are members representing the international crocodile skin industry. They keep us focused on attainable goals, make sure our concerns about trade are valid, and offer sound advice and a wealth of experience when required.

The proceedings of the 19th Working Meeting of the CSG is a unique compendium of current information on problems, research and new ideas about crocodilian conservation and management. It is both a source book and a reference book. We take this opportunity to thank the organizing committee for their efforts in getting the proceedings published in a timely way.



Profesor Grahame Webb,
Chairman, IUCN-SSC Crocodile Specialist Group

## 19th CSG Working Meeting Summary

The 19th Working Meeting of the IUCN-SSC Crocodile Specialist Group (CSG) was held in Santa Cruz, Bolivia, from 3-7 June 2008, and was preceded by a CSG Steering Committee meeting on 2 June.

The meeting was hosted by the Beni Department Prefecture, and the CSG is extremely grateful to them and the organising committee (Karina Sauma, Alfonso Llobet, Silvia Ovando, Alvaro Velasco, Alejandro Franulic, Ana Karina Bello, Francisco Aguilera, Omar Rocha, Aleida Justiniano) for their considerable support for the meeting. Karina Sauma and Alfonso Llobet were the chief co-ordinators for the meeting, and expended considerable effort in the months leading up to and during the meeting to ensure that everything ran smoothly – they did a wonderful job.

Professor Grahame Webb (CSG Chairman) welcomed 220 participants from 27 countries (Australia, Argentina, Bolivia, Papua New Guinea, Japan, Thailand, USA, Mexico, Panama, Colombia, Venezuela, Paraguay, Guatemala, Uruguay, Brazil, Germany, Netherlands, Spain, Hong Kong, Italy, France, United Kingdom, Costa Rica, Ecuador, Madagascar, Guyana and Denmark). In particular, there was good representation from Latin America.

CSG working meetings, held every two years, are the primary international meeting dedicated to crocodilian conservation, management and research. They have become the major forum for discussion of conservation issues, presenting new findings and new directions, and the 19th meeting was no exception.

A number of important issues were addressed by the CSG Steering Committee, and a range of topics were later covered by oral presentations over the 4-day working meeting, organised into the following sessions:

- Conservation, management and sustainable use of crocodiles in Bolivia
- Conservation, management and sustainable use of crocodiles in Latin America
- Trade and impacts on crocodiles
- Systematics, taxonomy and genetics of crocodiles
- Genetics and immunology of crocodiles
- Breeding in crocodiles
- Population status of crocodiles
- Endangered species actions
- Miscellaneous.

A series of workshops were also held:

1. Sustainability criteria (environmental, social, economic) for the success of national management programs.
2. Local organisations in conservation and management of crocodilians.
3. Aspects affecting the sustainability of crocodilian trade.
4. Management plans as conservation tools in Latin America.

In addition, the CSG's Veterinary Science (Vice Chair, Paolo Martelli) and Zoos and Community Education (Vice Chair, Kent Vliet) groups met during the course of the meeting. Merlijn van Weerd took the opportunity to convene a meeting of interested people to discuss conservation and management of the Philippine crocodile.

The deliberations of each workshop and thematic group were summarised at the end of the working meeting.

No CSG meeting would be complete without the various social activities, and this meeting was no exception. Monday's welcome function featured classical "baroque" music and singing by the "Coro y Orquesta de San Javier", and the following night's dinner included rock band "Track". On Wednesday, typical Bolivian cuisine featured at "La Casa del Camba", where traditional dancers provided entertainment.

After participants had dined on a range of local foods (and drinks) at La Casa del Camba, auctioneers Joe Wasilewski and Carlo Piña "extracted" $US3003 for various articles donated by people for the CSG auction. These funds will be contributed towards important research being undertaken on Indian gharial to better understand the cause of the recent mass mortality in the Chambal River. The auction is proving to be a popular event, and is set to become a permanent feature of future CSG working meetings.

Crocoland S.R.L., the first crocodilian farm established in Bolivia, was visited during the field trip on 7 June. About 29 km out of Santa Cruz, Crocoland has been in operation since September 2006. It started with 1600 female and 400 male adult *C. yacare* extracted from the Bolivian Pantanal. The breeding stocks are held in 8 lagoons. Farm stocks are derived from captive breeding and ranching of eggs. The visit was hosted by owners Jorge Baldivieso V., Jorge Baldivieso O., Carlos Ormachea and Alejandro Franulic. It was preceded by a magnificent barbeque at the adjacent San Juanito, giving participants a chance to "wind-down" and enjoy more Bolivian hospitality.

Zilca Campos from Brazil became the latest recipient of the Castillos Award for her significant contribution to crocodilian biology, management and conservation dedication and significant contribution are summarised by long-time colleague Alejandro Larriera:

"When one sees Zilca Campos at some international meeting, it is easy to mistake her for just another enthusiast and young researcher who attends workshops and conferences trying to learn something, and to know more about the "great" researchers of the CSG. Even if you speak with her, she will ask more about your work and research and be more interested in your results and successes, rather than being proud of her own successes, of which there have been many.

For over 20 years Zilca has worked enthusiastically, as much in the field as in the office, for the conservation of South American caimans. At times she has studied species of economic importance such as *Caiman yacare*, with her results being applied to programs of use and management. Yet she has also directed a great deal of effort to species with no commercial value, such as *Paleosuchus*.

Whatever her line of work, the common denominator has always has been her professionalism, stability, capacity to learn, and ability to work in a team - and luckily for all of us, her ability to teach. In this regard, it is worth clarifying that Zilca not only teaches us what she knows, but much more importantly, she serves as an example not only for young people starting in these activities, but for all of us. I hope there is still time to follow her lead."

The 20th CSG Working Meeting will be held in Manaus, Brazil, in September 2010.

## Host, Sponsors and Donors

Host Organization

· Prefecture of Beni Department

19th CSG Meeting Organizing Committee

· Karina Sauma, Fundación Amigos de la Naturaleza
· Alfonso Llobet, Asociación Boliviana de Conservación
· Silvia Ovando, Fundación Amigos de la Naturaleza
· Alvaro Velasco, Crocodile Specialist Group
· Alejandro Franulic, Moxos tannery and Crocoland SRL
· Ana Karina Bello, Prefecture of Beni Department
· Francisco Aguilera, Prefecture of Santa Cruz de la Sierra Department
· Omar Rocha, General Direction of Biodiversity and Protected Areas, Minister of Rural Development, Farming and Environment
· Aleida Justiniano, Museum of National History Noel Kempff Mercado

Mayor Sponsors

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· Programa Nacional de Biocomercio Sostenible (PNBS) - FAN Bolivia.
· Prefectura del Departamento del Beni.
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· Fundación Amigos de la Naturaleza.
· CIENSA
· Curtiembre Moxos.
· Bolivian Leathers and Food
· Bolivian Croco

Additional Sponsors and Donors

We are very grateful to the following people and/or organizations for their financial and in-kind support of the meeting:
· IUCN-SSC Crocodile Specialist Group
· Bunganvillas Hotel

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Chairman of the CSG, Prof. Grahame Webb. Included in the background are Charlie Manolis, Javier Castroviejo and Rafael Antelo (Photo: Mariana Escobar).



Joseph Wasilewski holding an excellent reproduction of a Gharial skull during the auction to raise funds for Gharial conservation (Photo: Alfonso Llobet).



Members of the Organizers Committee and some sponsors (Prefectura de Santa Cruz, Bolivian Leathers & Food, Bolivian Croco, Prefectura del Beni, Crocoland, Curtiembre Moxos, ICEA) of the 19th CSG Working Meeting (Photo: Mariana Escobar).



Some participants of the CSG 19th Working Meeting during the barbeque at Crocoland (Photo: Alfonso Llobet).




Alvaro Velasco being interviewed for a local news channel during the closure of the meeting (Photo: Mariana Escobar).



Charlie Manolis (one big croc), Alfonso Llobet and Tom Dacey (another big croc), happy after having a fine CSG Working Meeting (Photo: Mariana Escobar).

# Results of the first harvest of *Caiman yacare* by Takana communities in northern Bolivia: implications for sustainability and harvest regulations.

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**ABSTRACT:** *Caiman yacare* harvest is an important economic activity of the Takana people. The construction of the management plan for spectacled caiman harvest began in 2001, through an alliance between the Takana representative organization CIPTA and the Wildlife Conservation Society, which supported a student thesis and research on the abundance and distribution of caiman in the Takana indigenous territory. On the basis of this information a management plan was approved in August 2007 which permitted the harvest of 524 individuals. These individuals where harvested from 23 water bodies of the 34 originally planned. The majority of individuals where harvested from lagoons (260), followed by streams (136), lake Moa (65) and the Beni river (63). 78,8 % of hunted individuals had total lengths of over 180 cm, but 100 % of harvested individuals produced waist coats of at least 90 cm in length, as established by the harvest regulations. In the harvest process 136 individuals where reported as injured and lost. As a result of the harvest 11 females where removed of which 4 had measurements above 180cm. Accidental harvest of 5 black caiman *(Melanosuchus niger)* was also reported. The presentation of these results aims to guide the improvement of harvests throughout Bolivia and perhaps other countries. We consider that there is a wealth of information which could guide the revision and improvement of the regulation guiding caiman harvest in Bolivia and that monitoring efforts of this and other initiatives will continue improving the program to ensure that viable populations of *Caiman yacare* continue to be available as a sustainable harvested resource for future generations.

**RESUMEN:** El aprovechamiento de *Caiman yacare* es una actividad económica importante del pueblo tacana. La construcción del plan de aprovechamiento se inició el 2001, a través de una alianza entre la organización representativa de los tacanas (CIPTA) y Wildlife Conservation Society (WCS) la cual apoyó la ejecución de una tesis e investigaciones sobre la abundancia y distribución de lagartos en la TCO Tacana. En base a estos estudios, se elaboró un plan de manejo que fue aprobado en agosto de 2007 permitiendo la cosecha de 524 individuos. Estos individuos se cazaron en 23 cuerpos de agua de los 34 originalmente previstos. La mayor cantidad de individuos cosechados provino de lagunas (260), seguida de los arroyos (136), lago Moa (65) y del río Beni (63). El 78,8 % de los individuos cazados superó los 180 cm de largo total, pero el 100 % de los individuos cosechados produjeron chalecos iguales o mayores a 90 cm, conforme a lo establecido en el reglamento. En el proceso de la cacería se han reportado 136 individuos heridos y no recuperados. Como un efecto de la cacería 11 hembras fueron cazadas, de las cuales 4 tenían medidas superiores

a los 180 cm. También se ha reportado la caza accidental de 5 caimanes negros *(Melanosuchus niger)*. La presentación de estos resultados y análisis tienen como fin que estas experiencias sirvan como referencia para el mejoramiento del aprovechamiento en la zona así como en otras del territorio boliviano, y por que no en otros países de la región. Consideramos que existe mucha información y muchas implicaciones que podrían servir como base a para una potencial revisión del reglamento de aprovechamiento de lagarto en Bolivia y que los esfuerzos de monitoreo de ésta y otras iniciativas continuarán enriqueciendo a este programa con el fin de asegurar que poblaciones viables de *Caiman yacare* sigan constituyéndose en un recurso aprovechado sosteniblemente y esté disponible para muchas más generaciones.

# Conservation and Sustainable Use Program of Yacare Caiman *(Caiman yacare)* in Beni, Bolivia

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**ABSTRACT:** The Conservation and Sustainable Use Program of Yacare *(Caiman yacare)* has been the first management and harvest program of wildlife on a large scale in the Beni Department of Bolivia. With an experimental beginning in 1997 -1999, the Program has been adjusted to the biological and cultural characteristics of the social sectors that participate in it: (indigenous, farmers and ranch owners). At present, in the Beni Department, its develop a big strategy to correct some deficiencies observed inside the Yacare Program through the implementation of specific Management Plans, that include new actors, like Municipalities and NGOs, and a direct participation of Departmental and National Authorities. The Prefecture of Beni Department conscientious that the Yacare program needs a feedback that permit adapt to the new trade, biological and climatic changes, are organizing themselves to respond more efficient and practical to the new challenges, through make better the control and monitoring the yacare wild populations.

**RESUMEN:** El Programa del Conservación y Aprovechamiento Sostenible de Lagarto *(Caiman yacare)* ha sido el primer programa de aprovechamiento y conservación de fauna silvestre a gran escala en el Departamento del Beni. Con un inicio experimental en el periodo 1997 -1999, el Programa se ha ido ajustando a las características biológicas y culturales de los sectores sociales que participan de él: indígenas, campesinos y ganaderos. En la actualidad, en el Departamento del Beni se esta haciendo un gran esfuerzo por corregir algunas deficiencias observadas dentro del Programa de Lagarto a través de la implementación de Planes de Manejo específicos para esta especie, resaltando el involucramiento de nuevos actores al programa, como Municipios y ONGs, y la mayor participación más directa de Autoridades Departamentales y Nacionales. En el futuro, concientes que el Programa Lagarto se debe retroalimentar para adaptarse a los cambios del mercado, biológicos y climáticos que repercuten directa e indirectamente en los avances del Programa, la Prefectura del Departamento del Beni se está organizando para responder a esto nuevos retos de una manera más práctica y eficiente, realizando los esfuerzos para lograr un buen control y monitoreo del estado poblacional del *Caiman yacare*.

# Assignment of harvest quota of *Caiman yacare* in Bolivia

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**ABSTRACT:** The Program for the Conservation and Sustainable Use of spectacled caiman *(Caiman yacare)* foresees the annual harvesting of 45000 adult males, mostly in the Beni and Santa Cruz states of Bolivia. The present paper reviews the different systems that have been used in the past to assign harvest quota. Strengths and weaknesses of the different assignment systems are discussed and recommendations to improve these systems are provided.

**RESUMEN:** El Programa para la Conservación y el Uso Sostenible del *Caiman yacare* contempla la caza anual de 45000 machos adultos, primordialmente en los departamentos del Beni y Santa Cruz (Bolivia). Este estudio tiene como objetivo el de discutir los sistemas de asignación de cupos de caza que se han aplicado en el pasado. Se presentan las fortalezas y debilidades de los distintos enfoques y se formulan recomendaciones para mejorar los métodos.

# Analysis of models to assign harvest quotas and to estimate habitat potential of Yacare caiman in Bolivia

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**ABSTRACT:** In the framework of the Bolivian caiman *(Caiman yacare)* conservation and sustainable harvesting program one model was developed in 2004 to assign harvest quotas for each registered property and another model was developed in 2005 to estimate the relative potential of caiman habitats at the micro-watershed level. However, none of them was validated due to a lack of enough population data. With the development of 14 caiman management plans in indigenous lands, municipalities and protected areas, a significant census effort conducted between 2004 and 2007 produced enough data to analyze and validate the models. As a product of this analysis, we discuss the need to adjust and improve the existing models, as well as the usefulness of developing a new model to estimate in a more precise and reliable way the harvesting quotas for Bolivian caiman.

**RESUMEN:** En el marco del Programa Nacional para el Aprovechamiento Sostenible del Lagarto *(Caiman yacare)* en Bolivia, se avanzó en el desarrollo de un modelo para la determinación de cupos de cosecha de lagartos a nivel de predios, así como en otro modelo destinado a analizar el potencial de hábitat de la especie a un nivel de microcuencas. Sin embargo, ninguno de estos modelos pudo ser validado por falta de información confiable sobre datos poblacionales de la especie en el campo. Actualmente, con el desarrollo de 14 planes de manejo para el aprovechamiento del lagarto en tierras indígenas, municipios y en áreas protegidas, entre los años 2004 y 2007, se ha desarrollado un importante esfuerzo de muestreo que permitió contar con la información de campo suficiente para poder encarar un proceso de análisis y validación de los mencionados modelos. Producto de este análisis, se discute la necesidad de ajustar y mejorar los modelos existentes, así como la pertinencia de desarrollar un nuevo modelo que permita calcular de forma más precisa y confiable los cupos para el aprovechamiento sostenible del lagarto en Bolivia.

# Indigenous vision about the management of *Caiman yacare*: changing attitudes based on our reality

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**ABSTRACT:** The management of *Caiman yacare* on Bolivia has already started before the National Programme of *Caiman yacare* (PNASL) begins but without transparent to assure the sustainable management and with no real benefits for indigenous communities. Once the *C. yacare* Programme in Bolivia began, was recognized the right of local people to obtain benefits trough the use of this resource in their areas; however, instead of to have the rights in the more richness zones for de specie, the Government didn't give them the right to decided over the gestion of the Programme, with all this problems they didn't feel the benefits. With the indigenous experience in the territorial management they change the attitude changing the pression over the Government for a new way of development norms. In this way the PNASL moved on to a new sustainable management based in the development of management plans, where the indigenous are the leading actors. This new model wants to improve the social management to assure the sustainability of the harvest, and to promote the generation of responsibility over the taken actions. At the same time, strengths the role of the indigenous in the productive chain, avoiding manipulations from other actors and looking for better information to adjust de management in an adaptive approach.

**RESUMEN:** El manejo del lagarto en Bolivia comenzó antes del programa nacional, pero sin reglas claras ni criterios para asegurar un manejo sostenible, y sin que existan beneficios reales para los indígenas. Una vez que inició el Programa Lagarto en Bolivia, se reconoció el derecho indígena de obtener beneficios de este recurso en sus territorios; sin embargo, a pesar de tener derechos sobre las zonas con mayor potencial de aprovechamiento, siguieron marginados para poder decidir sobre la gestión del programa, y no recibían beneficios justos en un Programa Nacional donde no estaban claramente asumidos derechos y responsabilidades de los diferentes actores.

Con la experiencia indígena en la gestión territorial, se inició un cambio de actitud con el que se dejó de lado las medidas de presión para ser escuchados y se planteó el desarrollo de normas y criterios de manejo construidos participativamente, con base en la realidad local y con mejores posibilidades de aplicación. De esta forma, el Programa Lagarto evolucionó hacia un manejo bajo planes, en el que el sector indígena es el principal protagonista. Este modelo busca mejorar el manejo social para asegurar la sostenibilidad del aprovechamiento y promover la apropiación del recurso y del propio manejo, generando conciencia de responsabilidad sobre las acciones que se toman. Al mismo tiempo, se fortalece la participación de los indígenas en la cadena productiva, evitando las manipulaciones a las que estuvieron sometidos y obteniendo mejor información que permite ajustar el manejo bajo una visión de gestión adaptativa.

# Tools to promote caiman management plans with local communities of eastern Bolivia

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**ABSTRACT:** The yacare *(Caiman yacare)* skins have a great economic potential for the indigenous peoples of eastern Bolivia. The revenues from this resource should strengthen the communities and stimulate their responsibility on the wild caiman populations they harvest following management plans. In addition to the market for Class IV skins, there are options for handicrafts and other skin products. The communities with best access to buyers can also sell fresh or dry meat. The opportunity presents the challenge of reaching fair and long-term stable markets. There is enough knowledge and technical experience in the academy and the local people to reach a sustainable production of caiman in Bolivia. However, there is little experience in the communal management of this promising resource. In this document we share some experiences developed by indigenous peoples and tools applied to promote the active participation of hunters in the sustainable harvest and control of skins produced in their management areas.

**RESUMEN:** Los cueros de lagarto *(Caiman yacare)* tienen gran potencial economico para los pueblos indígenas del oriente de Bolivia. Los ingresos provenientes de este recurso deben fortalecer las comunidades y estimular la responsabilidad de producir su cosecha, aunque son extraídos de vida libre siguiendo un plan de manejo. Además de vender el cuero salado, existen mercados para artesanías fabricadas de cueros completos y pedazos no vendibles. Para las comunidades con acceso a los compradores, tienen potencial de vender la carne fresca y salada. El resultante reto de toda esta oportunidad es para lograr mercados justos y estables en el tiempo. Existe el conocimiento y experiencia técnica para lograr la producción sostenible de lagarto en las tierras bajas de Bolivia, en el mundo académico y en la sabiduría local. Lo que falta es mayor experiencia local en el manejo comunitario de este recurso promisorio. En este documento se busca compartir algunas experiencias adquiridas por los pueblos indígenas y algunas herramientas para promover la participación activa de los cazadores en la cosecha sostenible y el control de los cueros que salgan de sus áreas de producción.

# Spatial models of management: Strengths, threats, weaknesses and opportunities in the Spectacled Caiman Management Plans

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**ABSTRACT:** The Spectacled Caiman *(Caiman yacare)* National Program of Conservation and Sustainable Use, in operation in Bolivia since 1997, applied the assumptions of the Venezuelan model of biological sustainability for the use of this crocodilian species. Because problems arisen with the assignment of quotas to the properties that are annually registered in the Program, the participation of the hunters in the productive process, the distribution of benefits and other, from the year 2001 they have been a development of management plans in Beni and Santa Cruz. The spatial differences, the stakeholders, ownership of the land, organization, participation in the Program, history of use, and other outline problems and different opportunities, that are fundamental for the definition of the management system proposed and the design of management plans.

The experience demonstrates that all the proposed management models are viable, but their strengths, threats, weaknesses and opportunities shows that some previous conditions are key in the design of the management plans, and they demand follow-up systems to foster previously and to achieve the execution of a management plan.

# Equitable Sharing of Benefits in the Caiman Productive Network.

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**ABSTRACT:** The Equitable Sharing of Benefits (ESB), besides being a principle of bio-commerce, is one of the up-to-date postulates in the world of business with social responsibility. ESB is the sum of activities with the purpose of obtaining equitable benefits for all the actors involved in a productive chain as a whole; the distribution of benefits should be closely related to the effort and outcome of each actor. In Bolivia the productive chain of spectacled caiman *(Caiman yacare)* is being developed within the frame of the Program for Conservation and Sustainable Exploitation of Spectacled Caiman, implemented by the Bolivian State since 1997. The Program operates on the basis of harvesting wild animals observing an annual quota according to CITES recommendations. One of its goals is to offer economic opportunities to the actors of the productive chain, consisting of native communities, peasants, stock farmers, merchants and enterprises (tanneries). The weakest links in the chain are, beyond any doubt, the resources' providers, i.e. the numerous native and peasant communities who hunt caimans to generate income for their households since the beginning of the Program. Available records allow us to question the Program efficiency which, up to the date, has failed one of the main goals: to offer income opportunities to the resources' providers. In general producers, hunters and/or gatherers are exploited by merchants or enterprises who take for themselves a major share of the benefits. In general the producers (hunters) they have not improved their situation of poverty since the middlemen or companies are taken most of the benefits.

On the other hand, we can not ignore the entrepreneurial sector, who has open its own way by overcoming several difficulties and performing important financial efforts to carry on this business, and now claims for support from the State to obtain profit according to its effort. Other actors of the productive chain are the public institutions (national and local government) in charge of controlling and monitoring the Program implementation. These institutions represent the Bolivian State, owner of the natural resources, and do not profit from the ESB because they do not even have enough assets to accomplish their tasks, therefore it is unavoidable to look for their financial sustainability. Watching over the interest of all the actors involved, public and private, to attain the Program's sustainability, we propose the implementation of the following five tools to achieve the ESB in this productive chain, whose bigger threat is the inequity in the distribution of benefits:

a) To develop relations based upon confidence, dialogue and mutual benefit;
b) To improve commercial and legal capabilities of the resources' providers;
c) To establish fair and equitable commercial relations;
d) To obtain a wide cooperation between different actors to promote local development and to preserve biodiversity and traditional knowledge;
e) To generate a permanent exchange of transparent information.

**RESUMEN:** La Distribución Justa y Equitativa de Beneficios (DJEB), además de ser uno

de los principios del biocomercio, es uno de los postulados que está en boga en el mundo de los negocios con responsabilidad social. La DJEB es el conjunto de actividades que tiene la finalidad de lograr beneficios equitativos para los actores que intervienen en una cadena productiva, esta distribución deberá estar directamente relacionada con el esfuerzo y aporte de cada uno de los actores. En Bolivia la cadena productiva del lagarto *(Caiman yacare)* viene desarrollándose en el marco del Programa de Conservación y Aprovechamiento Sostenible del Lagarto (PCASL), implementado por el Estado desde el año 1997. Este Programa tiene como base la cosecha de animales silvestres a través de cuotas anuales de extracción de acuerdo con lo estipulado en CITES. Uno de sus objetivos es el de brindar oportunidades económicas a los actores de la cadena, que son comunidades indígenas, campesinas, ganaderos, intermediarios y empresas (curtiembres). El eslabón más débil de la cadena es, sin duda alguna, el de los proveedores del recurso, nos referimos a la gran cantidad de comunidades indígenas o campesinas que desde el inicio del Programa cazan lagartos para generar ingresos económicos para sus hogares. Antecedentes disponibles nos permiten cuestionar la eficiencia del Programa, en el que hasta ahora no se ha cumplido uno de los objetivos fundamentales, que es el de brindar oportunidades de ingresos económicos para los proveedores del recurso. En general los productores, cazadores y/o recolectores de la materia prima no han mejorado su situación de pobreza ya que los intermediarios o empresas se llevan la mayoría de los beneficios.

Por otro lado no se puede ignorar al sector empresarial, que se ha abierto camino en este rubro superando muchas dificultades y realizando grandes esfuerzos económicos para llevar adelante este negocio y reclama, de parte del Estado, el respaldo y apoyo que le permita obtener rentabilidad en función a su esfuerzo. Otro actor de la cadena productiva son las instituciones públicas (gobierno nacional y gobiernos departamentales) encargadas de controlar y supervisar el cumplimiento del Programa. Estas instituciones representan al Estado boliviano, propietario del recurso, y tampoco gozan de una DJEB, ya que ni siquiera cuentan con los medios económicos necesarios para desarrollar sus tareas, por lo que resulta indispensable buscar su sostenibilidad financiera. Velando por el interés de todos los actores, públicos y privados, en aras de la sostenibilidad del Programa, se plantea la alternativa de implementar las siguientes cinco herramientas que permitan una DJEB en esta cadena productiva, en la que la gran amenaza es la desigualdad en la distribución de beneficios:

a) desarrollar relaciones basadas en confianza, diálogo y mutuo beneficio;
b) mejorar las capacidades comerciales y legales de los proveedores del recurso;
c) establecer relaciones comerciales justas y equitativas;
d) obtener amplia cooperación entre los diferentes actores para promover desarrollo local y conservar la biodiversidad y conocimientos tradicionales; y
e) generar un intercambio constante de información transparente.

# PNBS strategy for a sustainable use of *Caiman yacare* in Bolivia

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**ABSTRACT:** Classified as Lower Risk category by the UICN, spectacled caiman (*Caiman yacare*) is currently commercially used in Bolivia. The value chain of this crocodilian involves principally indigenous communities, local communities and private companies. There are difficulties between these actors about the resource use and these difficulties threaten the sustainable use of the specie.

The Biotrade National Program (known in Spanish as Programa Nacional de Biocomercio Sostenible - PNBS) is a government of Bolivia initiative, implemented by 'Fundación Amigos de la Naturaleza' and supported by the governments of the Netherlands and the Swiss Confederation. The Program facilitates the trade of products and services of native biodiversity, produced with ecologic, social and economic sustainability criteria and thereby to generate income for the country.

Some of the products that the PNBS has selected to support are spectacled caiman leather and meat. The biotrade initiatives related with these products, were evaluated in order to meet the Biotrade Principles and Criteria. Combined with local workshops, these assessments allow identifying the needs of different actors and allow defining the PNBS's strategy for this value chain. This line of action identified the elaboration of management plans like a key activity. Because of, the PNBS has supported the elaboration of eleven management plans, which were designed in collaboration with the actors.

# Population Status of Spectacled Caiman *(Caiman yacare)* in areas under Management Plans for sustainable use of the specie in Beni and Santa Cruz, Bolivia

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**ABSTRACT:** Within the National Program for Sustainable Use of Spectacled Caiman in Bolivia, management plans are being developed on indigenous lands, protected areas, and municipalities for improving key aspects of the program; such plans are improving organization, establishing local controls, transparency of the reliable management and information about the specie. In this aspect, data were collected on the abundance and population structure of crocodiles in 419 sites in 10 Indigenous Lands (TCO), one protected area and one municipality. The data of abundance presents a major variation from places with 0 ind/km of shore (no animals) up to 1945.45 ind/km of shore (as in artificial pounds) on cattle ranches. The population structure also presents variations among areas, suggesting different degrees of pressure over the specie. Our data shows that on a national level, the population of the species is good enough to be subject of a sustainable management of the species as long as local controls are improved to stop the hunting of the animals that are too small or not during hunting season. However, the individual analysis per area shows that in some places the maximum amount of animals to be harvested could be some thousands of animals, while in other areas there are not enough potential to face the harvesting model in a sustainable way.

**RESUMEN:** En el marco del Programa Nacional para el Aprovechamiento Sostenible del Lagarto (PNASL) en Bolivia, se están desarrollando Planes de Manejo en tierras indígenas, áreas protegidas y municipios para mejorar aspectos clave del aprovechamiento como: fortalecimiento organizativo, establecimiento de controles locales, transparencia del manejo e información biológica confiable sobre la especie. En este último aspecto, se tomaron datos de abundancia y estructura poblacional del lagarto en 419 puntos de muestreo que corresponden a 10 Tierras Comunitarias de Origen (TCOs), un área protegida y un municipio. Los datos de abundancia presentan una gran variación, desde lugares que fueron registrados como 0 ind/km (sin animales) hasta 1945.45 ind/km de orilla en estanques artificiales en ranchos ganaderos. La estructura poblacional igualmente presentó grandes variaciones mostrándose estructuras por clases de tamaños en forma de escalera ascendente, escalera invertida y estructuras piramidales. Nuestros datos sugieren que a nivel nacional la especie se encuentra con un estado poblacional que permite llevar a cabo el aprovechamiento de manera sostenible, siempre y cuando se mejoren los controles locales para evitar la cacería de animales fuera de talla o de temporada. Sin embargo, el análisis individual por predios muestra que en algunos lugares los cupos de cosecha pueden representar varios miles de animales, mientras que en otros no se cuenta con el potencial necesario para que dicha actividad se lleve a cabo de forma sostenible bajo el actual modelo de manejo.

# Lessons learned from the Pilot Sustainable Management Plan for Spectacled Caiman *(Caiman yacare)* in the San Matias Protected Area

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**ABSTRACT:** The San Matías Protected Area is a conservation unit of national and global importance, with the objective to compatibilize Chiquitano Dry Forest and Pantanal ecosystem conservation with the sustainable development of the local people/communities. There are 26 communities living in the protected area, as well as 60 cattle ranches. The indigenous communities living in the protected area subscribed to the National Program for Sustainable Use of Spectacled Caiman in 2003, being represented by the Provicial Chiquitano Organization (*Central Indígena Reivindicativa de la Provincia Angel Sandoval* CIRPAS) and the National Ayoreo Organization (*Central Ayorea Nativa del Oriente Boliviano* CANOB). In the San Matías Protected Area, on request of the communities interested in using this resource and due the lack of a specific management plan, during fiscal year 2005, a pilot sustainable management plan for caiman was implemented in order to use this species legally within the framework of the General Regulations for Protected Areas and the National Caiman Regulations. As a result of this pilot experience, the following lessons can be drawn: local stakeholders as direct beneficiaries learned about the functioning of the National Program of *Caiman yacare*. Moreover, this enterprise of use within a protected area contributed through good caiman management, and avoiding conflicts and divisions within their traditional organizational structures, to broadening the positive effect of sustainable use and - on the other hand – to generating additional income for their families.

# Social Participation in the TIPNIS Caiman Management Program

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**ABSTRACT:** The Isiboro Sécure National Park (1965) was recognized as the Indigenous Territory of the Mojeño, Yuracaré and Chiman in 1990. Since 2004, the TIPNIS has a General Management Plan that includes instructions, guidelines, and policies for managing the Protected Area and Indigenous Territory. One of the strategic objectives is to promote increased social and economic development of local communities, based on the sustainable management of natural resources. Within this framework, social and organizational processes are being carried out since 2003, around the protection and management of spectacled caimans (*Caiman yacare*). These processes have in the design of a Caiman Management Plan, focused on developing short, medium and long-term community-based management and conservation and local capacity building strategies to avoid illegal use of this specie. Since 2005, TIPNIS is part of the National Sustainable Management Program for Spectacled Caiman. Commercial harvesting of *C. yacare* carried out on three separate occasions, has already produced economic benefits for the families of 30 indigenous communities. Species conservation is supported by participatory protection schemes, community organizational strengthening for caiman management, and biological and social monitoring of the specie.

**RESUMEN:** El Parque Nacional Isiboro Sécure (1965) fue reconocido como Territorio Indígena de los pueblos mojeño, yuracaré y tsimane en 1990. Desde el 2004 cuenta con un Plan General de Manejo que brinda directrices, lineamientos y políticas para la gestión integral del AP/TCO donde uno de los objetivos estratégicos apunta a que las comunidades logren un mayor desarrollo socioeconómico en base al manejo sostenible de los recursos naturales. En este marco, desde el 2003 se han desarrollado procesos sociales y organizativos en torno a la protección y aprovechamiento del lagarto *(Caiman yacare)*, culminando en el Plan de Manejo del Lagarto como instrumento que enfatiza el desarrollo de estrategias comunales de aprovechamiento y conservación y la generación de capacidades locales a corto, mediano y largo plazo y así evitar el aprovechamiento ilegal del lagarto. Desde el 2005 el TIPNIS participa en el Programa Nacional de Aprovechamiento Sostenible del lagarto, con 3 cosechas realizadas que han generado beneficios económicos para familias de 30 comunidades indígenas y apuntalado la conservación de la especie mediante sistemas participativos de protección, el fortalecimiento de la organización intercomunal para el manejo de lagarto y el monitoreo biológico y social de la especie.

## INTRODUCTION

One of the characteristics of Bolivia's Protected Areas is the resident populations (it is currently estimated that some 200,000 people live and develop economic activities in the 22 National Protected Areas). A large percentage of these are indigenous and/or native peasant populations, with ancestral territorial rights and traditional knowledge of land and natural resource management. Additionally, the protected areas coincide with or are adjacent

to 14 indigenous territories. As a result, two of the protected areas have also been formally recognized as indigenous territories.

Considering this reality, and within the framework of its authority, the Strategic Management Agenda (SERNAP, 2005) states that *"… Based on this new general political and regulatory framework, protected areas are to be conceived and managed as spaces 'with people'. This means recognizing not only the explicit right of their inhabitants to live in the areas, to own the lands, and to sustainably use the renewable natural resources …"*. The Agenda also establishes "sustainable economic and social development" as a strategic objective. According to this objective, managing biodiversity resources will not only generate income, employment and socioeconomic benefits for the local populations, but will also support biodiversity conservation in the PA's, and improve their acceptance and recognition.

More recently, and in a new political and social context, SERNAP has adopted a new management model to introduce the concept of **"Protected Areas of the people"**, which highlights the social and economic function of protected areas, focusing on increased ownership by resident populations, and proposes to discuss new roles and responsibilities for joint management of the country's protected areas and their service areas.

## SOCIAL PARTICIPATION IN THE TIPNIS CAIMAN MANAGEMENT PROGRAM

The Isiboro Sécure National Park and Indigenous Territory is located in the central part of Bolivia (north of Cochabamba and south of Beni), in a transition zone between the Andes and the Amazon. The park stretches over hills, mountains and plains, with altitudes ranging from 3,000 to 180 meters above sea level. The TIPNIS has more than 170 lakes, the majority of which were created as a result of meanders. These bodies of water are particularly rich in hydrobiological resources, including the spectacled caiman. In normal years, 70% of the alluvial plains (representing 40% of the total surface area of the park) is under water for 3 to 5 months.




The Isiboro Sécure National Park was created in 1965, when there were no institutions or regulations dealing with protected area management. In 1990, the Park is formally recognized as an Indigenous Territory, home to the Mojeño, Yuracaré and Chiman tribes, and becomes the Isiboro Sécure National Park and Indigenous Territory (TIPNIS). The TIPNIS is managed under a co-administration agreement signed by SERNAP and the indigenous organization (TIPNIS Sub-Central).

Historically, the TIPNIS has undergone extensive plundering of its natural resources. There is evidence of illegal commerce of spectacled caiman and black caiman (*Melanosuchus niger*) hides between 1960 and 1985, with dramatic consequences for the populations of both species, particularly the black caiman, which was in danger of becoming extinct. In the 90's, as a result of the enactment of the Decree of ecological pause or indefinite Prohibition (1990), the spectacled caiman population started to recover. However, in 2001-2003, poachers have started to hunt the spectacled caiman in the TIPNIS, where the practice is illegal according to the National Sustainable Management Program for Spectacled Caiman. Hunting is legal, within the framework of CITES, in cattle ranches, and indigenous and peasant communities in Beni and Santa Cruz. Unfortunately, authorities lack the capacity to enforce regulations prohibiting hunting in the TIPNIS.

**a) The Regulatory Framework**

Although Bolivian legislation provides for the sustainable use of renewable natural resources by local populations within the country's protected areas and indigenous territories, in the case of the TIPNIS, the path to obtaining authorization to do so was a long one. Efforts were undertaken to raise the express prohibition to harvest spectacled caimans in the TIPNIS, and, in 2005, the Ministerial Resolution approving the Caiman Management Program in the Isiboro Sécure Protected Area and Indigenous Territory was issued. A second Ministerial Resolution approved the Addendum to the Plan, in 2007, extending the scope of application to the other management areas.

Management of the spectacled caiman in the TIPNIS is protected by law and feeds back into the law, particularly with respect to the mechanisms that ensure that harvesting is not excessive (preventive principle) and that its economic benefits reach all the families of the indigenous communities involved in management activities.

Another regulatory instrument is the General TIPNIS Management Plan. It is precisely during the initial stages of the participatory development of this plan (during the sub-regional workshops), that the spectacled caiman was identified as a natural resource having a potential for sustainable use. It was decided to develop a spectacled caiman management plan, and thus both planning processes ran parallel to each other. For this reason, the strategic objective of the General TIPNIS Management Plan "increased socioeconomic development, based on the sustainable management of natural resources in the communities of the protected area, the indigenous territory and its surroundings" supports economic initiatives, such as spectacled caiman harvesting, and other productive activities carried out by the indigenous communities.

**b) The TIPNIS Caiman Management Program**

Developing the TIPNIS Caiman Management Program was a long (2002-2005), complex process. The indigenous communities of the lower areas of the TIPNIS participated extensively in the process. In addition to local knowledge, the planning process was based on aquatic habitat suitability models, interviews with hunters, and preliminary night counts, showing that this area of the TIPNIS contained a large population of spectacled caimans.

The TIPNIS Caiman Management Program represents a new resource management model in Bolivia. In addition to biological monitoring of the state of the spectacled caiman

population, the Plan incorporates social participation elements through the strengthening of indigenous organizations; monitoring and evaluation of the processes comprising the entire spectacled caiman production chain; the division into zones, taking into account the general division of the protected area into zones but creating new ones (three management zones and 2 protection zones are considered); and particularly when it stipulates that annual caiman harvesting quotas should be based on criteria such as the relative abundance of spectacled caimans, economic viability studies, and assessment of the social benefits to be obtained.

Spectacled caiman management in the TIPNIS is generally in line with the natural resource conservation approach integrating two interrelated dimensions: resource protection and resource sustainable management. One of the characteristics of the integral management of the spectacled caiman is its adaptability. The management plan can be adapted to new scenarios or as a result of progress made, as well as to changes in the environmental and/or social conditions. A second characteristic is the diversification of spectacled caiman management strategies.

**c) Implementing the TIPNIS Caiman Management Program**

Notwithstanding the fact that the TIPNIS Caiman Management Program, approved by a ministerial resolution, provides a legal framework for the Indigenous Territory, one of the privileged areas of social monitoring has been the development of internal regulations. The process focused on encouraging local participation in activities that, while not unknown to the protected area's resident populations, are organized differently in terms of the actual work performed (e.g. group hunting using appropriate techniques). Other areas of focus included marketing (public auctions), distribution of benefits to all inhabitants without exceptions, and a more participatory resource protection system.

An important element when implementing the Spectacled Caiman Management Plan, was the consolidation of an institutional structure comprising the Intercommunal Spectacled Caiman Management and Marketing Committee, representatives of traditional authorities at community level (*corregidores*) and supracommunal authorities (*Subcentral del TIPNIS*). The institutional structure also recognized both national level (DGB and SERNAP) and regional level (*Prefectura del Beni*) public entities. The institutional strengthening of local organizations and the Intercommunal Committee necessitated training in organizational, technical, economic and administrative issues to enable the organizations to control the different stages of the spectacled caiman production chain, and the resource protection system.

**d) Main Results and Outlook**

One of the main results has been implementing a transparent system for marketing spectacled caiman hides through public auctions. In 2005 and 2006 harvesting took place in the Ichoa river catchment basin area only (10 communities) but in 2007, the three TIPNIS zones (30 communities) were involved. The following table provides a summary:

| | 2005 | 2006 | 2007 |
|---|---|---|---|
| Number of hides sold | 535 | 591 | 1,368 |
| **Income from sales (US$)** | **13,652** | **12,128** | **36,346** |
| **Local currency equivalent (Bs)** | **109,770** | **97,000** | **278,773** |
| Distributed to communities (Bs) | 55,500 | 50,000 | 160,973 |
| Reserve for following year's operations (Bs) | 29,000 | 20,000 | 80,000 |
| Expenses (hunters and others) (Bs) | 25,770 | 27,000 | 37,800 |

The funds distributed directly to the communities (approximately US$ 800 per year) have been used to finance a series of local initiatives, such as, purchasing cattle for communal cattle raising, setting up local grocery stores, and others. The idea is to improve social interconnexion based con common projects.

Taking into account global figures for the three years, we are talking about a total injection of funds of more than US$ 60,000 (approximately US$ 1,000 per family) into an impoverished indigenous economy where the estimated annual per capita income is US$ 300. Still, it should be noted that spectacled caiman management is considered a complementary economic activity in the TIPNIS, and the income generated is in addition to the population's traditional income.

There are other aspects to be considered when reviewing the results of spectacled caiman management implementation. Apart from the economic results, spectacled caiman management facilitates local and regional capacity building and strengthening around a given natural resource. At the local level, under a participatory management system, with an integral approach to conservation (oriented toward protection and controlling illegal hunting, which seems to have succeeded as the number of hides seized has gradually declined in recent years), pointing to the biological sustainability of a natural resource the management of which involves individual sacrifice. At the Indigenous Territory level, spectacled caimans (and wood) have been identified as one of the strategic resources whose sustainable use should guide the integral management of the TIPNIS for the benefit of the indigenous communities, and the conservation of the protected area.

### e) Lessons Learned

There is evidence of support and ownership by the communities involved. In fact, in 2006, the Intercommunal Committee was able to organize hunting and marketing activities with practically no external assistance. In order to consolidate these developments, there is a need to promote increased training and technical advisory services at community and Intercommunal Committee level. At the regional TIPNIS level, efforts should focus on developing knowledge and tools for appropriate decision-making (regulations, monitoring data, etc.), and at the community level, on building technical and integral resource management capacities (self-monitoring, production, marketing).

As with any process, there are also weaknesses. These weaknesses can entail risks, particularly when there are signs of possible conflicts between the communities due to the way in which benefits are distributed among the communities (in equal shares), which is unfavorable to the more populated communities. Also, decisions as to the destination and use of funds could create problems within the communities.

## CONCLUSIONS

Some general learning can be obtained from this experience, including:

- The processes involved are long-term processes, where social monitoring is critical and involves technical (productive and administrative) training and advice on social and organizational matters.
- In terms of sustainability, social and economic developments are encouraging. The challenge is the environmental issue. The biological monitoring to be conducted at the time of the fifth harvest (2009) will show the actual impact of the activity on the resource, and its biological sustainability. So far, monitoring data show a positive trend.
- The experience has had a positive impact on protected area management, as shown by the results of the implementation of the "social perception monitoring" tool. The social perception among the TIPNIS resident populations, according to interviews conducted with different age groups, is that natural resource management is important both for the population involved –because of the economic benefits- and the protected area itself, in view of the participatory protection and conservation of the resources.
- In the socio-political sphere, successful natural resource management experiences support the strengthening of social organizations from the bottom up, which in turn stimulates the emergence of new leaderships in communal, supracommunal and regional organizations.
- The TIPNIS pilot project can serve as a model for other protected areas or indigenous territories.

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# Spectacled caiman *(Caiman yacare)* hunting in the low basin of the river Ichoa in the Indigenous Territory and National Park Isiboro Secure (TIPNIS), Beni-Bolivia.

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**ABSTRACT:** The hunting of spectacled caiman in the Amazon region of Bolivia has been registered since the beginning of the XX century, with particular peaks in the 60's. Less or nothing is known in Bolivia about the hunting of caimans in the wild. In the low basin of the river Ichoa places of hunting (tectonic lagoons fluvial and meandered, streams, rivers and "yomomos"), sex, length (ventral and total), length of the head, distance between the eyes, distance between the nose and the eye, weight, hunting and skinning effort were registered during the hunting of 191 spectacled caiman. 56% of the aquatic places visited were tectonic fluvial lagoons where 80% of the total animals were taken out, 33% were streams where they got 14% of the total *C. yacare* and 11% were yomomales where they got 6% of the animals. 100% of the hunted animals were males. An average of the measures of the head, ventral and total of *C. yacare* were of 29 (± 3.2), 113 (±7,3) y 220 (±16,9) centimetres respectively. The distance between eyes was 7, 6(±0,7) centimetres and between nose- eye was 20 (±2,1) centimetres. Average weight was 48 (±8.9) Kg. Hunting effort was of 9 (± 2.9) hours/2 men and the skinning time was of 34 (±12.6) minutes for 4 (±1.4) men.

Key words: Hunting, *Caiman yacare*, TIPNIS, Bolivia, "yomomos"
Yomomo: is a marsh like type of aquatic habitat in the amazon region.

# Hunting Evaluation of Spectacled caiman *(Caiman yacare)* in San Matías, Santa Cruz – Bolivia

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**ABSTRACT:** in order to evaluate the yield of the effort that the *Caiman yacare* hunt gives, in 2005 one carries out the pursuit to 4 hunters groups and 32 events carried out in 210.83 hours, in a locality called San Matías, being observed that the yield gives spectacled caiman hunted hourly was 1.27 individuals of a total 737 individuals, which 66.45% were bigger at 1.80m., 6.74% gave measure but it was not employee to present mishaps in its flanks, 13.7% was among 1,70 1,79 (no allowed by the regulation), 4.53% was not recovered of the water after being shooted, and 8.55% it was hunted to be sold in Brazil' s hatcheries. These results indicate us that exists a 33.42% surplus hunt on the total quotas granted by the government, if we added to this result the existence an internal smuggling leathers harvested outside of season, it is possible that the harvest in some areas wouldn't be sustainable, but with trainings and a rigorous control we could improve this situation.

## INTRODUCTION

Latin America has the highest wealth of crocodilians species that can be in any comparable area in the world; twelve taxa (including subspecies), are distributed from Mexico to the Argentina. The vast area gives wetlands and immense riverside systems this portion of the continent provides an extensive habitat for caiman and crocodiles, reason for which the number of these animals in the region is probably the highest gives the world, although at the moment the real figures continue being ignored (Messel et al. 1995). This situation makes that the crocodilians represents a resource of considerable ecological valor and with a great economic potential (Pacheco 1996).

The Latin America region has supported the biggest crocodilians exploitation in the world. Historically, the over-exploitation for the international trade of skins has caused a serious decline and local extinctions of some species; even though ironically still in the ´90 decade the region provided more than half of the skins crocodilians in the world trade (Messel et al. 1995).

In Bolivia the development of a pilot program for sustainable use of the *Caiman yacare* began in 1995 with the project "A Programme for the Sustainable Utilization and Management of Cayman in Bolivia" (King 1995), which gave cause so that in 1997 the Regulation was promulgated for the Conservation and Use of yacare *(C. yacare)* for Santa Cruz and Beni Departments (D.S. 24774, July 31 of 1997). At the same time, the DNCB (current General Direction of Biodiversity and Protected Areas–DGBAP), elaborated the National Program for Conservation and Sustainable Use of Caimans (PNASL), in which provides the evaluation and the populations monitoring for caiman and other crocodilians species (Llobet and Aparicio 1999).

Starting from that moment, due to the lack consent among the different actors, they were raised a series inconvenient that impeded the good development of the program up to 1999, year in the fact that a new legal mark was granted for the management wildlife, allowing the rising of the prohibition for susceptible species of sustainable use (D.S. 25458). The same year an Interim Regulation was approved with exception character that allows the use of yacare during 1999 and the crop and storing of 36500 skins (D.S. 25555, Ministerial Resolution No 307/99, Ministerial Resolution No 330/99). During the year 2000, the DGB with support of CESO/SACO (Canadian Executive Service Organization) began a echo-regionalization process of the Program through the use geographical information systems (GIS), like a proposal to be adjusted in function of new information gathered in the field, at the same time that they were carried out new monitoring in Beni, Santa Cruz and Pando Departments (MDSP 2002, you PEEP 2001). With the purpose of evaluating the development of the process and the operation of the yacare program, the year 2002 it was carried out the Evaluation of the Sustainable National Program of Caiman *(Caiman yacare)* (Llobet 2002).

The Santa Cruz department started the use of yacare from the year 2000 with a shared quota among the municipalities of Guarayos and San Matías. When having the town San Matías a high potential of caimans (MHNNKM, 2005), exists the risk that during the harvest some animals could be discarded (the small individuals or those that don't present an appropriate physical constitution) increasing the number of hunted caimans to reach the quota.

The present work seeks to quantify the hunt effort of the San Matías residents and to verify the harvest of the species, determining the real amount of hunted caimans, constituting this way in a tool that provides data to be used for the success of the yacare program.

**Study Area**

The study area corresponds to the San Matías municipality, county Ángel Sandoval located in the east end of Santa Cruz Department (figure 1). It presents two main physiographic units: 1) the Brazilian or Chiquitano Precambrian Shield, with slightly wavy, very eroded landscapes or cutleries give silts, and Pantanal that constitutes the floodplains of the Paraguay river. The floors are sub-hydrical type and have fine texture, result of the recent fluvial deposition silts in a surface area with scarce or null pending (Prevails et al. 2000, Rivero 2003).

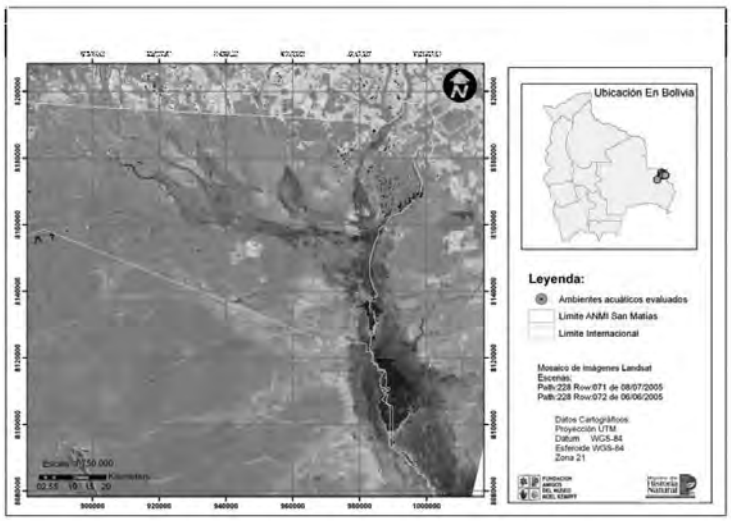



**Figure 1.** Study Area and sampling points inside and outside the ANMI San Matías, county Ángel Sandoval.

## METHODS

The field work had duration of 40 days distributed in the months of August, September and October. The habitat where the evaluation of the caiman hunt was carried out was: 9 artificial ponds, 1 river and 6 different areas within the swamp “Curichi Grande” in the Bolivian Pantanal (figure 1). The hunters were accompanied by direct way in the river Bella Vista and the Curichi Grande, and indirectly (I waited on the shore) in the ponds, because of these water habitat don't present perimeters bigger than 300 m.; when concluding the event I consulted to the hunters about the caimans shooted but not recovered.

### Hunt Registry:

For each hunt event they registered the following variables:

- **beginning Hour (HI):** registered starting from the moment of beginning of the hunt.
- **completion Hour (HF):** registered in the moment of finish hunt.
- **Hunting time (T):** it was calculated starting from the equation T=HF-HI (final Hour–Hour gives beginning).

For the classification of water habitat the classification that was used proposed by the MHNNKM (2004), described hereinafter:

- **Tectonic lagoons:** they are water bodies generally isolated from the rivers, they have not fluvial origin, and we consider them when they have a superior perimeter to 1 km.
- **Fluvial lagoons:** they have fluvial origin, connected temporarily with the river, many are abandoned meanders
- **Lagunetas:** lagoons minor to 1 km of perimeter.
- **Artificial ponds:** artificial ponds, in the border roads or for the livestock.
- **Rívers:** water courses that are continuous in the images Landsat (30 m of pixel) although variable wide that should be verified in the field. They are considered white waters (rivers those that are born above the 500 m in the Andes and clear waters from the basin of the Iténez).
- **Streams:** water courses that are not continuous in the Landsat images, and they are born in the plains (the ‘streams Andean ' is considered gulches, and they would not be excellent for the caimans).
- **Swamp lagoons (Curichi):** they are areas annually permanent or lingering flood, they include “yomomo” areas (floating vegetation).

To obtain information on the logistics employed in the hunt and characteristics of the trade, I carried out informal interviews directed to different actors (hunters, transport, middlemen, etc.).

The obtained data was organized in field schedules designed by the MHNNKM (2004) for such an effect, where they were considered some specific data like: habitat’s names, beginning hour and completion hunt; number of hunters that intervened and type of used weapon.

**Measures and sex determination:**

The taken measures to the hunted individuals were: total length (LT), measured ventrally tip of the mouth until the end of the tail; head-body longitude (BL), from the tip of the mouth until the later end of the cloacae opening; longitude head (LCab), from the tip of the mouth until the trailing edge of the cranial chart; and weight (P) (Llobet 2002).

The sex determination was carried out for direct observation of the penis or clitoris. In the individual class II had to bend to the animal lightly and to exercise a lateral pressure in the seer region, for the profitable adults it was necessary the introduction of the little finger inside the cloacae (Llobet 2002).

**Results Analysis:**

The results analysis began with a normality test for the data (Shapiro–Wilk´s), and when not following a normal distribution, statistical not parametric was used to analyze the information (statistical Statsoft programs - Statistica 6.0). A test Kruskal Wallis was applied to compare the corporal longitudes so much among the different water habitats, as effort/hour/man (h/men) and yields for individuals and vests (Ind/h/men = total gives individual hunted hourly and Flank/h/men = total gives utilized vests hourly) for each water habitat type lastly, was carried out an analysis correspondence among the sizes vests and the different valued hábitats (Ji–square).

They were also carried out correlations and lineal regressions (Spearman) among the hunt effort in hours/man and the different yields.

## RESULTS AND DISCUSSION

The caiman hunt in Ángel Sandoval County (San Matías) is given in three types of properties:

a) In private properties where the proprietor is the same one and give the bindings is made by the services a contractor that takes the responsibility to mobilize the hunters for the share extraction assigned by the Government. The contract is only by word of mouth, and in him the proprietor fixes the price for salted leather. The election of the sizes depends on the contract that the proprietor has with the tannery, always inside the normative one effective: vests give 110 cm, 115 cm and 125 cm (R.M. 147/02). The case is also given in the one that the contractor becomes buyer of the leathers or intervenes in the intermediation of the proprietor and the tannery.
b) In the communities the use is carried out by the leaders, which take charge give to hire the hunters with experience in San Matías town and give to sell the leathers with bindings to the tanneries. They also exist community that carry out this task in a secret way and they sell the leathers to the middlemen without binding.
c) In the ANMI San Matías (where the TCO is CIRPAS and private properties), the use was carried out legal way and only give experimental form the year 2005 (in previous years it was illegal hunted form on the part of the leadership and strange hunters without the knowledge forest keeper). During this year the hunt was directed by the president the TCO through the recruiting external hunters to the communities. These hunters were controlled by the forest keeper, responsible besides placing the bindings to the extracted leathers.

**Ecological description of the hunt places evaluated:**

11 environments water were analyzed that correspond to 9 ponds, 1 river, and 1 Curichi, which was evaluated in 6 different points (Chart 1).

The ponds are artificial water environments with a maximum perimeter of 0.34 km and a minimum of 0.16 km, waters slightly sour greenish color (pH 6) and not very oozy sandy substrate whose depth varies from 1 m to 1.5 m. These water environments don't dry off in low water time due to the presence underground pipes that extract water from the first phreatic table.

The valued River during the hunt presented waters clear brown color and pH 6, with oozy sandy substrate. The onsite registered maximum depth gives the hunt was 1.5 m. During the time of low waters this river it doesn't present current giving place to the formation of isolated puddles where the caimans concentrate. The perimeter gives hunt was of 4 km bank.

The Big Curichi, border division between Bolivia and Brazil, was evaluated in the high basin, being presented two areas gives hunt: the “Capon del Tigre”, with marshy substrate with enough deposition of organic silts, it presents a depth of 30 centimeters for his navigability in the hunt moment and very hot waters 35°C; and the “Capon Garrapatilla”, with crystals waters up to 2 meters depth and loamy substrate - sandy not very stony. The surface hunt area was 6 Km bank for the “Capon Garrapatilla” and give 2 km bank for the “Capon del Tigre”.

Chart 1. Town, environment's name, geographical location (UTM) and environments water types evaluated during the hunt of caimans *Caiman yacare* in San Matías.

| Town | Environment's Name | Environment water Type | Dates | X | Y |
|---|---|---|---|---|---|
| Porvenir (Calvario) | Isla verde | Stagnate | 27 08 2005 | 302578 | 8164442 |
| Porvenir (Calvario) | S/N (Calvario) | Stagnate | 28 08 2005 | 303086 | 8167343 |
| Porvenir | Pirañas | Stagnate | 29 08 2005 | 322403 | 8157338 |
| San Luis | El Salero(Ponds 1) | Stagnate | 30 08 2005 | 315293 | 8138166 |
| San Luis | Rodeo (Ponds 1) | Stagnate | 31 08 2005 | 306939 | 8140923 |
| San Luis | Rodeo (Ponds 2) | Stagnate | 01 09 2005 | 306651 | 8141206 |
| San Luis | La Pampa | Stagnate | 02 09 2005 | 317581 | 8143248 |
| San Luis | Garcero | Stagnate | 01 09 2005 | 310566 | 8140740 |
| San Luis | Labrador | Stagnate | 03 09 2005 | 317442 | 8142044 |
| AMNI San Matías | Good View | River | 08 09 2005 | 267461 | 8097238 |
| Cambará | Capon del Tigre | Curichi | 17 10 2005 | 339325 | 8143881 |
| Cambará | Capon del Tigre | Curichi | 17 10 2005 | 339325 | 8143881 |
| Curichi Grande | Capon Garrapatilla | Curichi | 18 10 2005 | 343284 | 8142663 |
| Curichi Grande | Capon Garrapatilla | Curichi | 18 10 2005 | 343284 | 8142663 |
| Curichi Grande | Capon Garrapatilla | Curichi | 19 10 2005 | 341335 | 8143595 |
| Curichi Grande | Capon Garrapatilla | Curichi | 19 10 2005 | 341335 | 8143595 |
| Curichi Grande | Capon Garrapatilla | Curichi | 20 10 2005 | 341335 | 8143595 |
| Curichi Grande | Capon Garrapatilla | Curichi | 20 10 2005 | 341335 | 8143595 |
| Curichi Grande | Capon Garrapatilla | Curichi | 21 10 2005 | 341335 | 8143595 |
| Curichi Grande | Capon Garrapatilla | Curichi | 21 10 2005 | 341335 | 8143595 |
| Curichi Grande | Capon Garrapatilla | Curichi | 22 10 2005 | 342479 | 8143188 |
| Curichi Grande | Capon Garrapatilla | Curichi | 22 10 2005 | 342479 | 8143188 |
| Curichi Grande | Capon Garrapatilla | Curichi | 22 10 2005 | 342479 | 8143188 |
| Curichi Grande | Capon Garrapatilla | Curichi | 23 10 2005 | 341514 | 8142730 |
| Curichi Grande | Capon Garrapatilla | Curichi | 23 10 2005 | 341514 | 8142730 |
| Curichi Grande | Capon Garrapatilla | Curichi | 23 10 2005 | 341514 | 8142730 |
| Curichi Grande | Capon Garrapatilla | Curichi | 24 10 2005 | 340968 | 8144791 |
| Curichi Grande | Capon Garrapatilla | Curichi | 24 10 2005 | 340968 | 8144791 |
| Curichi Grande | Capon Garrapatilla | Curichi | 24 10 2005 | 340968 | 8144791 |
| Curichi Grande | Capon Garrapatilla | Curichi | 25 10 2005 | 340968 | 8144791 |
| Curichi Grande | Capon Garrapatilla | Curichi | 25 10 2005 | 340968 | 8144791 |
| Curichi Grande | Capon Garrapatilla | Curichi | 25 10 2005 | 340968 | 8144791 |

**Hunt Aspects:**

**Individual hunted number**

During the 32 hunt events evaluated, they were hunted a total of 737 *Caiman yacare* individuals, 514 leathers took advantage like court type vest, 102 didn't give the allowed measure according to regulation, 55 individuals didn't serve to present wounded in the flanks and to have the damaged leather, and 35 caimans were shot but they could not be rescued of the water, for what you/they were not considered in the total hunted individual, but yes in the percentages use. Finally an illegal category use was presented for Bolivia ("rabudinhos"), conformed by individuals among the class II and III, giving a total of 66 hunted individuals (figure 2).

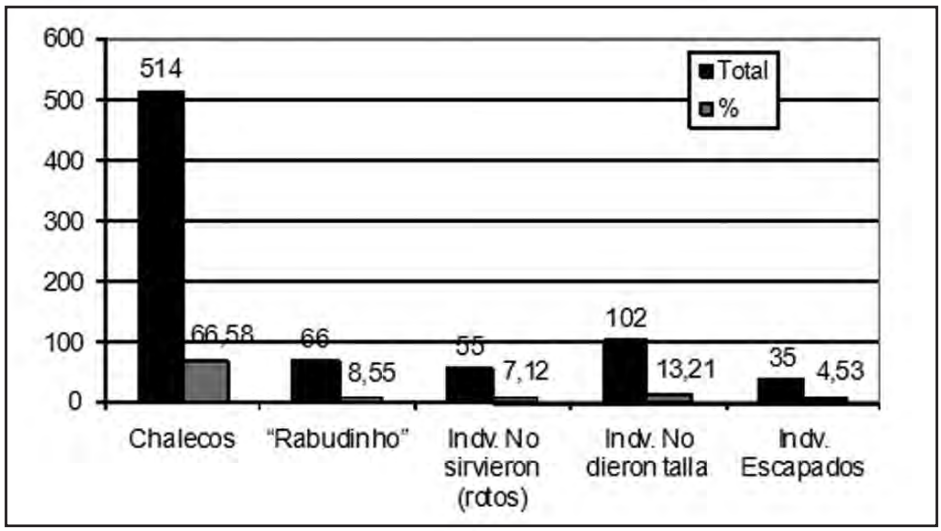



**Figure 2.** Graphical representation of the total hunted individual (for: flanks, rabudinhos, broken or damaged, that didn't give size and escaped), in the three types environment with use, indicating the percentages of each one of these.

Of the total percentage individual that were hunted, 66.58% took advantage as leather type flank, 8.55% was "rabudinhos" and 24.86% didn't arrive to be taken advantage of in its entirety (to some they were extracted the leather of the line, other they were simply discarded) (it figures 3). If we added the percentages that are not taken advantage of for the National Sustainable Program Use of the Caiman (PNASL) we have that the allowed share for the hunt in the area rises in 33.41%.

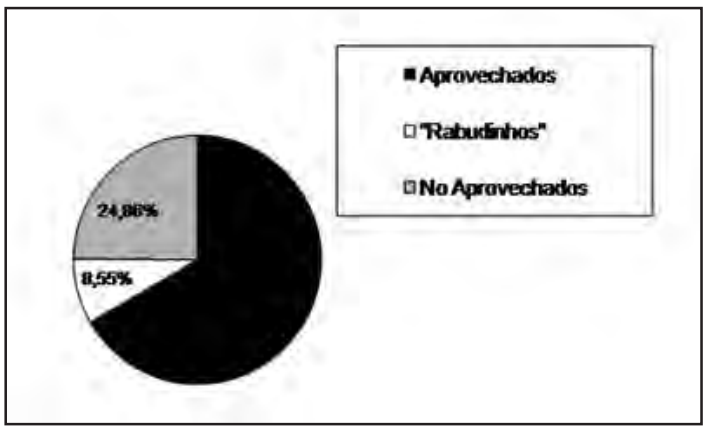



**Figure 3.** Percentages give individual taken advantage of, not utilized and "rabudinhos."

**Use for sizes classes**

Of the 737 harvested individuals, 605 belonged to the class IV forming 81.09%; 105 caimans were of class III corresponding to 14.25%; and 27 individuals give the class II, reaching 3.66% (Chart 2).

**Chart 2.** Absolute and relative abundance gives the utilized individuals according to classes.

| Classes | Nº Individuals | % |
|---|---|---|
| Class II | 27 | 3.66 |
| Class III | 105 | 14.25 |
| Class IV | 605 | 81.09 |
| **Total** | **737** | **100** |

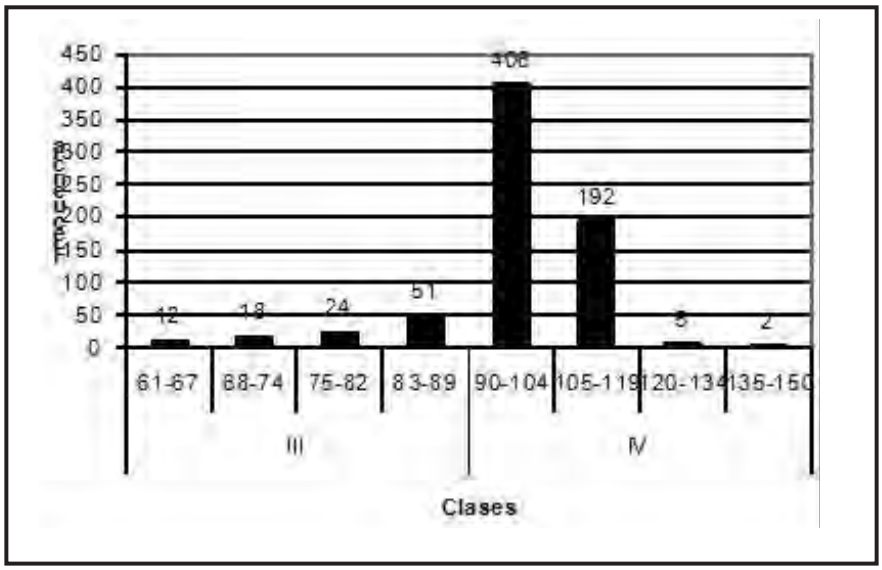



**Figure 4.** Frequencies Distribution of the Corporal Longitude (cm.) of the individuals belonging to the Classes III and IV.

The percentage of hunted caimans belonging to the class III are affected by the caimans discarded by the class IV in 48.57% (51 ind.) this percentage is inside the category 83-89 cm. BL. 51.43% (54 ind.) remaining, understood among 61-82 cm BL are taken advantage as “rabudinhos”, jointly with the entirety those belonging to the class II. (figure 4)

Of the total hunted caimans (without considering those escaped) 95.9% was male and 4% was female. 8.96% of the hunt were “rabudinhos.”

The percentages give use for sex show us that of 30 hunted females, 76.66% was hunted for “rabudinho”, and only a 6.08% of the males total (figure 5).

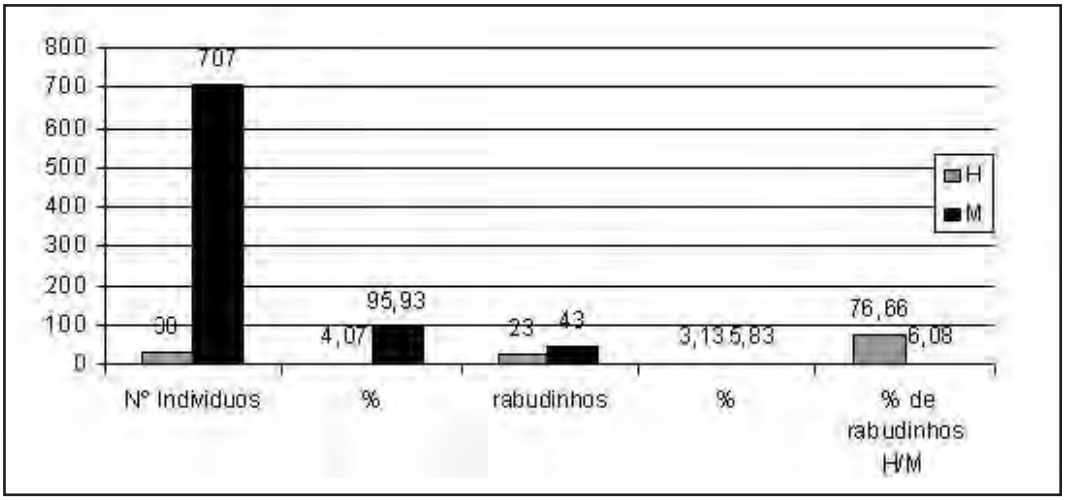



**Figure 5.** Absolute and relative caimans abundance according to the sex.

The discards of caimans hunted for “rabudinhos”, is subject at grade ossification level that these present in the ventral boards and number besides the damages that these can present in the leather.

**Effort and Product hunt for type environment water**

For the caiman hunt in San Matías participate groups of two or five people (conformed by one or two triggers (‘proeros' is the person that takes charge to hire the pilot, function is to get places with good potential caiman for the hunt and during this to light, to shoot, to retire, to finish off dislocate de neck and to carry up to the canoe the depressed caimans), and one or two pilots (´singa ' or oar, function is to drive the canoe in the hunt moment, as well as to count the dead caimans on the canoe to determine the capacity of this), besides a cook in case they are 5 people) these data were obtained of four hunt groups. The groups are directed by a contractor that takes charge of food, service and hardwares hunt for the hunters, enable that it is discounted later on of the leathers obtained by these. It is also inside their responsibility the transport (going and turn) of the hunters and the canoe to the places where will be carried out the hunt.

In the ponds (private properties), with a total 9 hunt events, were an average of 6.51 hours for event, with an effort average of 1.63 h/men. The product for this environment water was of 27.14 Ind/h/men and 18.38 Flank/h/men (Chart 3a).

The event that bigger time lasted was in the Saltcellar, with a total of 10,75 hours and an effort of 2.69 h/men; while the smaller duration was registered in the Rodeo (stagnates 2), with 4,50 hours and an effort of 1.13 h/men. As for the prodruct Ind/h/men, the biggest detected valor corresponds to Isla Verde, with 36.71 Ind/h/men; however a great difference exists with the prodructs for flanks obtained in this water environment (17.65 flanks/h/men) this was due to that the hunt in this place (it was the only place) took place of stealthy way causing that 34.5% of the total hunt valued (737 Ind.) break down before being hides (“they didn't serve”) and 28.5% of this same one total doesn't recover from the water (“escaped”).

**Chart 3a.** Types environment water (Stagnate), total hunt time, Product hours/men and product flank for hunt event of *C. yacare* in San Matías.

| **Name of water environment** | **Total hunt time** | **Eff. h/men** | **Efficiency Ind/h/men** | **Efficiency Flank/h/men** |
|---|---|---|---|---|
| Isla verde | 5.67 | 1.42 | 36.71 | 17.65 |
| S/N (Calvario) | 8.17 | 2.04 | 26.45 | 22.53 |
| Pirañas | 3.00 | 0.75 | 30.67 | 12.00 |
| El Salero (Ponds 1) | 10.75 | 2.69 | 17.86 | 14.88 |
| Rodeo (Ponds 1) | 7.50 | 1.88 | 30.40 | 24.53 |
| Rodeo (Ponds 2) | 4.50 | 1.13 | 23.11 | 12.44 |
| La Pampa | 6.00 | 1.50 | 33.33 | 22.67 |
| Garcero | 5.50 | 1.38 | 27.64 | 23.27 |
| Labrador | 7.50 | 1.88 | 18.13 | 15.47 |
| Average | 6.51 | 1.63 | 27.14 | 18.38 |

The maximum product in flanks was presented in Rodeo (stagnates 1) with 24.53 flank/h/men. The securities minima as for the product was presented in Saltcellar, 17.86 Ind/h/men, and in the Pond Pirañas, with 12.00 flank/h/men.

In the Bella Vista river (ANMI San Matías) one could only witness an hunt with a duration of 2 hours and a product of 22 Ind/h/men and 14 flank/h/men (Chart 3b), not existing discards for the use of the hunters, but for the PNASL (of 11 Ind. hunted 4 were “rabudinhos”).

**Chart 3b.** Types of water environment (River), total hunt time, product hours/men and the product of flanks for hunt event of *C. yacare* in San Matías.

| Name of water environment | Total hunt time | Eff. h/men | Efficiency Ind/h/men | Efficiency Flank/h/men |
|---|---|---|---|---|
| Buena Vista | 2.00 | 0.5 | 22 | 14 |
| Total | 2.00 | 0.5 | 22 | 14 |

In Curichi (Curichi Grande) could evaluate 20 hunt events whose average in hours totals were of 6.83. During this time an effort was averaged of 3.41 h/men. The bigger event duration was the seventh, with a total of 9.50 h, and the minimum valor was registered in the event 1 of the Capon del Tigre with 3.25 h. As for the efficiency Ind/h/men, for the Curichi was of 4.68 and efficiency in flanks of 3.09 Flank/h/men (Chart 3c).

**Chart 3c.** Types of water environment (Curichi), total hunt time, product hours/men and the product of flanks for hunt event of *C. yacare* in San Matías.

| Name of water environment | Total hunt time | Eff. h/men | Efficiency Ind/h/men | Efficiency Flank/h/men |
|---|---|---|---|---|
| Capon del Tigre (even. 1) | 3.25 | 1.63 | 5.54 | 3.08 |
| Capon del Tigre (even. 2) | 4.00 | 2.00 | 10.50 | 4.50 |
| Capon Garrapatilla (even. 1) | 7.00 | 3.50 | 2.86 | 2.00 |
| Capon Garrapatilla (even. 2) | 7.00 | 3.50 | 4.29 | 2.57 |
| Capon Garrapatilla (even. 3) | 7.50 | 3.75 | 2.67 | 1.60 |
| Capon Garrapatilla (even. 4) | 7.50 | 3.75 | 2.13 | 1.60 |
| Capon Garrapatilla (even. 5) | 6.25 | 3.13 | 7.68 | 6.72 |
| Capon Garrapatilla (even. 6) | 6.25 | 3.13 | 6.08 | 5.44 |
| Capon Garrapatilla (even. 7) | 9.50 | 4.75 | 4.42 | 3.58 |
| Capon Garrapatilla (even. 8) | 7.50 | 3.75 | 6.67 | 4.27 |
| Capon Garrapatilla (even. 9) | 8.00 | 4.00 | 3.75 | 2.75 |
| Capon Garrapatilla (even. 10) | 8.00 | 4.00 | 7.25 | 3.75 |
| Capon Garrapatilla (even. 11) | 7.50 | 3.75 | 1.60 | 1.07 |
| Capon Garrapatilla (even. 12) | 7.50 | 3.75 | 6.40 | 5.07 |
| Capon Garrapatilla (even. 13) | 6.50 | 3.25 | 6.15 | 5.23 |
| Capon Garrapatilla (even. 14) | 5.50 | 2.75 | 1.82 | 1.09 |
| Capon Garrapatilla (even. 15) | 8.00 | 4.00 | 4.75 | 4.00 |
| Capon Garrapatilla (even. 16) | 5.50 | 2.75 | 2.91 | 1.82 |
| Capon Garrapatilla (even. 17) | 5.50 | 2.75 | 1.82 | 0.73 |
| Capon Garrapatilla (even. 18) | 7.50 | 3.75 | 4.27 | 3.47 |
| Capon Garrapatilla (even. 19) | 7.50 | 3.75 | 8.00 | 3.20 |
| Capon Garrapatilla (even. 20) | 7.50 | 3.75 | 1.33 | 0.53 |
| Average | 6.83 | 3.41 | 4.68 | 3.09 |

The event that smaller efficiency presented was the 20 with 1.33 Ind/h/men and 0.53 flank/h/men.

The variations among efficiency Ind/h/men and Flank/h/men, is because of the Curichis is the area where bigger hunt exists of “rabudinhos”.

**Comparison between effort and efficiency for the different water environments**

To determine type in which environments presents a bigger effort during the hunt the effort man hour was compared by water environment type being verified a significant differences (Kruskal Wallis H=18.528; p=0.0001) (Figure 6).

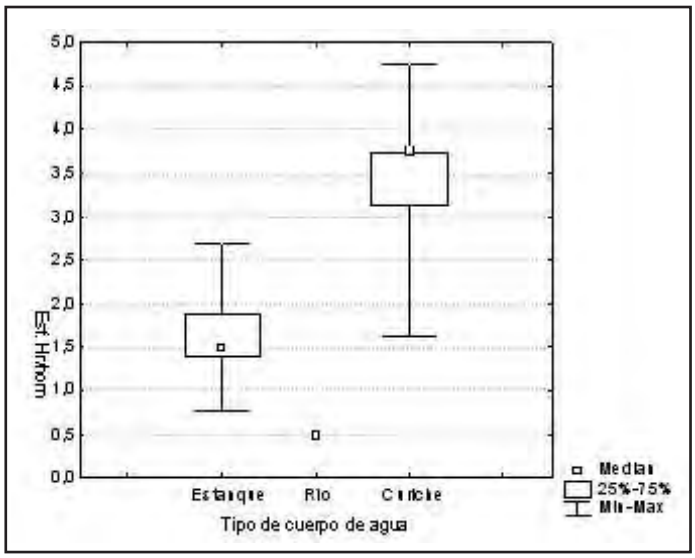



**Figure 6.** Comparison among the effort man hour (Eff. h/men) and the different water environment types.

In the same way this analysis was applied to compare the efficiency, as much ind/hours/men, as flanks/hours/men, among the different water environment types, finding for both cases significant differences (Kruskal Wallis H=20.082; p=0.0000) (Figure 7).

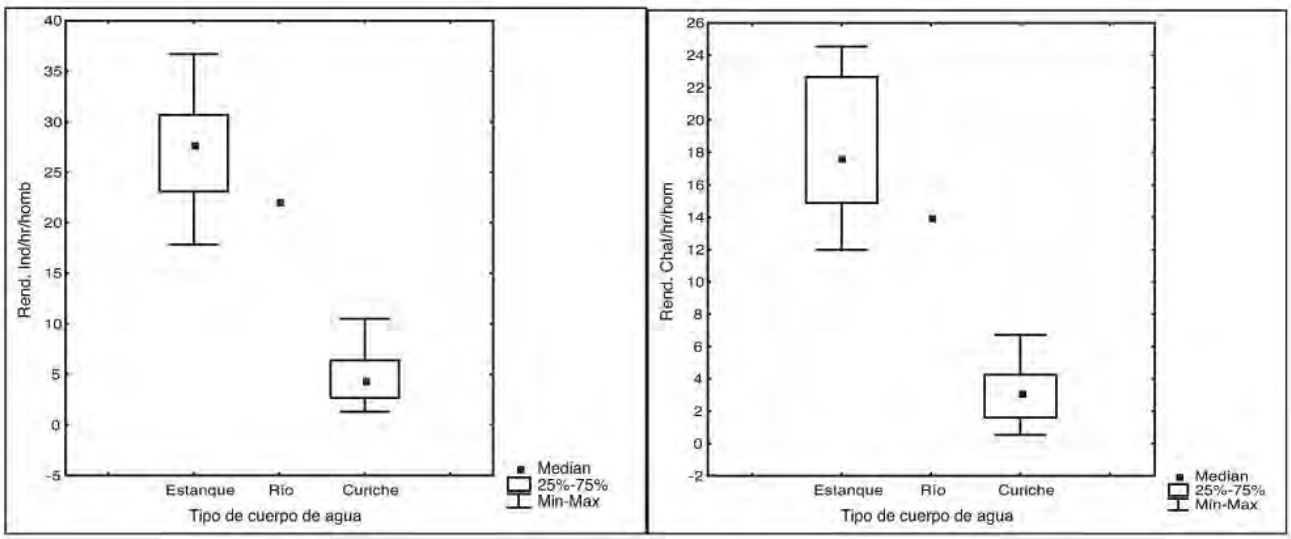



**Figure 7.** Comparison among the efficiency ind/hours/men (effic. Ind/h/men), flanks/hours/men (effic. Flank/h/men) and the different water environment types.

The effort realized for the caimans hunt was bigger in the Curichis, but the efficiency as much in individuals as in flanks was bigger in the ponds, however the comparison among these two environment water indicate that the ponds present 63.02% of discard (“they didn't serve”, “they didn't give measure” and “they were not found”) in front of 32.35% presented in the Curichi, this is because of in the ponds where there is a bigger concentration of individual exist a bigger pressure in the hunt (the caimans spread to leave out of environment water and to hide in the coast vegetation) and that’s why exists a high number of discard that don't give measure or that the skin breaks down.

**Sizes comparison for hunt for the different types environment water**

To compare the corporal longitudes with the different types environment water were carried out tests no parametric (Kruskal–Wallis), being observed that significant differences don't exist as much to the 95 as to 99% probability (H = 9.38; P = 0.0091) (Figure 8).

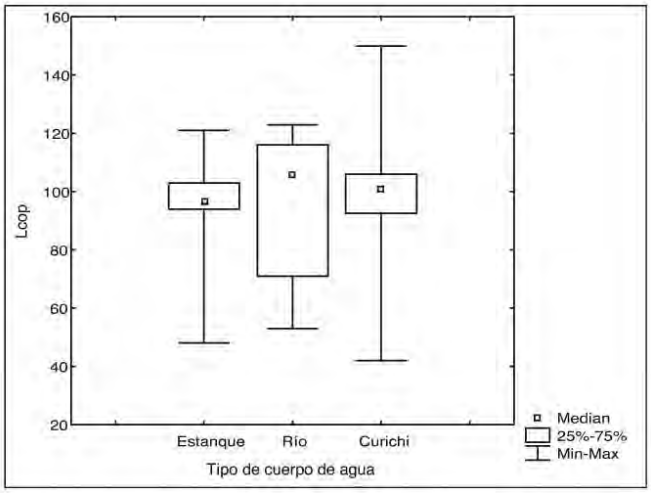



**Figure 8.** Comparison of the Corporal Longitude (BL) of caimans hunted by environments water types.

To compare the tendencies of use of different sizes flanks among the types environment water was carried out a correspondence analysis, finding significant differences among these two variables ($X^2$ = 74.3831; P < 0.000).

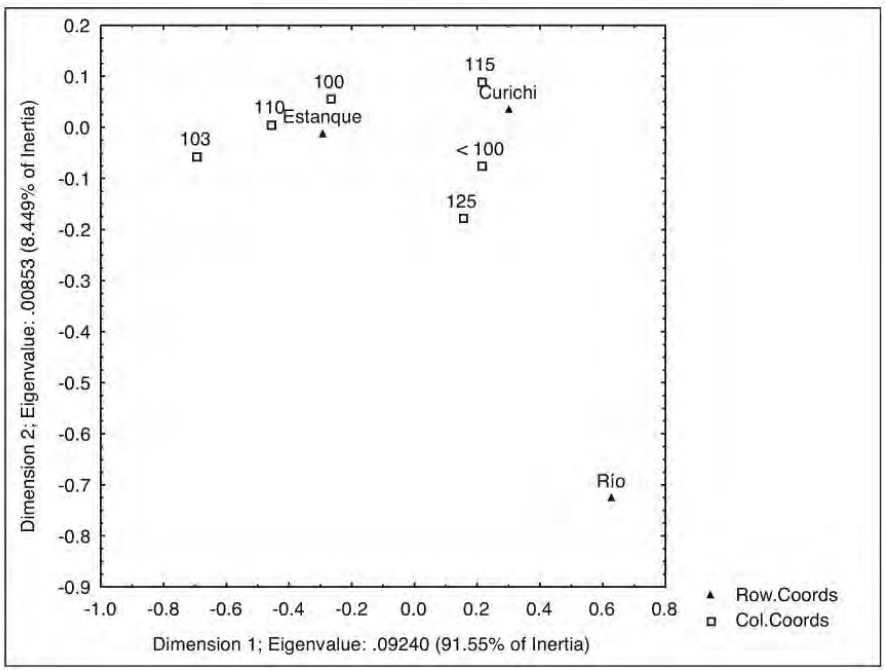



**Figure 9.** Preferences of use of flanks sizes by environment water Types.

In the figure 9 observed that the flanks sizes are presented bigger in the Curichi (115 and 125) while in the ponds a tendency exists of flanks use of 110 cm since the populational structures of the species doesn't allow a bigger size in flanks and the efforts to reach this

size is subject to the leather stretched in the moment of the drying (caimans from 95 cm. BL gives 110 in flank), something that doesn't happen if is not carried out this previous operation. In the Curichi caimans are looked for those have a BL from 100 cm. so that with this operation they reach 115 or 110 without more effort. However in Curichi is given the hunt mostly of “rabudinhos” (< 100) where 89.39% was extracted (59 ind.) from the total of rabudinhos hunted, while in ponds alone 4.54% (3 ind.) and in the river 6.06% (4 ind.) (Figure 10).

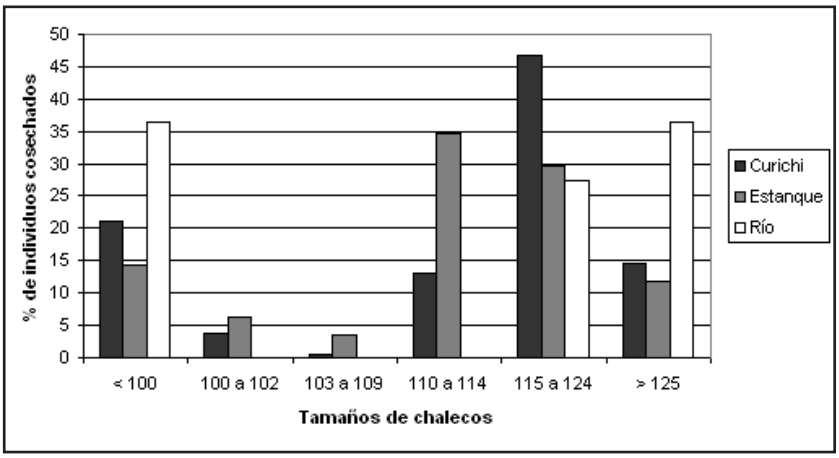



**Figure 10.** Frequencies Distribution among the percentages of individual harvested and the intervals of the flanks size for environment water type.

## CONCLUTIONS AND RECOMMENDATIONS

- San Matías presented an over exploitation of share hunt allowed by the National Government for the *Caiman yacare* use in Bolivia in a 33.42%. this increment is caused by the leathers smuggling for hatcheries from Brazil, but mostly because of individuals that don’t present sizes and the don’t taken out of water and shooted.
- In the ponds (Private Properties) the biggest caiman populations are presented, but the sizes for the use are on the inferior cut-off (190 cm. LT and 90 cm BL) that indicates the regulation of the caiman, producing a bigger effort in the hunters to reach the measures allowed in flanks, this also indicate the no execution of the D.S. 24774 (regulate of the Caiman Use where it indicates that alone 25% of the populations individual class IV can be taken advantage of) extracting year to year 100% of the reproductive males.
- The Curichi Grande is the area where is carried out the biggest leathers exploitation with no allowed sizes for Bolivia, besides being the place where leathers are extracted to cover shares of other domestic areas, along of all the leathers evaluated in this place to none was placed the national binding for the use.
- It is advisable to carry out bigger control by the competent authorities in the legal use of sizes for Bolivia, also that the recent plans being carried out in the area (ANMI San Matías and Private) include a strong training in used sizes in Bolivia and how the good ones practice of management take us to a good use of the resource, planning to a short term to eliminate excessive hunt as much for the international smuggling (“rabudinhos”) as local smuggling (bindings of Beni are placed in leathers extracted in San Matías) among to considerate these percentage in the time to calculate the share.

## ACKNOWLEDGEMENTS

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# Distribution, abundance and population structure of Spectacled Caiman *(Caiman yacare)* in the Natural Area of Integrated Management (ANMI) San Matias, Santa Cruz - Bolivia.

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**ABSTRACT:** We evaluated wild populations of spectacled caiman *(Caiman yacare)* between June and September 2007 during dry season within the Protected Area of San Matías. The relative abundance was determined using night counts data, collected in water bodies of communities and private properties within the protected area and its influence zone. The values of relative abundance vary from 1.98 individuals/kilometer of shore to 1945.45 ind/km of shore, showing maximum values in artificial pounds inside cattle ranches. The observed population structure was dominated by Class I individuals (25.70%), followed by Class III individuals (25.68%), Class IV individuals (20.28%) and finally Class II (14.00%) individuals. The population structure shows a well preserved population which could be subject to selective extractions of adult male individuals. Despite this, as the abundance is not uniformly distributed, certain areas would be excluded from harvesting. These data correspond to caiman population assessments in the context of the elaboration of the management plan for spectacled caiman *(C. yacare)* in ANMI San Matías; the main objective is to achieve sustainable use of caimans and to produce economic benefits that could improve the quality of life of local residents within the protected area.

## INTRODUCTION

Latin America is the region of highest species richness in crocodilians that can compare with any other region of the world, spread from Mexico to Argentina that due to the amount of wetlands and coastal systems found throughout the region (Messel *et al.* 1995). This situation makes the crocodilians represent a resource of considerable ecological value and great economic potential (Pacheco 1996), this is a great potential that the region that also has suffered the further exploitation of crocodilians in the world. However, currently there is a change in the trend of conservation of crocodilians around the world. The adoption of new strategies for sustainable use of crocodilians has provided new incentives for the conservation of these species and their habitats (Messel *et al.* 1995).

In Bolivia, to develop a program of sustainable use of *Caiman yacare* the chosen model was the harvest of wild animals, based on the model of exploiting Venezuelan *Caiman crocodilus* (King and Godshalk 1997, Thorbjarnarson and Velasco 1998, Llobet and CIPTA 2006). This system requires less economic investment and the biggest beneficiaries are the landowners (Velasco *et. al* 1995). Harvesting is based in the sexual dimorphism of the species, which is used to establish the boundaries of minimum size of the animals to be harvested, so the harvest is focused towards animals greater than 180 cm. in total length, which generally are male, thus protecting breeding females.

A situation that must be considered is that hunting in the wilderness is more difficult to

regulate and has a high risk of not being sustainable. Populations of crocodilians can be reduced easily by the removal of breeding adults, if this is compounded by the vast over-exploitation of stocks crocodilians which led to many species to critical levels in terms of its conservation, was due largely to direct hunting (King, 1989), it is easy to deduce that this require different control systems that serve to adjust and correct the flaws of the program, so that it can ensure compliance with the same main goal: achieving Effective conservation *Caiman yacare* in Bolivia (Llobet and CIPTA, 2006).

## METHODS

To evaluate the population of spectacled caiman of ANMI San Matías, we carried out four sampling campaigns in with night counts in water bodies, established under standardized methodologies in the crocodilians study.

ANMI San Matías was divided into 4 zones (North, Central, South and East), conducting a month of work in each area. The first campaign took place during the month of June in the northern zone, the second campaign was developed in July at the Center area, the third campaign was carried out into the south (August) and the fourth campaign was conducted in Parallel with two teams, one in the northern zone (in water bodies that could not be sampled during the first campaign) and other team in the eastern area.

We visited 13 communities (Bahía Negra, Candelaria, Corechi-Rincon del Tigre, Florida, Natividad Pozones, Puerto Gonzalo, San Fernando, San Miguelito, Santo Corazon, Tapera, Tornito and Villazon) and 30 private properties (Altamira, Bahía Grande, Buena Vista, Caribe, Cotoca, or Cotoca The Puquio, El Carmen, El Gato, El Junte, Espinal, Florida, Jesus, Esperanza, Union, Mojon, Motacú, Propiedad Barbosa, Curupau, Paraiso Santo Rosario, San Antonio, San Jose, San Miguel, San Roque, San Sebastian, Santo Tomas, Tel Aviv, Tres Hermanos, Urkupiña, and one name is not known for the absence of those responsible).

In all cases we worked with residents of the communities and/or workers of the properties. For some samples we obtained information from local perceptions, through informal talks with community members who worked as local technicians (trained in techniques of counting and estimating sizes of yacares). Additionally, several park rangers were trained in crocodile counts.

Censuses were carried out avoiding the presence of the moon and during the hours of more darkness (before the exit of the moon, after the exit of the moon or taking advantage of cloudy nights). During the counts we registered different variables as environment temperature, water temperature, wind speed, cloud cover, moon phase, aquatic vegetation coverage, as well as some characteristics of water bodies.

Analyses of abundance and population structure were conducted with the results obtained in all water bodies sampled. The index of relative abundance was calculated based on the number of animals recorded per kilometer from shore (without taking into account the individuals from Class I) (Aparicio 1997, Godshalk 1994, King and Godshalk 1997, Llobet and Aparicio 1999, Llobet and Goitia 1997, Pacheco 1993, Vasquez 1981). In those water bodies where the counting was extended by more than 6 hours and those in which for reasons of navigability we could not complete to circumnavigate all the perimeter, the relative

abundance obtained under the sampled section was applied to the rest of the water body.

The population structure of the caimans for the entire area, as well as for the different water bodies was calculated by taking the number of individuals positively identified and extrapolating this proportion to the rest of the population (identified as Eyes Only). We compared this structure between locations through a chi-square test ($X^2$) using contingency tables (Llobet 2002, Llobet 2005, Llobet and CIPTA 2006).

To guarantee compatibility of data generated in the monitoring of caimans in Bolivia, we used the water bodies' classification from the Museum of Natural History Noel Kempff Mercado (2005):

- Tectonic lagoons: isolated bodies which are not home river, and they are regarded when they have a perimeter more than 1 km.
- River lagoons: temporarily connected with the river, many are abandoned meanders.
- Small lagoons: bodies of water less than 1 km perimeter.
- Ponds: artificial water bodies, on the edge of roads or dug for watering livestock.
- Rivers: watercourses that are continuing in the Landsat images (30 m pixel) wide although variable that must be verified in the field.
- Streams: watercourses those are not continuing in the Landsat images, and born in the plains.
- Swamps: permanent flood zones.

To this classification was added:
**Bays:** Parts of the rivers which have special characteristics in terms of their morphology therefore resemble broad parts or corners of the river.

**Big "Pantanal" Lakes:** large lakes in the far eastern border of Bolivia (Mandioré, Gaiba and Uberaba). Despite the name of "lakes", these water bodies are in permanent connection with rivers.

## RESULTS

As a result of the four-month campaign, we sampled 88 water bodies of which most were Ponds (40), followed by segments of Rivers (27), lagoons (9), Bays (8) and Small lagoons (4), sampling a total of 189.43 km of shore in different bodies of water in ANMI San Matías (Table 1).

**Table 1.** Bodies of water sampled in the ANMI San Matias Body Type Water Quantity of sites distance travelled (km) range represented shore (km).

| Bodies of water | Number of sites | Distance traveled (Km) | Distance of shore represented (Km) |
|---|---|---|---|
| Bay | 8 | 3.5 | 4.29 |
| Ponds | 40 | 7.75 | 8.05 |
| Lagoons | 9 | 30.31 | 35.99 |
| Small lagoons | 4 | 0.78 | 0.78 |
| Rivers | 27 | 121.32 | 140.32 |
| **Total** | **88** | **163.66** | **189.43** |

The figure below shows all the sampling points visited during the 4 months of fieldwork, you can appreciate that the largest number of points is in the northern part of the protected area and its zone of influence because of the increased amount of bodies water that exist in these areas (Figure 1).

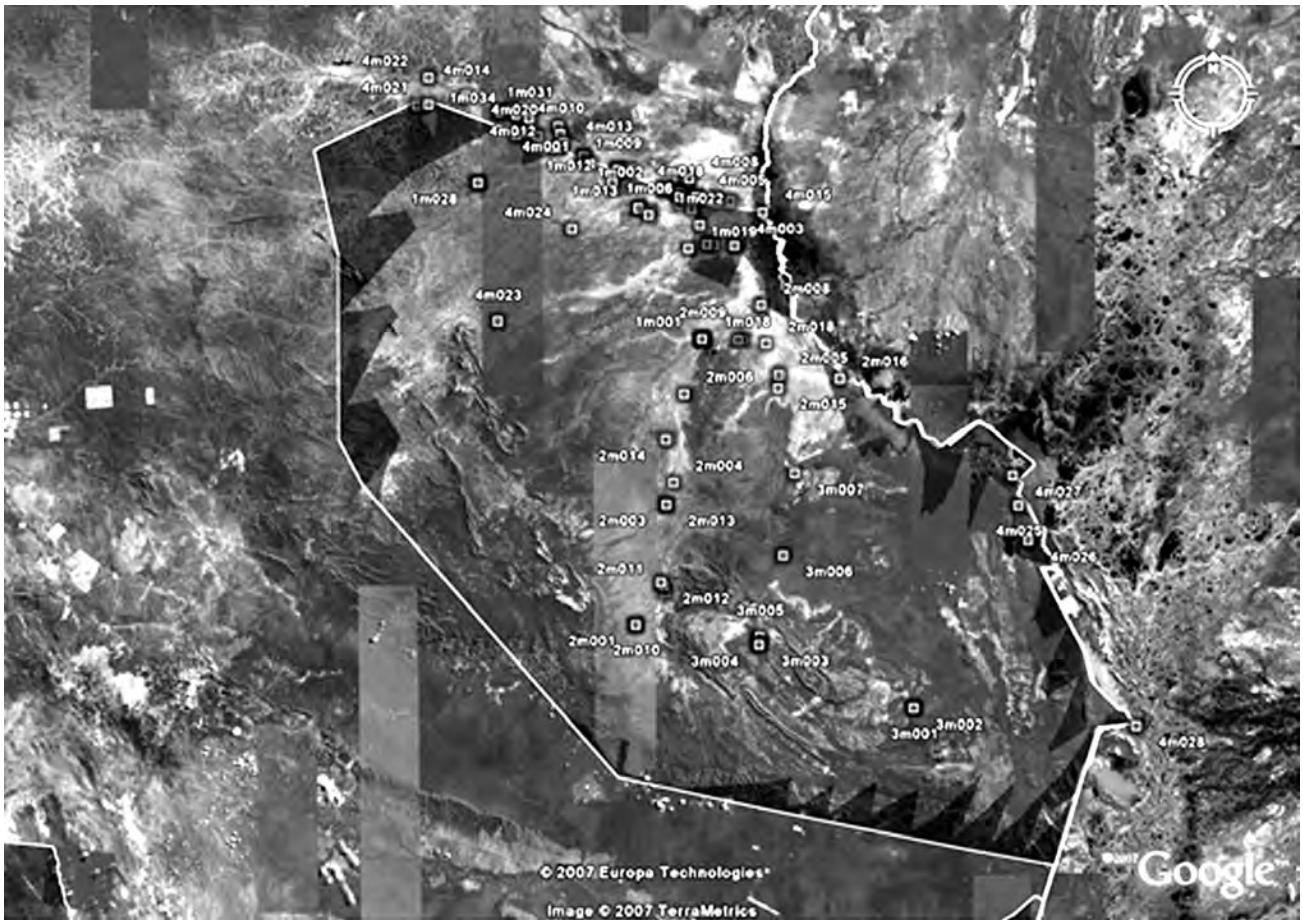



**Figure 1**. Points within the sampling ANMI San Matías and its zone of influence.

We counted 9245 individuals of different size classes from hatchlings (class I) to adult males (Class IV) including those individuals identified as' Eyes Only ' (OS).

The higher values of abundance were presented in the ponds of private property with values of 1945.45 ind / km of shore (San Antonio), followed by Tel Aviv 2 ponds with 1750.00 ind/km of shore and La Union 1 to 1482.35 ind / km of shore, while the lowest values were found in the river Santo Corazon, and a swamp near Villazon community with 0.0 individuals per kilometer of shore.

We made an analysis by grouping the values of abundance by type of water body, using five categories: Rivers (grouping all water flows, rivers and streams), Ponds (artificial water bodies), Bays, Small lagoons (those places whose perimeter is less than 1 km) and lagoons. We found statistically significant differences ($H = 37.81$, $P = 0.00$) (Figure 2).

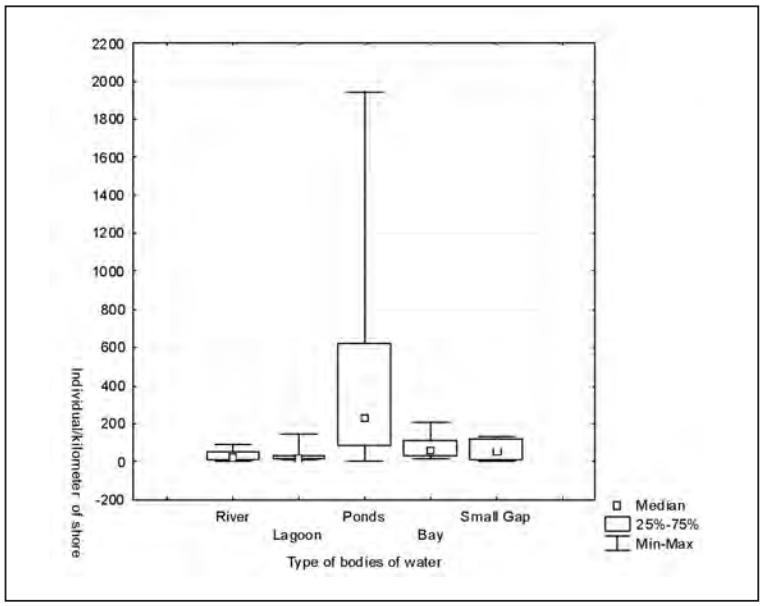



**Figure 2.** Changes in the values of abundance of spectacled caiman between different types of water bodies of ANMI San Matias.

Proceeds from the analysis can be seen that the ponds have a different behavior as they maintain the highest variation in abundance of all types of water bodies, hiding the differences that might exist in other categories. In this regard, in order to assess trends in different locations sampled was carried out further analysis, but excluding the ponds (Figure 3).

In this new analysis, although there are different types of trends in places, we can also see that the great variation in abundance in each type of water body makes the differences are not considered statistically significant at 95 and 99% of probability (H=6.59, P = 0.089). However, it is important to note that the Bays have a slight tendency to have higher values of yacare abundance than other water bodies (Figure 3).

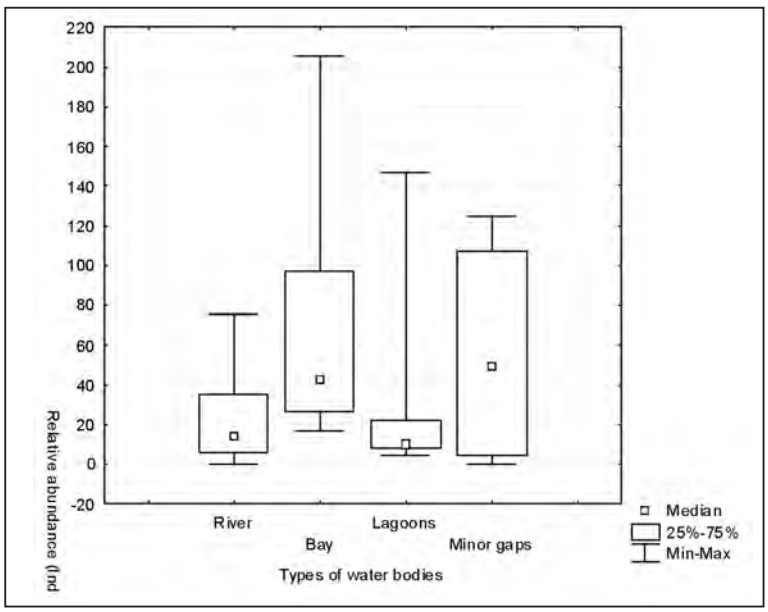



**Figure 3.** Abundance values of spectacled caiman between different types of water bodies of ANMI San Matías without taking into account the artificial ponds.

Concerning the population structure, many individuals observed correspond to the Class I (25.70%), followed by Class III (25.68%), Class IV (20.28%) and finally the Class II (14.00%) of individuals observed (Figure 4). We include in this distribution percentages also to those individuals identified as OS ("eyes only") finding that represent 14.34% of all animals observed that is that if we believe that could have a positive identification and estimated height of 85.66% of individuals observed, we can assume that the analysis of population structure will be highly reliable.

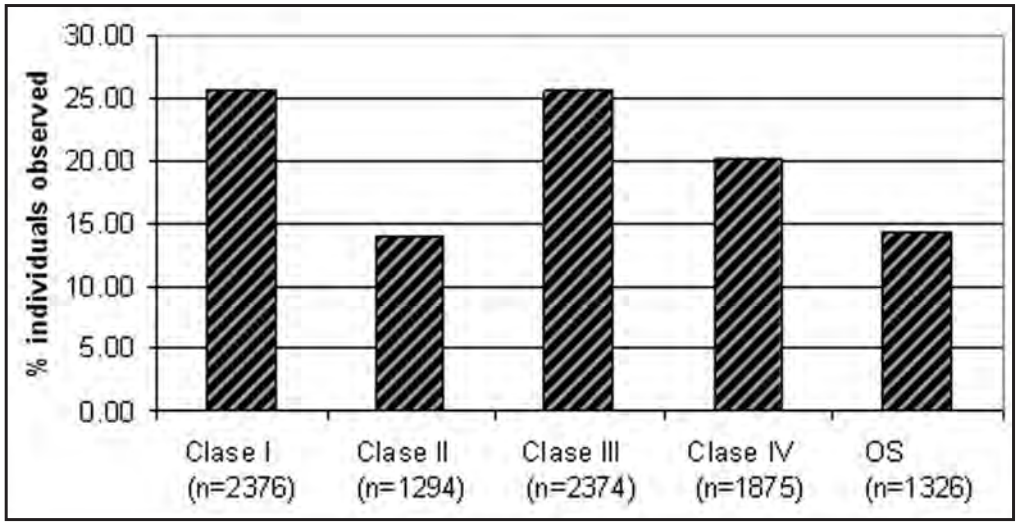



**Figure 4**. Structure population of the spectacled caiman of ANMI San Matías.

If we analyze the population structure only on Class II, III and IV, and following the criteria of Ayarzagüena and Velasco (1995) we observed that the structure correspond to a pyramidal structure with more than 15% of individuals Class IV, which represents a population with high potential for sustained harvests of adult males. Moreover, if we consider that under this type of analysis the percentage of spectacled caiman adult males Class IV of ANMI San Matías is 33.83%, an initiative to raise the use of spectacled caiman in the area accomplishes the National Regulations for the Conservation and Sustainable Use of spectacled caiman *(Caiman yacare)* which states that "permitted the harvest of spectacled caiman of 25% of the Group IV (animals with over 180 cm. in total length) in populations of good state of conservation" (Article 18), and which additionally states that "It is understood by population in a good state of preservation, when animals of group IV, exceeds 15% of total formed by groups II, III and IV".

But this population structure observed for ANMI San Matías, during sampling could be seen that some areas showed significant differences in terms of the proportion of individuals from Class IV. In this sense, looking at the population structure for each area inside ANMI San Matías, we note that there are highly significant differences ($X^2$=464.27, P <0.001), with a northern zone where the percentage of Class IV individuals is greater than the other classes (40.32%), an eastern area in which the Class IV is in second place (34.32%) after the Class III, a central zone in which the Class IV is in third place (16.07%) after Classes III and II, and finally the southern zone where the Class IV (3.70%) is well below Class II and III (Figure 5, Table 5).

**Figure 5.** Structure of the spectacled caiman population in areas at the ANMI San Matias.

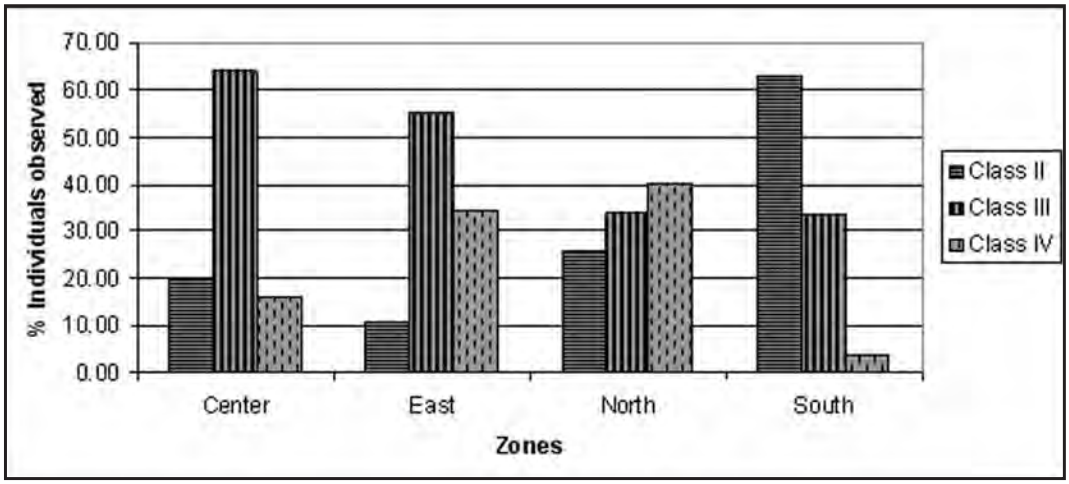



**Table 2.** Percentages of size classes in areas in the ANMI San Matías.

| Zone | Class II | Class III | Class IV |
|---|---|---|---|
| **Center** | 19.94 | 63.99 | 16.07 |
| **East** | 10.62 | 55.06 | 34.32 |
| **North** | 25.64 | 34.03 | 40.32 |
| **South** | 62.96 | 33.33 | 3.70 |

Regarding the population structure of the spectacled caiman for each type of water body, it was noted that the Bays have a very high proportion of Class IV individuals. Ponds maintained a high proportion of individuals Class III, followed by Class IV; lagoons also features a high proportion of Class III animals, but in this case followed by Class II and Class IV animals; smalls lagoons maintain the same pattern described by Llobet (2005) and Llobet and CIPTA (2006) for the TCO Tacana, with a very high proportion of individuals Class I, followed by Class II and Class III; finally we found in rivers is a high proportion of individuals Class I, followed by Class III, II and IV (Figure 6).

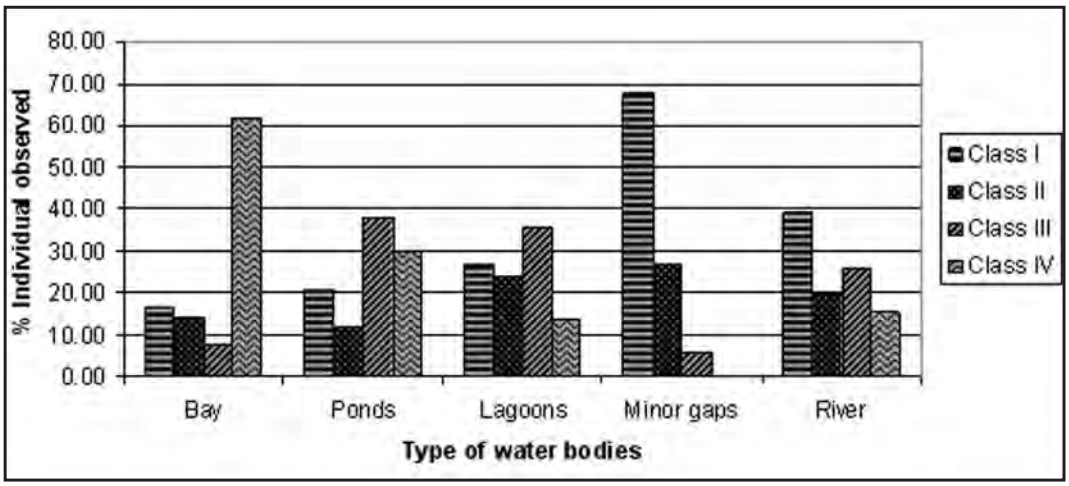



**Figure 6.** Structure of the spectacled caiman population by type of water body in ANMI San Matías.

The differences ($X^2$ = 1075.02, P <0.001) found on the population structure by type of water body may be because some of the small water bodies (in this case small lagoons) behave as breeding centers of the species. This segregation in the use of some water bodies represents an adaptive advantage to reduce the possibility of intraspecific predation.

## DISCUSSIONS

It is important to note that an accurate size of the population is very difficult to estimate, in addition the monitoring of crocodilians presents some problems that have been treated extensively in the literature (Woodward and Marion 1978, Messel 1981, Magnusson 1982, Larriera et al. 1993, Abercrombie and Verdade 1995, 1994 Pacheco, Pacheco 1996). Additionally, it is theoretically possible to control the effect that the majority of environmental variables have on the night counts by doing them under similar conditions, but is more difficult to control biological variables as the caution of caimans and population density (Pacheco 1994, 1996) In areas where it has been practiced (or even practiced) hunting of animals for different purposes, they will tend to be more timid which may hinder its observation and produce a bias in the calculation of abundance. This tendency to underestimate the population also occurs in cases of population with very low densities, and that significantly reduces the probability of observing an individual. Finally, the relative abundance indices will underestimate the true size of the population, because a portion of it usually remains without being detected and it is very difficult to establish the relationship between the index of abundance and the true density in the area (Hutton and Woolhouse 1989).

The environmental factor that can affect more the night counts of crocodilians is the water level (Woodward and Marion 1978, Messel et al. 1981, Llobet and Goitia 1997). However, this variable can also be related to changes in behavior that can also affect the results of the counts. Seijas and Chavez (2000) in the river system Cojedes (Venezuela) reported variations in the number of juvenile alligators observed as the dry season passed, on the other hand, these same authors also noted an increase in adult individuals observed (particularly females) at the beginning of the breeding season. However, despite variations that may exist as the dry season passes, it is recommended for future work, and for reasons of accessibility to water bodies, to carry out the monitoring of the caimans in the interval of time since June (when it is falling water levels) until September (before the water level reaches its lowest point), because at this time animals are confined in bodies water, and secondly, not having reached the point of minimum water level, it facilitates navigation to perform the counts. These two factors must be added that at this time and all were born offspring, so that we can obtain valuable information on trends of reproductive crocodilians.

Other factors that may explain the variability in the abundance indices have to do with differences between sectors of visibility, which can introduce errors into the results (Hutton and Woolhouse 1989, Da Silveira et al. 1997). In almost all places sampled was presented an extensive vegetation cover, what makes us assume that in all cases it was possible to identify a fraction of the population and that the calculations are underestimating the real size of the same one. Additionally, we must point out that visits to places of sampling were conducted sequentially rather than simultaneously, namely that the recent visits to sites of counting should be conducted until 3 months after the first visit to the sites, this factor may have results in two consequences: on the one hand counts have facilitated the past due to greater concentration of alligators in the water remaining (Seijas, 1986), or on the contrary

if in some places there were extreme drought, have done more difficult to locate individuals who take refuge in forested areas or hide en the mud, which hinders their location (Medem, 1983). In addition to these factors are also taken as a limiting factor during the counts in the vast territorial extension introducing the protected area (nearly 3 millions hectares), the limited possibilities of mobilizing between places and the presence of a large number of water bodies.

It is also clear that the population structure can be shaped by human activities in a given area (Seijas and Chavez 2000). Animals of larger sizes are more visible than small ones and people probably kill the first more frequently. If to this situation, we add that there is a relationship between the caution of animals and human pressure present in a given area (Pacheco 1996), we could expect to observe greater distances to animals (distance to which the individual immerses when approaching an observer) in areas under greater pressure. In this sense, the larger animals, probably more experienced on activities carried out directly toward their hunting or trapping, will show more timid that juveniles, and may produce a bias on the outcome of the structure of the population sizes.

The population of spectacled caiman in ANMI San Matías is not evenly distributed. Among the different bodies of water were observed differences in the abundance of spectacled caiman. In general (not counting the ponds and values of zero), the values of abundance of spectacled caiman ranging from 1.98 ind / km (in the channel of Puerto Gonzalo) to 206.03 ind / km (Altamira's Bay). While many values found in abundance in ANMI San Matías are similar, or are within the range of values reported for water bodies of the Department of Beni, most are significantly higher than those reported for the Chapare (Cochabamba) and Chaco (Tarija) (King and Godshalk 1997, King and Videz-Roca 1987, Llobet 1996, Llobet and Goitia 1997, Llobet and Aparicio 1999, 1993 Pacheco, Pacheco and Llobet 1998, Llobet 2005). The Museum Noel Kempff Mercado in 2005 in the area of San Matías found relative abundances of individuals to 100.62 per kilometer of shore in the Ponds of private properties, while in Rivers found an abundance of 50.09 ind / km, in the Gaps 14.08 ind / km, 91.5 ind/km in the swamps and only 37 Eyes Only in the stream sampled, these values fall within the range of values found in this work.

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# Nesting habits of spectacled caiman (*Caiman yacare*) in Natural Area of Integrated Management (ANMI) San Matías

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**ABSTRACT**: We evaluated some reproduction aspects of spectacled caiman (*C. yacare*) in the North zone of the Natural Area of Integrated Management (ANMI) San Matías, and part of its influence zone within five local communities (Candelaria, San Miguelito, Natividad, Villazón & Tornito) and cattle ranches (Cotoca, San Roque, El Gato, Altamira, Pueblo Viejo & San Sebastián). The search for nests was carried out during day time in the surrounding areas of different water bodies (swamps, floodplains and lagoons). We collected data of every nest (number of eggs, proportion of fertile eggs, nest composition, and habitat characteristics). The comparisons of this data between different zones suggest that the largest percentage of nests is found in the swamp (57.29%), then the lagoons (26.04%) and finally the Floodplains (16.67%). We found a relative abundance of 0.18 nests per kilometers (nests/km.), in the floodplain, 0.46 nests/km. in the curichi (lagoons) and 2.78 nests/km. in the swamp. An average of 24.11 eggs per nest was determined, with a proportion of 96.94% of fertile eggs. The composition of the nests vary according to the surrounding environment; in the floodplains nest are made of dry leafs, dry branches and dirt, meanwhile in the swamp nests are made of aquatic vegetation (*Eichornia* sp., *Salvinia* sp. & Cyperaceae) and few dry stems.

**RESUMEN:** Se evaluaron algunos aspectos reproductivos del lagarto (*Caiman yacare*) en la zona norte del Área Natural de Manejo Integrado (ANMI) San Matías y parte de su zona de influencia, comprendiendo cinco comunidades (Candelaria, San Miguelito, Natividad, Villazón y Tornito) y ranchos ganaderos (Cotoca, San Roque, El Gato, Altamira, Pueblo Viejo y San Sebastián). La búsqueda de nidos fue realizada durante el día en áreas adyacentes a cuerpos de agua (pantanos, llanuras inundadas y lagunas). Se colectaron datos de cada nido (número de huevos, proporción de huevos fértiles, composición de los nidos y características de hábitat). La comparación de nuestros datos entre diferentes zonas sugiere que el mayor porcentaje de los nidos se encuentra en las zonas de pantano (57.29%), seguido de lagunas (26.04%) y llanuras inundadas (16.67%). Se encontró una abundancia relativa de nidos de 0.18 nidos/km en las llanuras inundadas, 0.46 nidos/km en las lagunas y 2.78 nidos/km en el pantano. Se determinó un promedio de 24.1 huevos por nido, con un porcentaje de 96.94% de huevos fértiles. La composición de nidos varía de acuerdo al ambiente circundante; en la llanura inundada los nidos son construidos con hojas secas, ramas y tierra, mientras que en las zonas de pantano están compuestos por vegetación acuática seca (*Eichornia* sp., *Salvinia* sp. y Cyperaceae) así como por tallos secos.

## INTRODUCTION

Knowing the different aspects of the biology of a species is fundamental to the understanding of its position in the environment, their relations intra e interspecific, as well as its proper management and where appropriate use (Casas -Andreu 2003). The cocodrilians part of this

intricate tangle, occupying places higher in the food chain.

In Bolivia to develop a program of sustainable use of *Caiman yacare* the chosen model was the harvesting of wild animals, based on the model of exploiting Venezuelan *Caiman crocodilus* (King and Godshalk 1997, Thorbjarnarson and Velasco, 1998: Llobet, 2006). This system requires less economic investment and the biggest beneficiaries are the landowners (Velasco et. al, 1995).

Both the programme of conservation and sustainable use of *Caiman yacare* (PNASL) and the Integrated Management of Natural Areas (ANMI) San Matías processes are at significant changes designed to effectuate the sustainable development of settlers in the target PNASL as in the creation of the protected area. In the process of learning in the years leading operating PNASL, it has been noting the need to present alternative management to beneficiaries in the insertion of the lessons learned, mainly regarding the role of local actors as a key element for environmental sustainability, social and economic use of this resource.

Within the information it seeks to generate PNASL, and to explore new models for harnessing the species has raised the need to investigate the reproductive potential of the caimans, because studies of nesting habits are quite scarce. The study of nesting habits is of particular importance, since it allows many infer population parameters, including distribution, habitat selection and reproductive rates (Prado et al., 2001). That is why in order to broaden knowledge about the nesting habits of yacare and the absence of prior information about it, has developed this study could provide the residents of the protected area of information which could in the future serve to propose ranching plans in the area of San Matías ANMI. The objectives of this study are

- Identify yacare nests in the communities of San Matías ANMI, with the help of local technicians.
- To assess the efficiency of search method in relation to the effort.
- Evaluate environments nesting.
- Obtain information about the nesting habits and assessing habitat preferences, distribution and characteristics of the nests.

## METHODOLOGY

All search effort is concentrated in the northern zone of the protected area and the zone of influence, including the area of swamp in San Sebastian property. It worked in the communities of Candelaria, San Miguelito, Nativi, Villazón and Tornito. Also included private properties surrounding communities: Cotoca, San Roque, El Gato, Altamira, Pueblo Viejo and San Sebastian (Swamp) (Figure 1).

The search for nests was carried out through the help of local technicians in each community, in addition to the facilitators of the Management Plan of *Caiman yacare* and forest rangers of the protected area.

- Information collected in each of the nests found was:
  - Kind of environment,
  - Vegetati cover around the nest,

- Distance and type of water bodies associated with the nest,
- Dimensions of the nest,
- Material and composition of the nest,
- Presence and behavior of the female,
- Presence of predators.

• Type of search:
- Working during the day by looking around the waterways near communities and visited places.
- Location of places with fairly and vegetation that impede access to the nest.
- Georeference the place,
- Opening of the nest to record the amount of eggs, fertility rates, and size of the nest.

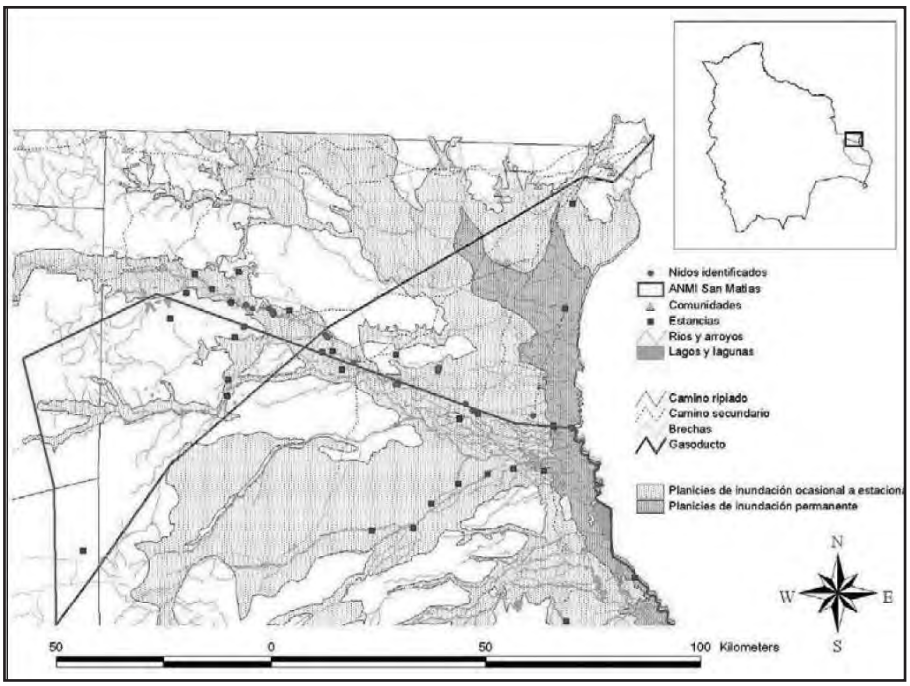



**Figure 1.** Yacare nests identified in the area of San Matías.

## RESULTS AND DISCUSSIÓN

Two rates were differentiated according to ecological environments, geomorphology of the area, the seasonal water and vegetation associated. Our observation in the field during the flood season confirms what has been described by Navarro and Maldonado (2002) established a clear differentiation of habitats according to the regime of flooding in the area, in this sense we can find:

Environments with vegetation of the shoals of seasonal waters:

- Curichi (lagoons): tackled water formed in the seasonally depressed parts of floodplains from the flooding of rivers, where there are two types of vegetation clearly associated with the geomorphology of the floodplain, savannas and clay soil seasonally flooded Várzea forests of clay soil (Navarro and Maldonado, 2002).
- Flat River flood: areas flooded by overflowing river, either with vegetation similar to curichi (lagoons), with the difference that the flood level of the river is always associated with lotic bodies of water.

Environments with vegetation of the shoals of water standing Swamp:

- Swamp: bodies of water that holds water all year or more than eight consecutive months a year. It develops in the most depressed areas of topographically floodplains flood (Navarro and Maldonado, 2002).

According to this classification, for further analysis will consider three types of water bodies already described, assuming that curichi (lagoons) and rivers, will be representing the behaviour of the area of temporary seasonal flooding, while the dam will represent the area of permanent flood.

There were 96 nests of which 45 nests (46.86%) have complete data and geo-referenced data on the position, number of eggs, material composition of the nest, and so on. There were 51 nests (53.13%) observed in the area of the swamp without obtaining data on the quantity of eggs from the position, that because of the impossibility of being able to approach the nests that were floating on aquatic vegetation.

Of the total nests found, 27.08% of the nests were found in communities, whereas 72.92% were found on private property. Of the nests found on the premises of the private properties, 75.71% was recorded in San Sebastian property, adjoining the border with Bolivia - Brazil, which coincides with the ongoing flood zone.

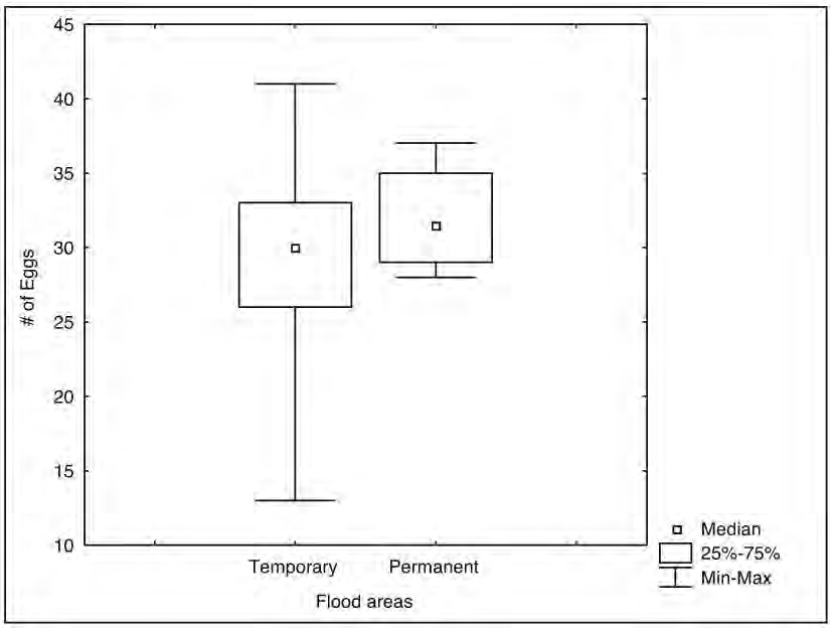



**Figure 2.** Number of eggs for nest in different areas of flooding.

We applied a test Kruskal - Wallis to compare the size of the position according to the types of flood areas. We found that there were no statistically significant differences (H=0.89, p=0.34); note that in the flood zone temporary shows greater variation in the number of eggs per clutch, while the area of permanent flood the range of variation is smaller (Figure 2). The clutches in general showed an average of 30 (SD ± 6.01) eggs per nest with a minimum of 13 eggs and a maximum of 41 eggs per clutch, these data were not taken into account those nests that were zero.

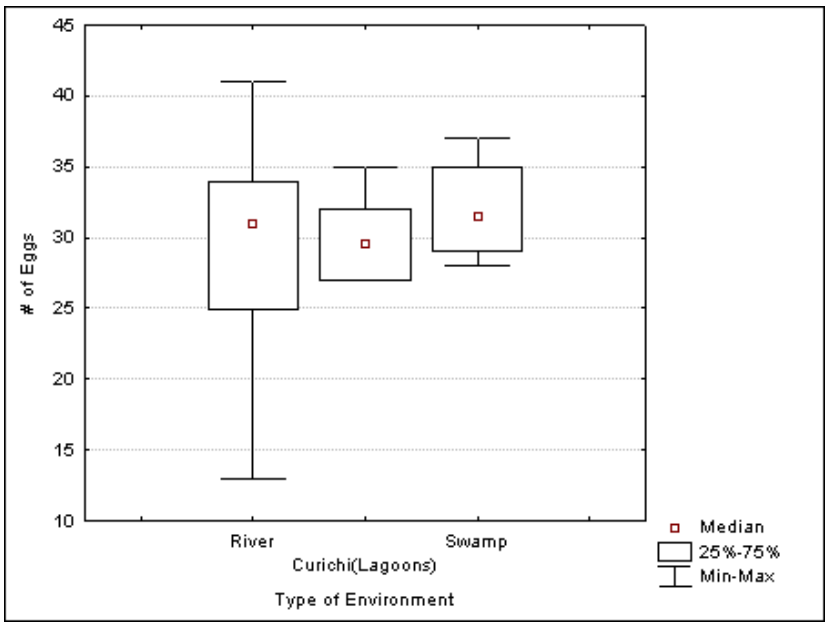



**Figure 3.** Number of eggs for nest in different types of water bodies.

It brought together the size of the clutches by type of water bodies: river, swamp and curichi (lagoons) in which they were found, showing that in rivers there is a greater variation in the number of eggs per nest, while in the curichi (lagoons) and the swamp is less variation (Figure 3) but the analysis ($H = 0.89$, $p = 0.64$) indicates that there is no statistically significant differences.

The dominant vegetation in search of places are for palm forests (*Copernicia alba*) and forests motacú (*Attalea phalerata*) sometimes forming islands of forests as part of the landscape group, along with other species known as ambaibo (*Cecropia polystachia*), mapajo (Kapok), with a predominant shrub layer around the islands of forests with forest scribbles (*Bromelia sp.*)

Generally nests were associated with the base of a tree with bushy vegetation around or below vines and creepers, even were in the midst of forests scribbles (*Bromelia sp.*) That hindered the registration of the position of the nest.

In the area of swamp nests have other characteristic that offers the atmosphere, which every year is water. The nests are mainly floating on the thick layer of aquatic vegetation, which is dominated by species tarope (*Eichornia sp*), pochi (*Salvinia sp*) and several species of Cyperaceas.

Within the environments identified was found that 16.67% of the nests was found in rivers, 26.04% are found in the area of curichi (lagoons) and 57.29% of the nests was observed in the swamp area.

The distance from the nests to water bodies are differentiated by the environment in which the nests were found, where the nests were found in rivers 4.11 meters away until the nearest

bodies of water, but two nests found in community Villazón discovered 50 meters from the nearest bodies of water, nests in environments curichi (lagoons) were found at a mean distance of 9.84 meters to the nearest bodies of water and nests in the marsh area were floating on the aquatic vegetation, which was considered as the minimum distance to water (Figure 4).

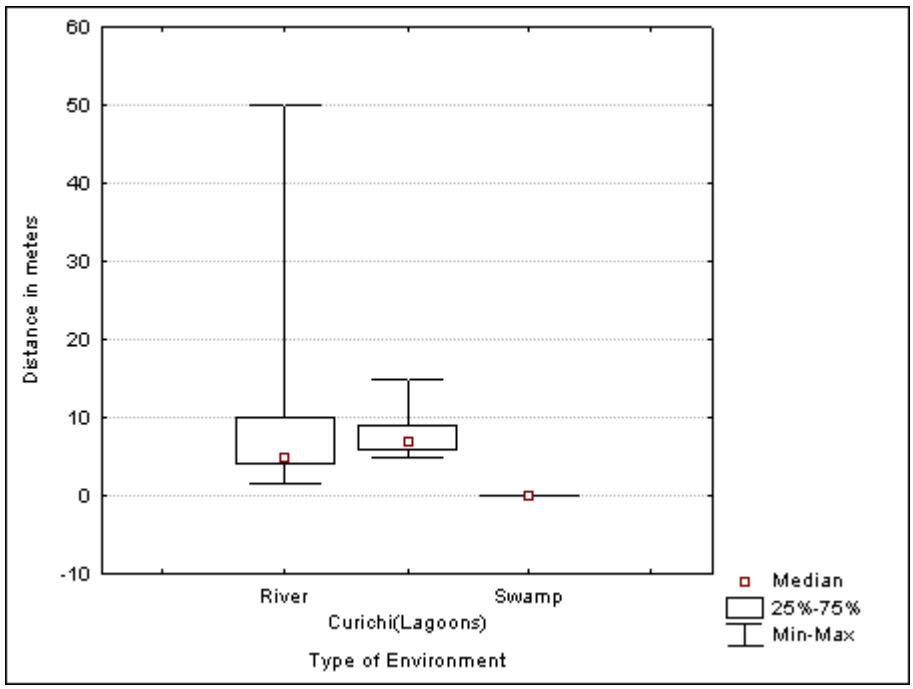



**Figure 4.** Distance from nests to the nearest bodies of water by type of environment.

The greatest distance ranges are found in curichi (lagoons) as these are not associated with permanent bodies of water, but rather are seasonal training. The rivers have lower ranks to be associated with water bodies, while the nests observed in the swamp are floating in the river Curichi Bravo. These trends distances showed highly significant differences (H = 18.85, p = 0.0001).

The composition of the nests varies with the environment in which they find themselves, because the nests were observed in the area of swamp (permanent flood area) are composed mainly of aquatic vegetation (*Eichornia sp., Salvinia sp.* and Cyperaceae) while the nests observed in the mainland (temporary flood area) are composed of dirt, sand, stems and nuts, including leaf litter scribbles (Bromeliaceae) nests were discovered in these environments and rivers curichi (lagoons) (Figure 5).

The width of nests varies between 65 cm. and 164 cm. with a median of 99.67 cm. (D.S. ± 23.54). The length of the nests ranged between 50 cm. and 150 cm. long with a median of 98.50 cm. (D.S. ± 23.78). While the height of the nests had a variation between 23 cm. and 46 cm. tall with a median of 33.50 cm. (D.S. ± 5.75).

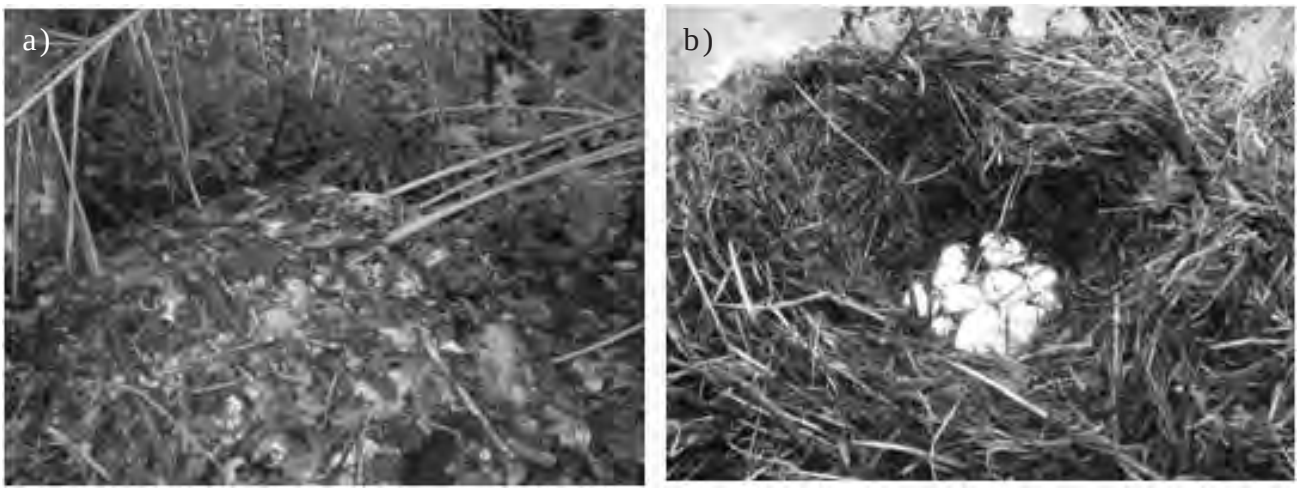



**Figure 5.** (a) Nest in the area of temporary flooding. (b) Nest in the area of permanent flood.

The nests recorded had approximately a half spheroidal shape. The nests use a surface that ranges from 0.26 $m^2$ to 1.93 $m^2$ getting a median area of 0.68 m2 (SD ± 0.34). We pooled the surfaces of the nests as the environment where they were discovered them, finding no significant differences (H = 3.77, p = 0.15). In the nests were recorded volumes between 0.06 $m^3$ and 0.52 $m^3$ obtaining a median of 0.15 m3 (SD ± 0.09).

We analyzed the volume of clutches according to the flood area in which there are (H = 0.63, p = 0.42), demonstrating that there are no significant differences, however, is a small trend of smaller area and volume in the area permanent flood (Figures 6 and 7), perhaps this is because these nests are found floating in the swamp and to be composed of aquatic vegetation take up less volume than those who are on land which have more space and provision of vegetation to build nests.

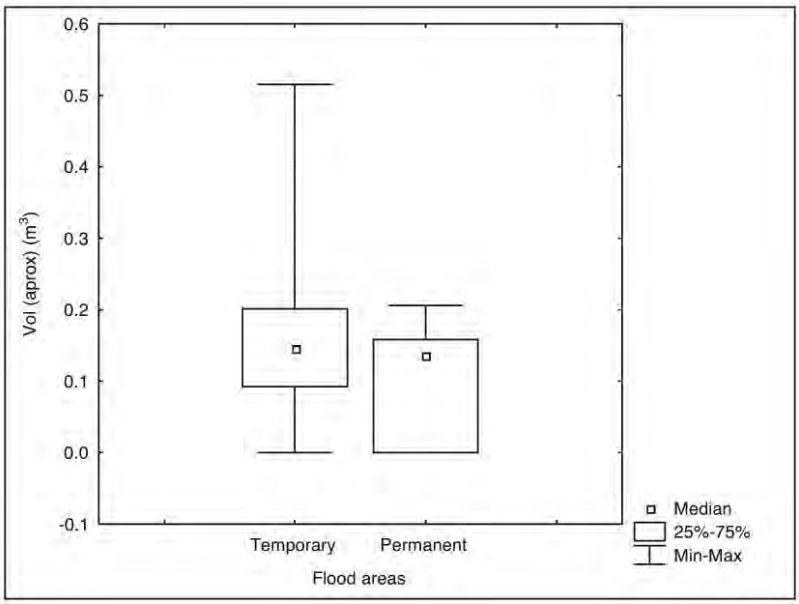



**Figure 6.** Volume of nest according to the Flood Areas.

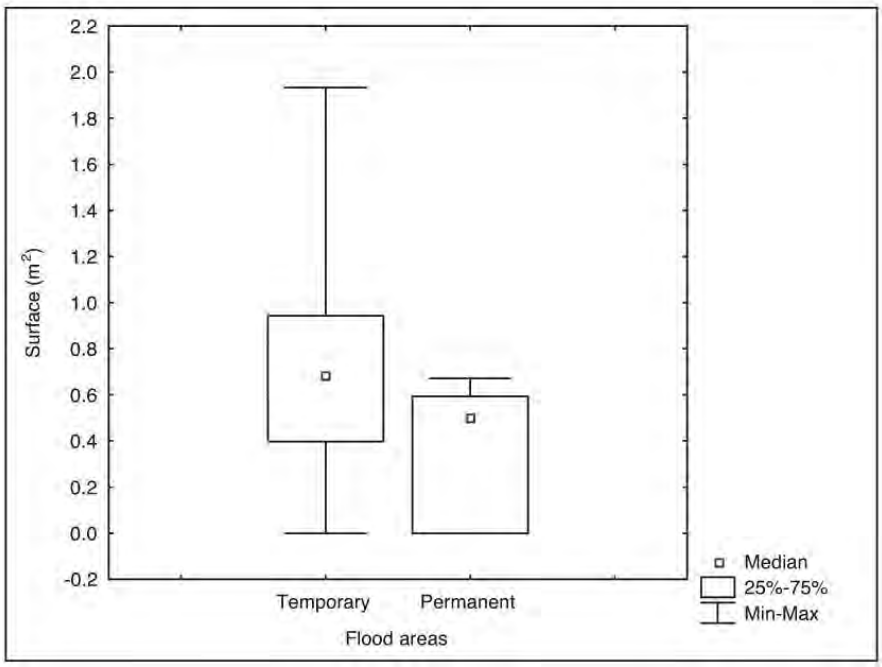



**Figure 7.** Surface of nest according to the Flood Areas.

We found the same patterns to make an analysis by type of water bodies, where the tendency of nests found in rivers would have a greater surface regarding the other two environments identified, however the number of nests discovered in environments curichi (lagoons) and in the swamp is less than the river (Figure 8).

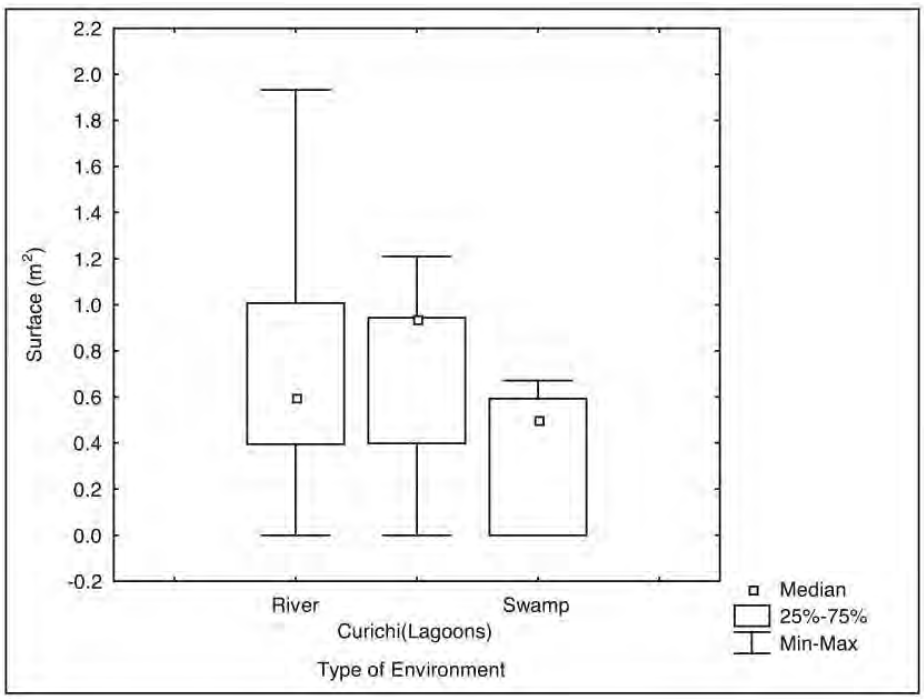



**Figure 8.** Areas of nests depending on the type of water bodies.

Nests were grouped according to the environment in which they were identified, found no significant differences ($H = 0.95$, $p = 0.62$) (Figure 9).

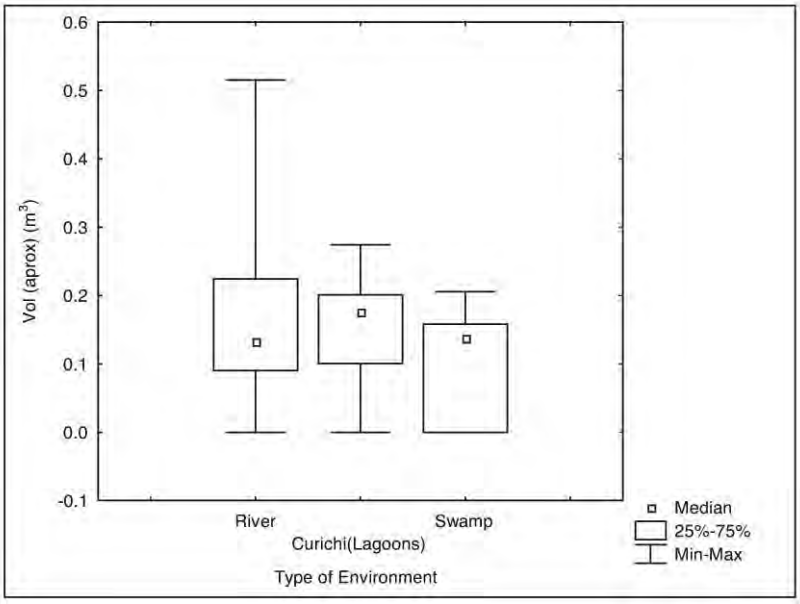



**Figure 9.** Volumes of nests in environments identified.

The highest values of abundance are presented in the swamp area reaching up to find nests 5.07 per hour search, while the lowest were found in the river flood plane with zero nests for the communities of Candelaria and Tornito.

Although it is noteworthy that the values observed in the swamp may have greater variations, since these nests were observed only and not as open nests found in both environments nesting identified. By accessing the nests discovered hours of search or the reverse could increase the value of relative abundance will decrease due to increased time used to record data clutches, as in the nests of other nesting environments.

As we see again the highest values of abundance are presented in the swamp, although there were no statistically significant differences (Figures 10 and 11), if we note that the swamp is a trend to greater abundance of nests that identified the two other environments. The highest values were found in abundance in the swamp with 2.82 and 8.72 nests per kilometre, while the lowest were found in the river with zero kilometers per nest.

In general the trend of relative abundance of nests using any of the two indices is the same as the swamp shows most successful gathering of nests in nests per hour search for nests and mileage.

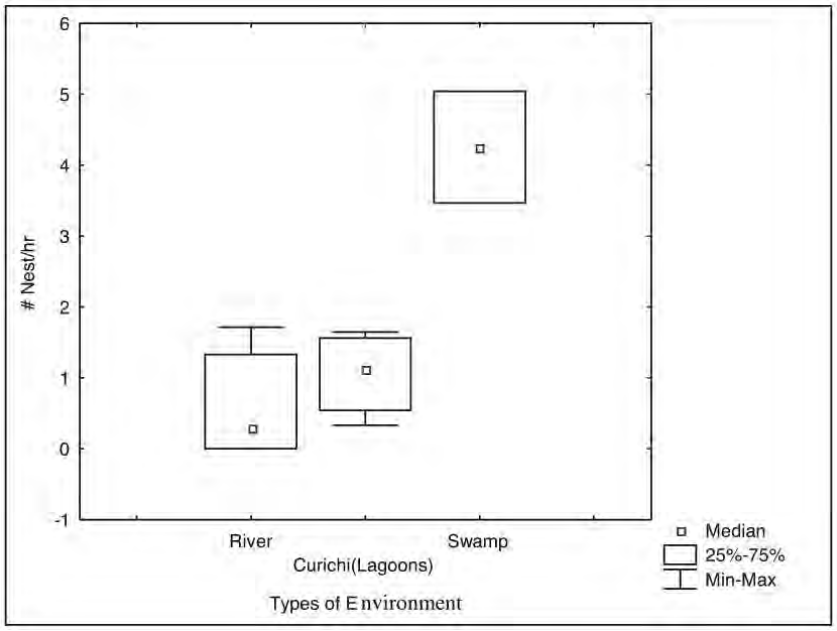



**Figure 10.** Relative abundance of nests (Nests/hours of search) in 3 environments.

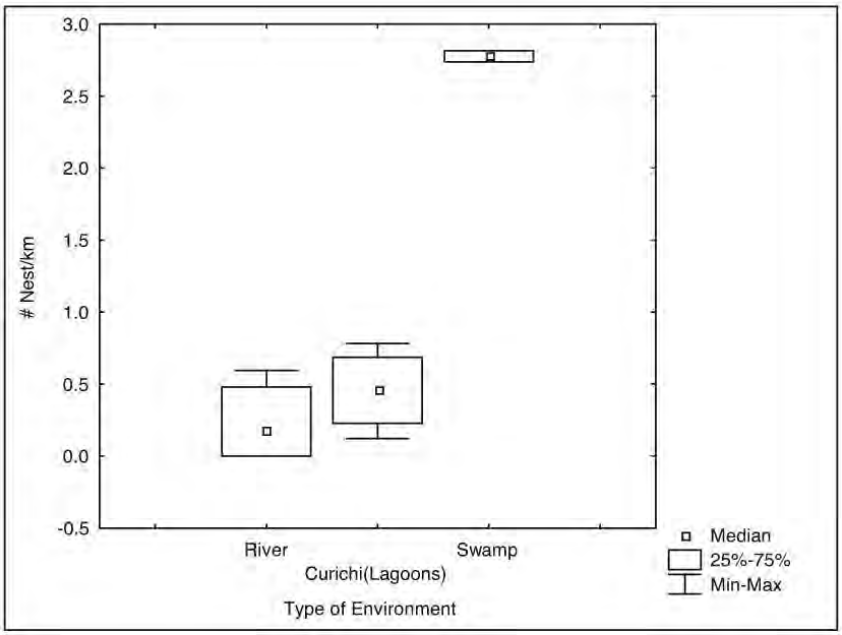



**Figure 11.** Relative abundance of nests (Nest/km of search) in 3 environments.

## CONCLUSION

We found and opened 45 nests in the 3 environments already described; clutches generally showed an average of 30 (SD ± 6.01) eggs per nest with a minimum of 13 eggs and a maximum of 41 eggs per clutch, these data are not took into account those nests that were zero. We obtained a rate of 96.94% of fertile eggs.

The material of which are composed nests varies with the environment in which they find themselves, in enclosed environments such as flood plains occupy as construction material nest dry leaves, nuts, stems and dry land. In places open in the swamp used as aquatic vegetation (*Eichornia sp.*), (*Salvinia sp.*), (Cyperaceae) and some dried stalks.

In the northern part of the protected area is clearly differentiated 3 environments nesting grouped into two areas:

- Area Flood permanent represented by the swamp area.
- Area Flood temporary or seasonal represented by curichi (lagoons) and river.

We appreciate that the swamp area shows a greater amount of nests observed, showing a great similarity in what has been described by Prado (2001) which states that the yacares show a greater affinity for nesting in open places, this place shows a larger problem of accessibility to nests because of the abundant aquatic vegetation. For this reason we have data from nests observed unregistered size of the position. These difficulties of access make the likelihood is that a nest predators is less than that of a nest that is located onshore.

Within the environments identified was found that 16.67% of the nests was found in rivers, 26.04% are found in the area of curichi (lagoons) and 57.29% of the nests was observed in the swamp area.

In terms of the number of eggs per flood zone and by type of water bodies could be seen that there were no statistically significant differences, but in showing that the area flood temporary shows greater variation in the number of eggs for nest, while the flood zone permanent the range of variation is smaller but we must note that our sample size is smaller. As for the types of bodies water, we find that in rivers there is a greater variation in the number of eggs per nest, while in the curichi (lagoons) and in the swamp is less variation.

Regarding the distance from the nests to the nearest water bodies, revealed that nests in rivers can have a very broad range of distance (4.11 to 50 meters) of the nearest bodies of water, while nests in environments curichi (lagoons) were found at a distance minors (average 9.84 meters) and nests in the area of marsh were floating on aquatic vegetation.

In data collected from surfaces and volumes of nests, although we found no statistically significant differences, however the results show a slight tendency for nests of swamp (permanent flood zone) to occupy areas and smaller volumes that nests temporary flood zones. This trend can interact with both the availability of material as with the physical space available for building the nest. In this sense it is clear that in the swamp area there are major constraints in these two aspects presenting a very particular dynamics of nests on floating aquatic vegetation in this area. This characteristic shows an interesting adaptation which in turn can enhance the reproductive success of the kind (to be inaccessible to most nests terrestrial predators.

The trend of relative abundance of nests, making the analysis number of nests per hour sampling and numbers of nests per kilometre, which was obtained using either of the two indices is the same as the Swamp shows most successful meeting nests in nests per hour search for nests and mileage. However under the field experience, the values of abundance of nests by mileage better suited for analysis of nests in the marsh area, as with the index

of abundance of nests per hour search could mask differences might exist from the sampling effort or carrying out other activities.

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# Potential nesting status and prospection for egg harvesting of *Caiman yacare* in the region of San Matías, Santa Cruz, Bolivia

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**ABSTRACT:** Potential nesting estimation and biological evaluations allows us to establish an egg harvest quota that will ensure the conservation of natural resources and cover the production needs of the farm. To estimate nesting population size we made daily journey over 10 days, from 6:00 am to 18:00 pm, marking off the nest spots on a GPS and registering the hatching data. During daylight reconnaissance over 19.26 km$^2$, we collected 424 nests, registering a nest density of 19.93 nests/km$^2$. Mean number of eggs per nest was 27.64 ± 4.03 of 22 nests observed, with a mean egg viability of 24.23 ± 5.5. The mean egg damage was 0.14 ± 0.07 and mean egg infertility was 27 ± 1.26. The mean temperature of the egg storage pod was 32.2 ± 2.31 ºC. Mean hatching exploitation found in our study represents the 54.95%, 32.07% of the nests were not collected, but have shown signs of viability, and the other 12.97% of the nests represent the natural lost, either because of flooding or natural depredators. We estimate the nesting potential equal to 1250.21 for the 62.706 km$^2$ of the permanent flooding zone. For the year 2008, we registered 10500 eggs bought from the CIRPAS Indigenous Communities equivalent to 379.88 nests, and the harvest of 920.12 nests in the private stock farms, obtaining a total of 1300 nests equivalent to 35932 eggs for Crocoland farm.

## INTRODUCTION

The legal exploitation in Bolivia is trying to reach steady levels in the use of species and generate economical benefits that could improve the level of life in rural areas, diminishing the usual economic activities over the environment (Aparicio y Rios 2004).

Even though, the department of Santa Cruz is passing through a process of environment degradation with no precedents in Bolivia, because of the irresponsible use of natural resources without any control over the impact that it causes. That is why, Crocoland SRL. is using private inversion in order to help with the National Strategy of Environmental Conservation (Franulic 2006).

The environmental management project – Crocoland SRL, proposes an alternative way on captivity breeding and sustained management of *Caiman yacare* that could be viable ecologically, socially and economically. We also have the advantage of working with a specie that shows great endurance to commercial exploitation, and because of its economical importance, it has become in one of the best models of management (Palacios 2006).

The success in this program depends on: a) technical support and capacity to lay out the foundations for abundance, density and population structure of the specie in all the regions involved in the Management Program, b) adequate techniques of breeding and c) activities to control every program in this project.

Even though, little is known about reproductive ecology of the specie in our country. All the information could be summarized on the studies about population estimations in Beni (Liceaga et al. 2001, Hombre y Naturaleza 2001, Salvatierra et al. 2001), on TCO Tacana of the department of La Paz (Ríos 2003), of the indigenous lands and Isiboro Secure National Park (Méndez y Van Damme 2004), and the evaluations in Guarayos, Santa Cruz (Paredes y Maldonado 2003) and San Matías (Romero 2004) (Rumiz y Llobet 2005).

That is why, in order to diminish this lack of information, Crocoland pretends to establish the basic foundations of densities and the nesting potential in the authorized regions for the egg harvesting in San Matías and to improve the Yacare National Sustained Management Program with relevant information.

## METHODS

The evaluation and the trademarks of the nests on the GPS were done on February, in the property of "Cambará", "Santa María", "Santa Rosa", "Bella Vista", y "Cascabel", in the municipality of San Matías, located between 16º36' – 18º36' South, and 57º26' – 59º40' West, in Angel Sandoval county town, Santa Cruz department.

We controlled the quantity of harvested eggs in the CIRPAS indigenous communities of San Manuel, San Francisco, Cañón de Fátima, San Joaquín, y Santa Clara, during january, and the ones that were collected by Crocoland farm, according to the sell-buy receipts that the farm give to the workers.

During the field work, we count the nests over 10 days, since 6:00 to 18:00, with break periods of half an hour. The journey was done in a canoe, registering every nest on a GPS, and the biological harvesting data. We only considered the nests that were collected in the properties of "Cambará" and "Santa Maria" (Figure 1) in order to estimate nests density, because guides told us that the harvesting effort is concentrated in this region.

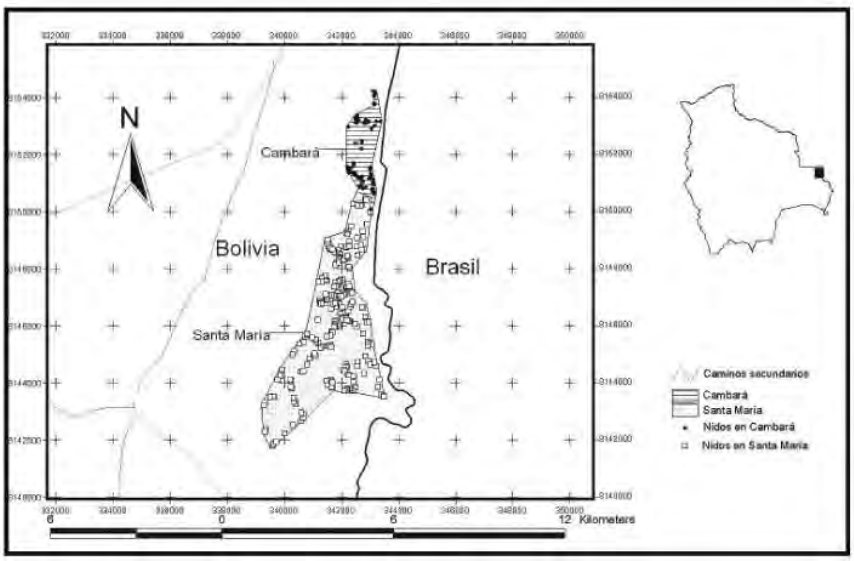



**Figure 1.** Location map of the nests that were collected during the evaluation in Cambará and Santa María properties

We calculated the surface by marking a perimeter according to all the GPS nests marks and forming a “minimal convex polygon” (Figure 2).

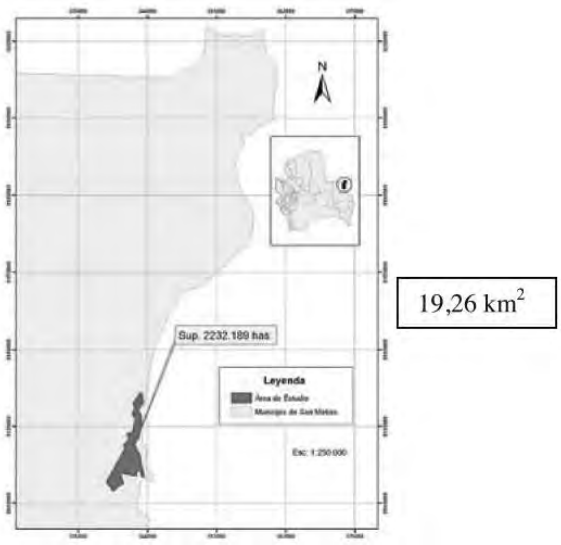



**Figure 2.** “Minimal convex polygon” that represents the total surface of the evaluation.

To calculate the “potential nesting status” in this area, we use the total surface of the permanent flooding zones reported for the region of San Matías by the UOT – BID (2002), but not including the ANMI San Matías (Integrative Management Natural Area), and we extrapolate the real densities obtained during this evaluation in the region of “Cambará” and “Santa María” (Figure 3).

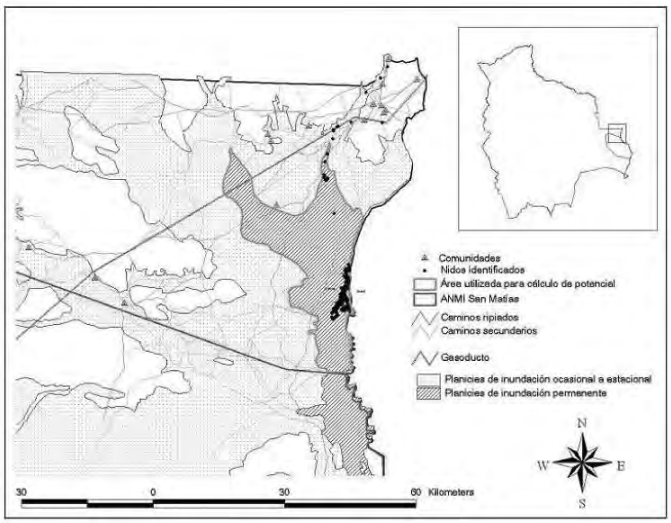



**Figure 3.** Permanent flooding zone used to calculate the nesting potential status.

RESULTS

a) Private properties evaluations:
We marked 424 nests in the prívate properties “Cambará”, “Santa María”, “Santa Rosa”, “Bella Vista”, y “Cascabel”, but the harvesting effort was focused in two of them (Table 1): “Santa María” with an evaluated surface of 16.23 km$^2$ and a density of 7.92 nests/km$^2$; and

"Cambará", with an evaluated surface of 3.03 km$^2$ and a density of 30.69 nests/km$^2$.

**Table 1.** Results for the nests counting in Cambará and Santa María.

| PROPERTY | NESTS |
|---|---|
| Santa María | 291 |
| Cambará | 93 |

We evaluated 19.26 km$^2$ among Cambará and Santa María, obtaining a final densitiy of 19.93 nests/km$^2$.

**b) Indigenous Communities harvesting (CIRPAS):**
According to the sell-buy receipts delivered to the farmers, Crocoland bought 10500 eggs, and using the mean number of 27.64 ± 4.03 eggs/nests obtained during this research, those 10500 eggs are equivalent to 379.86 nests collected in CIRPAS Indigenous Communities (Table 2).

**Table 2.** Total quantitiy of eggs bought from Indigenous communities.

| COMMUNITY | BOUGHT EGGS | EQUIV. IN NESTS (27.64 eggs/nests) |
|---|---|---|
| San Manuel | 2800 | 101.3 |
| San Francisco | 3140 | 113.6 |
| Cañón de Fátima | 1500 | 54.26 |
| San Joaquín | 560 | 20.26 |
| Santa Clara | 2500 | 90.44 |
| **TOTAL** | **10500** | **379.86** |

**c) Harvesting and eggs collection during this research:**
Depending on the characteristics of the evaluated nests, they were classified:

- Previously harvested nests: Nests with no eggs, with the nesting material removed from the upper section of the nests, and there are no biological data of the harvest.
- Viable nests: Natural nests that show no removal of the nesting material.
- Harvested nests: Nests where the eggs were removed from, and have the biological harvest data.
- Half-sunken nests: Nests with the half of their structure under the water level. They lean to be natural lost.
- Sunken nests: Nests with all the structure under the water level.
- Depredated nests: Nests with the nesting material removed. There are still not well developed embryos out of the eggshell.
- Natural hatching nests: Nests with the nesting material removed from the side and with eggshells out of the egg storage pod.

The evaluation checked 424 nests (Table 3).

**Table 3.** Classification of the nests.

| CLASSIFICATION | CANT. |
|---|---|
| Previously harvested nests | 211 |
| Viable nests | 110 |
| Harvested nests | 22 |
| Half-sunken nests | 49 |
| Sunken nests | 5 |
| Depredated nests | 1 |
| Natural hatching nests | 26 |

We registered the biological data of the 22 **"harvested nests".** The quantities are resumed on table 4:
**Table 4.** Registry of the harvest.

| REGISTRO DE COSECHA | CANT. |
|---|---|
| Harvested eggs | 608 |
| Viable eggs | 533 |
| Damaged eggs | 3 |
| Unviable eggs | 72 |

According to this results, we obtained a mean number of 27.64 ± 4.03 eggs/nests, 24.23± 5.5 viable eggs/nests, 0.14 ± 0.07 damaged eggs/nests, and 3.27 ± 1.26 unviable eggs/nests. The mean temperature of the storage pod was 32.2 ± 2.31 ºC.

**d) Evaluation of the exploitation:**
We summarized the resource development in 3 categories:

- **Effective harvesting (54.95%):** Percentage of the nests that were collected, either during this evaluation or previous harvesting works.
- **Natural hatchings (32.07%)**: Percentage of the nests that contained either viable eggs or shown indication of previous natural hatchings.
- **Natural lost (12.97%):** Percentage of sunken or depredated nests.

**e) General summary of the harvest:**
The general exploitation is summarized in the next table:

**Table 5.** Final quantity of nests and eggs harvested in the year 2008.

| COMMUNITY | HARVESTED NESTS | HARVESTED EGGS |
|---|---|---|
| San Manuel | | 2800 |
| San Francisco | | 3140 |
| Cañón de Fátima | | 1500 |
| San Joaquín | | 560 |
| Santa Clara | | 2500 |
| Santa María * | 142 | |
| Cambará * | 37 | |
| Bella Vista * | 29 | |
| Santa Rosa * | 2 | |
| Cascabel * | 1 | |

* Private properties

According to this results, and with the mean number of 27.64 ± 4.03 eggs/nests used as an "precaution equivalent number" , we competed the quantity of nests and eggs missing in the table (Table 6):

**Tabla 6.** Estimation of the final quantities of the harvest using the "cautious equivalent quantities".

| COMMUNITY | HARVESTED NESTS | HARVESTED EGGS |
|---|---|---|
| San Manuel | 101.30 | 2800 |
| San Francisco | 113.60 | 3140 |
| Cañón de Fátima | 54.27 | 1500 |
| San Joaquín | 20.26 | 560 |
| Santa Clara | 90.45 | 2500 |
| Santa María | 142 | 3924.88 |
| Cambará | 37 | 1022.68 |
| Bella Vista | 29 | 801.56 |
| Santa Rosa | 2 | 55.28 |
| Cascabel | 1 | 27.64 |
| **TOTAL** | **590.88** | **16332.04** |

In order to complete the egg harvest quota of 1300 nests authorized for Crocoland, 709.12 nests must have been harvested in the private properties of Cambará and Santa María, equivalent to 19599.96 eggs. Therefore, the final results for this year are summarized on table 7:

**Table 7.** General summarizing of the harvest – 2008.

| HARVEST AREAS | NESTS | EGGS |
|---|---|---|
| CIRPAS Indigenous communities | 379.88 | 10500 |
| Cambara, Santa María, Bella Vista, Santa Rosa, Cascabel | 211 | 5832.04 |
| Private cattle-raising properties | 709.12 | 19599.96 |
| **TOTAL** | **1300.00** | **35932** |

According to the 90% of hatching success in the farm, Crocoland has the license to breed 32338.8 hatchlings for the 35932 harvested eggs.

**f) Estimation for the nesting potential status:**
The permanent flooding areas in San Matías cover 62.706 $km^2$, and according to the density of 19.93 nests/$km^2$, we get a nesting potential status of 1250.21 nests.

That is why, the 888.12 harvested nests in the prívate properties of Cambará and Santa María represent the 71.03% of the real nesting potential estimated for the permanent flooding area, not including the ANMI San Matías (Integrative Management Natural Area).

## DISCUSSION

The most reliable information about nesting ecology of this specie in Bolivia are the studies published by Rumiz & Llobet (2005). That is why, this results would be the first ones related to abundance and nests densities in this area. This information will be used to adjusts, execute and control the future programs of the management project Crocoland SRL.

According to Yanosky (1990), the most commons nesting places are the floating vegetation (named as “yomomos”) that not only gives an adequate incubation environment for temperature and humidity, but also protect the eggs from predators and inundation. During this study, we didn´t marked de nests located inside this vegetal formations, either because of the distance to distinguish them or the difficulty to get inside this formations. It is also good to remark that the GPS marks were taken tight next to a nests, so it didn´t matter if we located a nests, we also had to get next to it in order to check it in our data. We consider that the results underestimate the real nesting potential, because of the extensive surface of this vegetal formations and the difficulty to search for nests inside them.

Another relevant aspect related to this study is the one published by Larriera (1991) who says that high mortality levels during the incubation are the predators and inundation. We think that this percentage could increase because “110 viable nests that were not harvested” are vulnerable to weather factors.

The average of 27.64 ± 4.03 eggs per nests is different from the ones published by Larriera e Imhof (2000) who found 34 eggs per nest according to their studies since 1990 to 2003, or the ones published by Piña et al. (2002) of 37 eggs per nest. Therefore, the 35932 eggs obtained from 1300 nests underestimate the real average of eggs per nest. This safety margin and the “cautious equivalent quantities” are used to control the estimation of harvested nests from indigenous communities, because farmers don´t get the biological data at the moment for the extraction, so, we don´t know the real quantities of harvested nests. By using this underestimated average, we just “estimate” the quantity of nests by using the number of harvested eggs.

### Future prospection for the exploitation according to this study.-

There are a lot experiences of captivity breeding in alligators, like the ones published by Mphande (1987) "Only the 2% of wild eggs would survive and turn into adults"; or the proposal of Mozambique presented for the last CITES meeting (Lausanne): "The percentage of survival of wild hatchling is 3% to 5%; and finally Hutton and Jarsveldt (1988) who said that the mortality of wild eggs and hatchlings could be more than the 95%". In the other side, Prado (2001) suggested that the survival percentage in wild conditions is less than the 20% because of predators and low temperatures during winter, but the survival could be more than the 90% under controlled breeding conditions.

In our case, Crocoland, in agreement with the Natural History Museum Noel Kempff Mercado, is working with a harvesting system where we can only collect the 50% of the located nests, with a maximum quota of 1300 nests. That is why, we estimate the production for this year, considering aspects of wild repopulation and the survival percentages and mortality levels that were mentioned before. We also propose new harvesting systems of exploitation

considering the natural model of survival; in other words, we take account the percentages of lizards that would have survived in wild conditions to calculate the number of lizards that would be repopulated.

**a) Wild survival model:**

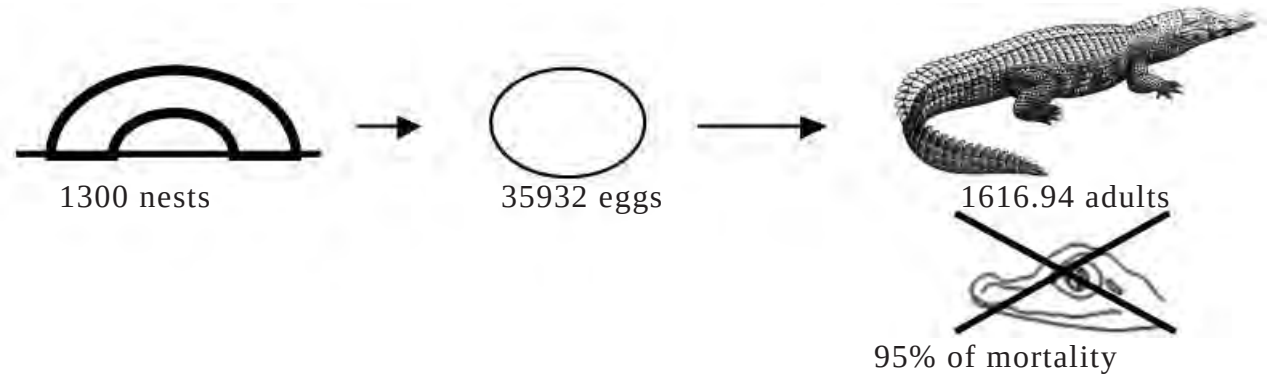



**Figure 4.** Wild survival model of 1300 nests.

According to this model, 35932 eggs are obtained from 1300 nests by using a mean number of 27,64 ± 4,03 eggs/nests, and only 5% will reach mature age because the inviability at embryonic development, weather factors, inundation and predators. Therefore, in wild conditions, we obtain 1616.94 adults from 1300 eggs.

b) Current harvesting model used by Crocoland farm.-

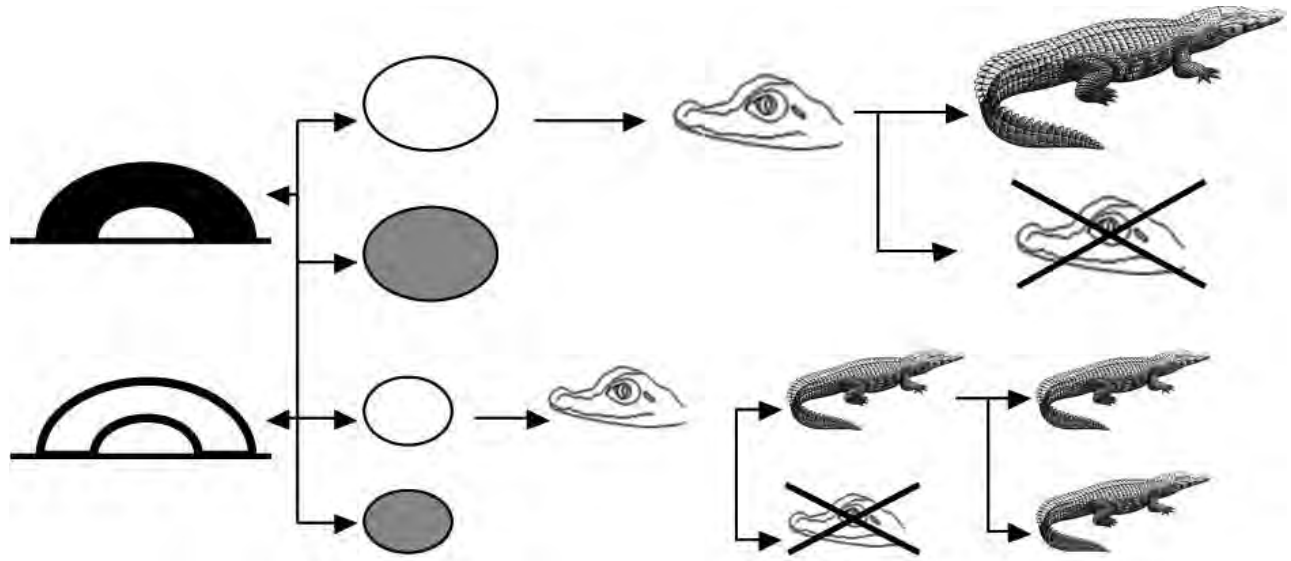



**Figure 5.** Current harvesting model in Crocoland and expectations for production.

In this model we are authorized to collect the 50% of the located nests, so, we should find 2600 nests in order to harvest 1300 nests. Under controlled consditions and acording to the resulst of this researching, we get 35932 eggs from 1300 nests; 5% shows inviability (1796,6 eggs), and another 10% shows young death (3593.2 hatchlings), therefore we obtain an 80% of success in the development of the animals that would reach mature age for the production of the farm (28745.6 adults).

From this 28745.6 adults, 1616.94 would be used to repopulate wild population, because this quantity would match with the real wild development success model. Under this system of repopulation, the quota of animals that would be repopulated takes as a parameter the quantity of the nests that have been harvested (nests as the sample unity) and the animals that would have survived and turned into adults from that quantity. So we establish the repopulation quota independently from the captivity breeding system. We consider that this is the only way to diminish to the minimum the wild impact of the harvesting. The production balance would be:

**Table 8.** Balance of the current harvesting model.

| | LOST | SURVIVAL | PRODUCTION | REPOPULATION |
|---|---|---|---|---|
| **1300 WILD NESTS** | 30721.86 | 1616.94 | - | - |
| **1300 HARVESTED NESTS** | 7186.4 | 28745.6 | 27128.6 | 1616.94 |

**c) Proposal for a new harvesting model for Crocoland farm SRL.**

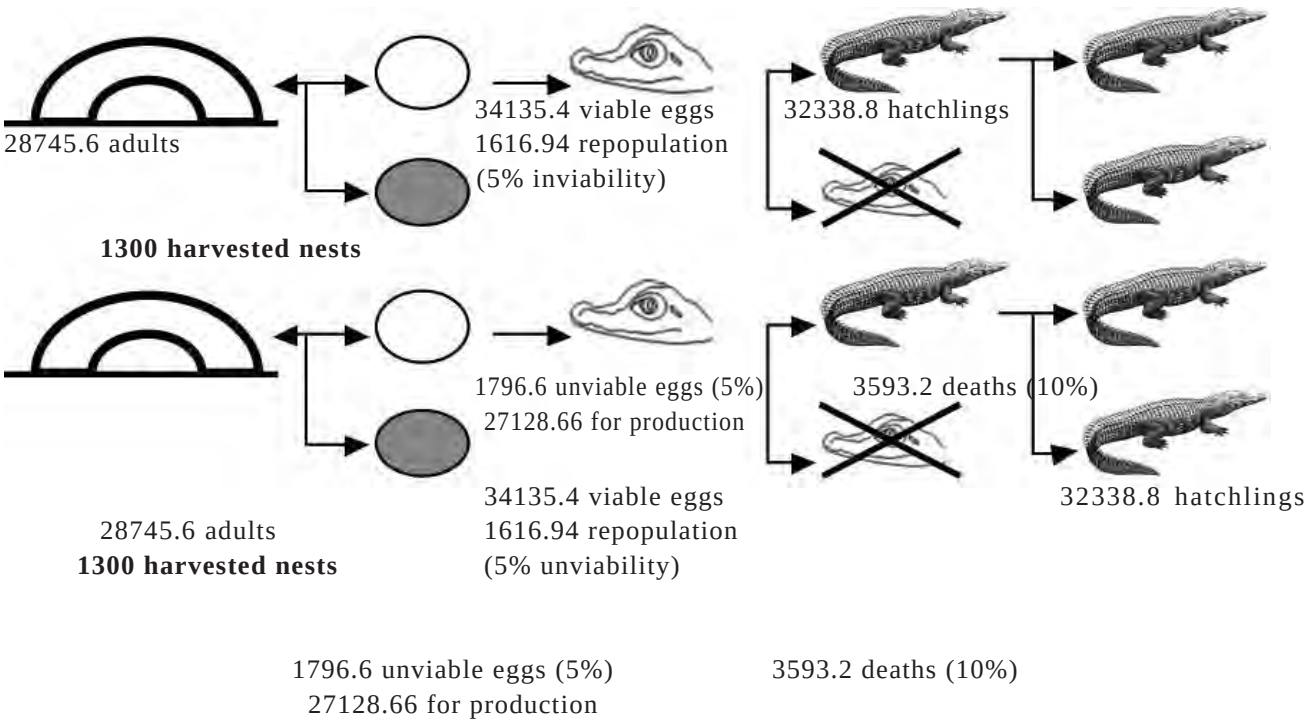



**Figure 6.** Harvesting proposal to increase production and keep stable wild population.

This proposal assumes the harvesting of 100% of the located nests, in order to diminish the searching effort, increase the production and to keep wild population with the minimum

impact as possible in the harvesting areas.

With this model we get an improvement in the balance:

**Table 9.** Balance of the new harvesting model.

| | LOST | SURVIVAL | PRODUCTION | REPOPULATION |
|---|---|---|---|---|
| 1300 | | | | |
| HARVESTEDNESTS | 7186.4 | 28745.6 | 27128.6 | 1616.94 |
| 1300 | | | | |
| HARVESTED NESTS | 7186.4 | 28745.6 | 27128.6 | 1616.94 |

## CONCLUSIONS

From 19.26 km$^2$ we get a density of 19.93 nests/km$^2$.

In this researching we harvested 22 nests, and we get a mean number of 27.64 ± 4.03 eggs/nest and a mean incubation pod temperature of 32.2 ± 2.31 ºC.

The harvesting exploitation in this study represents the 54.95 % of the total quantity of located nests, the other 32.07% are represented by the wild viable nests, and the 12.97% are the natural lost, either because of inundation or predators.

We calculate 62.706 km$^2$ of permanent flooding zones in San Matías and according to the current density of 19,93 nests/km$^2$, we estímate a nesting potential of 1250.21 nests in the entire area, but only 888.12 nests were harvested (exploitation of the 71.03%, including the harvested nests that were not found during this evaluation). This final potential does not include floating vegetation, and those areas area considered as mitigation areas for the exploitation.

For Crocoland, 1300 nests represent 35932 eggs. We expect to get 32338.8 hatchlings for this year (2008), with the responsibility to repopulate 1616.94 young-adult lizards according to the wild survival model.

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# Crocodile management, conservation and sustainable use in Latin America

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**ABSTRACT:** Latin America is one of the most diverse regions in crocodiles. The CSG Action plan in its 2dn edition recognizes 23 species around the world and in Latin America and we can find 10 of them. The status of each species in 1971 was nothing good; all are threatened or endangered, caused by over exploitation, illegal trade and uncontrolled harvest programs. Today this situation is completely different, through the conservation, management plan and sustainable use program implemented with all species. *Crocodylus moreletti* is recovering in it range distribution and is under captive breeding program. *Crocodylus acutus* is under conservation programs and in Colombia is under captive breeding and in Cuba it´s downlisted to Appendix II for commercial proposes. Only *Crocodylus intermedius* and *Crocodylus rhombifer* is not recovery completely. *Caiman crocodilus*, *C. c. fuscus*, *Caiman yacare* and *Caiman latirostris* are recovery and support wild harvest, ranching and captive breeding programs. *Melanosuchus niger* change recently the status in Brazil and would be support wild harvest. The *Paleosuchus* spp. status is unknown principally because this species do not have commercial interest. In 2006 the skins trade from Latin America was 1,000,442, dominated by Colombia with *C. c. fuscus* and very down in participation Venezuela and Bolivia. This quantity cold is change when the skins production from Argentina, Brazil and Mexico will be more open to international market, because in this moment they focus are in domestic market for Argentina and Brazil and Mexico downlisted the *C. moreletti* population.

**RESUMEN:** Latino América es una de las regiones más diversas en cocodrilos. La 2da Edición del Plan de Acción del CSG se reconoce la presencia de 23 especies en el mundo, de las cuales 10 de ellas se encuentran en Latino América. El status de esta especies en 1971 no era bueno, todas estaban amenazadas o en peligro debido a la sobre explotación, comercio ilegal y programas de cosechas incontrolables. La situación al día de hoy es totalmente diferente, gracias a la conservación, planes de manejo y la implementación de programas de uso sustentable en las todas las especies. *Crocodylus moreletii* se está recuperando y está bajo un programa de cría en cautiverio. *Crocodylus acutus* está bajo programas de conservación, en Colombia en cría en cautiverio y la población de Cuba fue transferida al Apéndice II de CITES con propósitos comerciales. *Crocodylus intermedius* y *Crocodylus rhombifer* no se han recuperado completamente. *Caiman crocodilus, C. C. fuscus*, *Caiman yacare* y *Caiman latirostris* se han recuperado y están bajo cosechas silvestres, rancheo de huevos y neonatos, y cría en cautiverio. *Melanosuchus niger* ha cambiado su estatus recientemente en Brasil pasado al Apéndice y puede soportar cosechas silvestres. Se desconoce el estatus de los *Paleosuchus* spp. debido principalmente a que no tienen valor comercial. En el 2006 el mercado de pieles desde Latino América fue de 1.000.442 unidades, dominado por Colombia con *C. c. fuscus* y con muy baja participación Venezuela y Bolivia. Esta cantidad de pieles producidas podría cambiar al abrirse al mercado internacional Argentina, Brasil y México,

debido a que en estos momentos Argentina y Brasil están más enfocados en sus mercados nacionales y México reclasifique la población de *C. moreletii*.

## INTRODUCTION

Latin America is the most diverse regions in crocodiles. The CSG Action plan in its 2dn edition (Ross 1998) recognize 23 species around the world and in Latin America we can find 10 of them (table 1).

**Table 1.** Species presented in Latin America region.

| Species | Countries |
|---|---|
| *Caiman crocodilus* | Brazil, Colombia, Costa Rica, Ecuador, El Salvador, Guyana, French Guiana, Guatemala, Honduras, Mexico, Nicaragua, Panama, Peru, Suriname, Venezuela (introduced in Puerto Rico and Cuba) |
| *Caiman yacare* | Argentina, Brazil, Bolivia, Paraguay |
| *Caiman latirostris* | Argentina, Brazil, Bolivia, Paraguay, Uruguay |
| *Melanosuchus niger* | Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru |
| *Paleosuchus palpebrosus* | Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Suriname, Venezuela |
| *Paleosuchus trigonatus* | Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname, Venezuela |
| *Crocodylus acutus* | Belize, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, Guatemala, Haiti, Honduras, Jamaica, Nicaragua, Mexico, Panama, Peru, Venezuela |
| *Crocodylus intermedius* | Colombia, Venezuela |
| *Crocodylus Moreletii* | Mexico, Guatemala, Belize |
| *Crocodylus rhombifer* | Cuba |

The status of each species in 1971 was nothing good; all are threatened or endangered (Thorbajarnarson 1992), caused by over exploitation, illegal trade and uncontrolled harvest programs. With the appearance of two institutions, the Convention of International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the Crocodile Specialist Group SSC/IUCN, institutions that help and produce guidelines to reduce the illegal trade, design strategies or tools to recover the wild populations and mechanism to control de international trade (Velasco 2005).

Now the situation is completely different, most of all species in Latin America region they status changes. The table 2 show the species status by IUCN red list and the CITES Appendix classification.

**Table 2.** IUCN and CITES Species status.

| Species | IUCN | CITES |
|---|---|---|
| *Caiman crocodilus* | Red list: Not Listed<br>(LC, Least Concern) | Appendix II,<br>except C. crocodiles apaporensis – Appendix I |
| *Caiman yacare* | Red list: Not Listed<br>(LC, Least Concern) | Appendix II |
| *Caiman latirostris* | Red list: Not Listed<br>(LC, Least Concern) | Appendix II in Argentina (Ranching)<br>Appendix I in all other countries |
| *Melanosuchus niger* | Red list: LR/cd<br>(Low Risk: Conservation Dependent) | Appendix II in Ecuador (Ranching)<br>subject to quota cero from 1997,<br>Appendix II in Brazil (Wild Harvest)<br>Appendix I in all other countries |
| *Paleosuchus palpebrosus* | Red list: Not Listed<br>(LC, Least Concern) | Appendix II |
| *Paleosuchus trigonatus* | Red list: Not Listed<br>(LC, Least Concern) | Appendix II |
| *Crocodylus acutus* | Red list: VU A.1. a. c<br>(Vulnerable) | Appendix I<br>Appendix II in Cuba (Ranching) |
| *Crocodylus intermedius* | Red list: CR A.1.c. C.2.a.<br>(Critically Endangered) | Appendix I |
| *Crocodylus moreletii* | Red list: LR/cd<br>(Low Risk: Conservation Dependent) | Appendix I |
| *Crocodylus rhombifer* | Red list: CR A.1.c. B. 1. 2c.<br>(Endangered) | Appendix I |

## Conservation and management programs

### Central America and Mexico

In Central America and Mexico, we can find 4 different species in 8 countries. The table 3 shows each country, species, programs and goals.

**Table 3.** Central America countries and Mexico species, programs and goals.

| Country | Specie | Program | Goal |
|---|---|---|---|
| Mexico | *Caiman crocodilus* | Captive breeding | Conservation |
| | *Crocodylus acutus* | Captive breeding | Conservation |
| | *Crocodylus moreletii* | Captive breeding | Trade and Conservation |
| Belize | *Crocodylus acutus* | Captive breeding | Conservation |
| | *Crocodylus moreletii* | | |
| Guatemala | *Caiman crocodilus* | Captive breeding | Local trade |
| | *Caiman crocodilus fuscus* | | |
| | *Crocodylus acutus* | Captive breeding | ? |
| | *Crocodylus moreletii* | | |
| Honduras | *Caiman crocodilus* | | |
| | *Crocodylus acutus* | Captive breeding | Trade |
| El Salvador | *Caiman crocodilus* | | |
| | *Crocodylus acutus* | Captive breeding | Conservation? |
| Nicaragua | *Caiman crocodilus fuscus* | Wild harvest | Trade |
| | *Crocodylus acutus* | | |
| Costa Rica | *Caiman crocodilus* | | |
| | *Crocodylus acutus* | | Translocation program |
| Panama | *Caiman crocodilus fuscus* | Captive breeding | Trade |
| | *Crocodylus acutus* | Captive breeding | Conservation |

Mexico

The Mexican government design The National Plan for Conservation, Research, Management and Sustainable Use of crocodiles and caiman in Mexico (Semarnap 1996). Under this National Plan, during 2002 – 2004 was developing surveys around *C. moreletii* distribution area to determinate the population status (Dominguez et al 2004). The result of this investigation is a document was summit to Fisher Wildlife Services of USA proposing a downlist in the Endangered Action List (Conabio 2005) and more recently to CITES Animals Committee proposing the transfer the specie from Appendix I to Appendix II (AC23 Doc. 18).

All species are under captive breeding program, principally for conservation goals, with the exception of *Crocodylus moreletii* that are under commercial proposes in the domestic market. For *C. moreletii*, 33 captive breeding farms we found in Mexico, but only 3 are registered in CITES.

Belize

Non official programs with crocodiles Belize have, only with *Crocodilus acutus* they have one captive breeding farm for conservation goals. With *C. moreletii* private researches are developing.

Guatemala

Three species are under captive breeding program, *Caiman crocodilus, Caiman crocodilus fuscus* and *Crocodylus acutus*. The *Caiman genus* shows a local trade. With *C. moreletii* private researches are developing.

Honduras

The government support a management plan that have like principal goals evaluate the status of *Crocodylus acutus* in the El Cajón Dan, involving the local communities in ecotourism activities (Espinal 2005). The first captive breeding farm registered in CITES is in Honduras, but not international trade is registered in this moment.

With *Caiman crocodilus* we do not have any information regarding conservation programs.

El Salvador

Any information about conservation program with crocodiles is in this country. With *C. acutus*, one captive breeding farm is in function, but the goals probably are for conservation.

Nicaragua

With *C. acutus* no conservation program is implemented. *Caiman crocodilus fuscus* is under wild harvest, until 2005 the export quota was 10.000 skins per year, in 2006 this quota reduce to 3.000 skins and the last two year is fixed in 1.500 skins per year (http://www.cites.org/eng/resources/quotas/index.shtml).

Costa Rica

No conservation program Costa Rica implemented. Until 2000 *Caiman crocodilus* export quota is in 2.000 skins per year, but now is close this trade (http://www.cites.org/eng/resources/quotas/index.shtml). With *Crocodylus acutus*, the government implements a translocation program with conflictive animals; also they have an ecotourism program around the principal rivers.

Panama

With the species *C. acutus* and *C. crocodilus* have captive breeding program. With *C. acutus* for conservation proposes and *C. crocodilus* for commercial goals.

## Caribbean

In the Caribbean, we can find 3 different species in 6 countries. The table 4 shows each country, species, programs and goals.

The status of all species in Jamaica, Haiti, Trinidad and Tobago, Dominican Republic and Puerto Rico is unknown. *Caiman crocodilus* is introduced specie in Puerto Rico and Cuba.

In the past, until 2002 conservation programs are implemented in Dominican Republic with *Crocodylus acutus* evaluation the population abundance and reproduction (Shubert & Mendez 2000 and 2002).

**Table 4.** Caribbean countries, species, programs and goals.

| Country | Specie | Program | Goal |
|---|---|---|---|
| Cuba | *Caiman crocodilus* | | |
| | *Crocodylus acutus* | Ranching | Trade and Conservation |
| | | Captive breeding | |
| | *Crocodylus rhombifer* | Captive breeding | Trade and Conservation |
| Jamaica | *Crocodylus acutus* | | |
| Haiti | *Crocodylus acutus* | | |
| Dominican Republic | *Crocodylus acutus* | | |
| Puerto Rico | *Caiman crocodilus* | | |
| Trinidad and Tobago | *Caiman crocodilus* | | |

Cuba

The Empresa Nacional para la Protección de la Flora y la Fauna (National Enterprise for the Protection of Flora and Fauna - Ministry of Agriculture) implemented the National Crocodile Program, divided in 3 sub programs, that are: a) Research and management of wild populations, b) captive management and c) environmental education (Soberon et al 2006).

Under the National Crocodile Program, are developments these researches:

1. Continuation of studies on *C. acutus* reproductive ecology and ethology, and management of crocodile nesting habitat at the Wildlife Refuge Monte Cabaniguan.
2. Follow-up monitoring of the re-introduced population of *Crocodylus rhombifer* in Lanier Swamp, Isla de la Juventud.
3. Population abundance and distribution of *C. acutus* at the Crocodile Conservation Units proposed by the Workshop American Crocodile Conservation Priorities (Gainesville 2002).
4. Genetic characterization of *C. acutus* and *C. rhombifer* Cuban populations (in collaboration with University of Habana - Faculty of Biology, WCS, Smithsonian Tropical Research Institute and Texas Technical University).

In 2008 during the CoP 13 in Bangkok, the *Crocodylus acutus* population was transfer to Appendix II, under ranching program. After the downlist the specie, the ranching program pass for different environmental success, hurricanes mostly that not permit to implement the ranching program like there wan. But the continued collecting from the wildlife and incubation in the different farms are in Cuba in 2007 with 373 hatchlings and in 2008 collected 23 nests (Regional report).

**South America**

All species are present in 12 countries, except *Crocodylus rhombifer* that endemic of Cuba. The table 5 shows for each country, the specie present, program implemented and goals. Only French Guyana and Surimane do not have any crocodile conservation program, and the *Paleosuchus trigonatus* and *P. palpebrosus* are the only species are not under conservation programs in South America countries.

**Table 5.** South America countries, species, programs and goals.

| Country | Specie | Program | Goal |
|---|---|---|---|
| Argentina | *Caiman latirostris* | Ranching | Trade |
| | *Caiman yacare* | Ranching | |
| Bolivia | *Caiman latirostris* | | |
| | *Caiman yacare* | Wild harvest, Captive breeding and Ranching | Trade |
| | *Melanosuchus niger* | | |
| | *Paleosuchus palpebrosus* | | |
| | *Paleosuchus trigonatus* | | |
| Brazil | *Caiman crocodilus* | | |
| | *Caiman yacare* | Ranching and Captive breeding | Trade |
| | *Caiman latirostris* | Captive breeding | Conservation |
| | *Melanosuchus niger* | Wild harvest | Trade |
| | *Paleosuchus palpebrosus* | | |
| | *Paleosuchus trigonatus* | | |
| Colombia | *Caiman crocodilus* | Captive breeding | Trade and Conservation |
| | *Caiman crocodilus fuscus* | | |
| | *Melanosuchus niger* | | |
| | *Paleosuchus palpebrosus* | | |
| | *Paleosuchus trigonatus* | | |
| | *Crocodylus acutus* | Captive breeding | Trade and Conservation |
| | *Crocodylus intermedius* | Captive breeding | Conservation |

| | | | |
|---|---|---|---|
| Ecuador | *Caiman crocodilus* | | |
| | *Melanosuchus niger* | Ranching | |
| | *Paleosuchus palpebrosus* | | |
| | *Paleosuchus trigonatus* | | |
| | *Crocodylus acutus* | | |
| French Guyana | *Caiman crocodilus* | | |
| | *Melanosuchus niger* | | |
| | *Paleosuchus palpebrosus* | | |
| | *Paleosuchus trigonatus* | | |
| Guyana | *Caiman crocodilus* | Wild harvest | Trade |
| | *Melanosuchus niger* | | |
| | *Paleosuchus palpebrosus* | | |
| | *Paleosuchus trigonatus* | | |
| Paraguay | *Caiman latirostris* | | |
| | *Caiman yacare* | | |
| | *Paleosuchus palpebrosus* | | |
| Peru | *Caiman crocodilus* | | |
| | *Melanosuchus niger* | | |
| | *Paleosuchus palpebrosus* | | |
| | *Paleosuchus trigonatus* | | |
| | *Crocodylus acutus* | Captive breeding | Conservation |
| Suriname | *Caiman crocodilus* | | |
| | *Paleosuchus palpebrosus* | | |
| | *Paleosuchus trigonatus* | | |
| Uruguay | *Caiman latirostris* | Captive breeding | Conservation |
| Venezuela | *Caiman crocodilus* | Wild harvest and Ranching | Trade |
| | *Paleosuchus palpebrosus* | | |
| | *Paleosuchus trigonatus* | | |
| | *Crocodylus acutus* | Captive breeding | Conservation |
| | *Crocodylus intermedius* | Captive breeding and Ranching | Conservation |

Argentina

Two species we found in Argentina, *Caiman latirostris* and *Caiman yacare*, both under ranching program for commercial proposes in different Provinces; Santa Fe with *C. latirostris*, Chaco, Formosa and Corrientes with *C. latirostri*s and *C. yacare*. Larriera *et al* (2008) show

an excellent compilation about the research, status and sustainable use of caimans in Argentina. The ranching program permit to reintroduce caimans into wildlife and from the beginning in 1991, more than 30,000 caimans of both species are reintroduced into wildlife (op. cited).

A new initiative in Formosa Province with *Caiman yacare* is develop, wildlife surveys (Sirosky 2004) are doing to evaluate the population status with the main goals to design a wild harvest for obtain skins and meat. The proposal is under revision.

Bolivia

In Bolivia occurs 5 species. *Melanosuchus niger* and *Caiman latirostris* had some private initiatives to determinate the population status in wildlife, with *Palesuchus sp*. nothing investigation was do it, and only the wild population of *Caiman yacare* is under a Conservation and Sustainable Use Program based in wild harvest from 1997 (Llobet 2004). This program is continually under changes, and the most recently are described by Llobet and Bello (2008). In 2004 began the reformulation of yacare program, and the principal results are: develop of monitoring protocols including habitat characterizations; protocols to evaluate the annual harvest; evaluation of ecological data that include surveys, harvest and habitats with administrative information based in a GIS tool and develop a criteria or baseline to design and apply experimental management plan by each locality (Rumiz & Llobet 2005). At this moment two management plan are implemented in indigenous land, eleven are finished and in evaluation processes by the Management Authority and one in progress and in 2007 implement the first experimental harvest (Llobet & Bello 2008)

From 2006 the Cites Administrative and Scientific Authority of Bolivia published a new resolution that permit to implement a ranching and captive breeding activities with *Caiman yacare* for commercial proposes (Velasco 2008).

Brazil

Is one of the most diverse crocodile country in South America with 6 species, but not all are under conservation, management or sustainable use programs. *Caiman yacare* is the oldest specie under captive breeding and ranching program for commercial proposes. *Caiman latirostris* is under captive breeding private operation in an experimental phase.

In 2003 has established a nationwide research and development programme, named Programme for Biology, Conservation and Management of Brazilian Crocodilians (Coutinho & Luz 2008). The goals are determinate the population status through surveys, estimating size structure and sex ratio, habitat characterization, reproductive biology and nesting ecology.

More recently, in CoP 14 the *Melanosuchus niger* population was transfer from Appendix I to Appendix II and the government would be implement a wild harvest in Sustainable Development Reserves (Ibama 2007). The main goal of this program is obtain skins and meat.

Colombia

Of all species only the *Paleosuchus* spp. is not under conservation program. *Crocodylus intermedius* Conservation plan is under revision by the Ministerio del Ambiente, Vivienda y Desarrollo Territorial (Ministry of Environmental, Housing and Territorial Development - MAVDT), at this moment the activities is a captive breeding program. *Melanosuchus niger* have also a conservation program from 1996, but not many action are made. In this moment is under review and redesign the conservation plan.

*Crocodylus acutus* the conservation program is under revision, especially the captive breeding farm relation with the conservation strategies. In other hand, the Cispata project continued implemented through the eggs collection and realizing juveniles in natural habitat, population survey, biological research and local people participation (Ulloa & Sierra 2006).

In relation with *Caiman crocodilus crocodilus* and *Caiman crocodilus fuscus*, are the two species under captive breeding program since 1980. Medrano & Gómez (2008) publishing the history of genus Caiman conservation and harvest, make emphasis in a big description of all processes regarding the use of *Caiman crocodilus fuscus*. In 2007 the MAVDT began to design a National Programs for Conservation and Sustainable Use of Crocodylia in Colombia.

Recently, two the Regional Autonomous Corporations are implementing a conservation program, Atlantico (CRA 2006) and Del Sur de Bolivar (Mercado & Palacios 2006). The main goal is the local population participation in growing hatchling for realize in wild habitat evaluation the population status and habitat characterization to design conservation plans for *C. c. fuscus.*

Ecuador

The population of *Melanosuchus niger* is in Appendix II to implement a ranching program with quota cero for commercial proposes. This program present during the time, different problems that not permit to be successful, was analyses by Hines (2002). In 2008 the ranching program is closed.

With the other species (*Caiman crocodilus, Crocodylus acutus, Paleosuchus trigonatus* and *P. palpebrosus*) only private initiatives are develop.

French Guyana

Private initiatives are develop with *Melanosuchus niger* through captive breeding programs and ecotourism activities.

Guyana

Only *Caiman crocodilus* are under a conservation program through wild harvest.

Paraguay

In 1996 began the wild harvest program with *Caiman yacare*, until 2003, year that Paraguay implemented a voluntary moratorium on the utilization of wildlife species, following recommendations from the CITES Secretariat. After this decision, the government are design a management plan for crocodiles species in the country.

Aquino & Scott (2008) present an excellent paper about the history and dynamics of crocodiles management in Paraguay, with emphasis in *Caiman yacare*, showing the mistake and propose mechanism to solve the situation.

Peru

In this country 6 crocodiles species occurs, but only have a conservation program with *Crocodylus acutus* with a captive breeding facilities in Tumbes region. This farm was under the guide by the government, but knows the manage in a local university close the facilities and the new goals would be presented in the 19 Working Meeting of CSG through 2 papers.

Uruguay

Is the minor diverse crocodile country in South America, with one specie *Caiman latirostris*. A private initiative is implemented; the farm "Criaderos de yacare Cerros Azules" began its activities 6 years ago with *Caiman latirostris*. The main goals are reproduction of the species and the reintroduction of hatchlings into wild habitat, and in the future to produce skins and meat for commercial purposes. It is a private initiative which now has Government support and permits. At this stage breeding stock consists of 20 adults, in addition to some 330 other caimans. To date they have reintroduced about 100 caimans into the wild. This program has the governmental support.

Venezuela

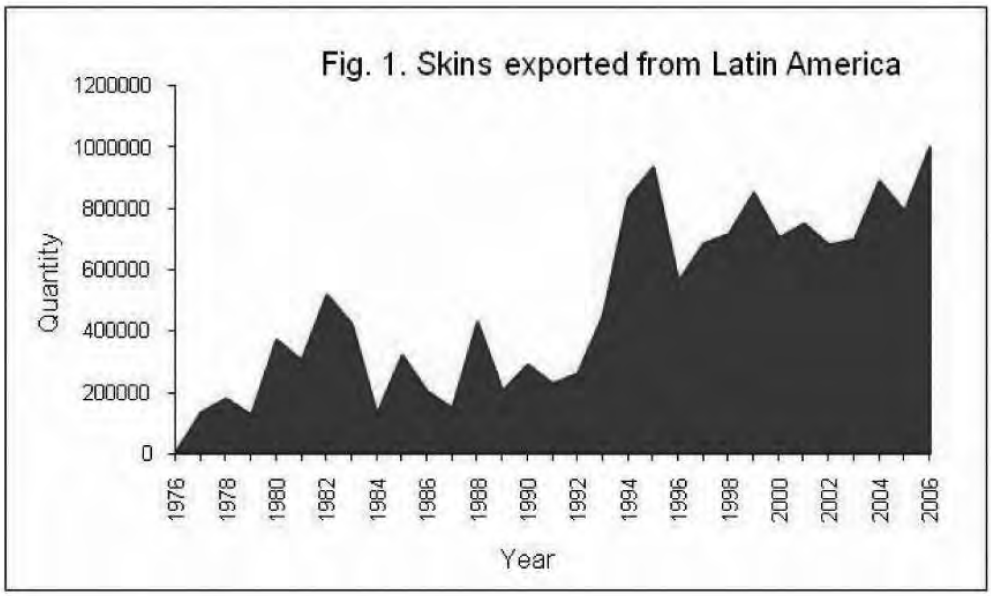
Only the *genus Paleosuchus* is not under a conservation program. With *Crocodylus acutus* and *Crocodylus intermedius*, the government implements the National Plan for Conservation both species (MARN 2003) jointly with different NGO´s, universities and the private sector. The main goal of both national plan for conservation is "Recovery the wild populations through eggs collection, growing in captivity for reintroduced the juveniles into natural habitats, involving farms and local people. In 18 years (1990 – 2007) 5,620 Orinoco crocodiles was realize in 14 different localities and one of them in Caño Guaritico Wildlife Refuge in Apure State, is recognize like a new population (Ayarzaguena et al 2007). The last two year the Ministry of Power Popular of Environment began to collect hatchling in two different areas to growing and reintroduced in natural habitat (Babarro in press). With American crocodile, from 1996 to 2007, 628 crocs were reintroduced in 9 natural localities.

*Caiman crocodilus* is under wildlife harvest program from 1986 and is the oldest sustainable program in South America. The annual harvest is based on the old male caimans (Class IV) longer that 1.80 total length (Velasco & De Sola 1999). The harvests on the natural populations have produced an improvement in the population characteristics, such as their abundance and structure of sizes classes, verified by continuous populations monitoring under harvests,

and comparing them with populations not harvest, which shows the sustainability (Velasco *et al* 2003).

**Skins production and trade**

Latin America is the region in the world that produces more skins to the international market. In 1999 the world production was estimate in 1,179,539 skins and from Latin America are 851,326, which represent the 72.14% of the total export. Now the situation is not different, the figure 1 shows the skins exported from Latin America from 1976 to 2006.

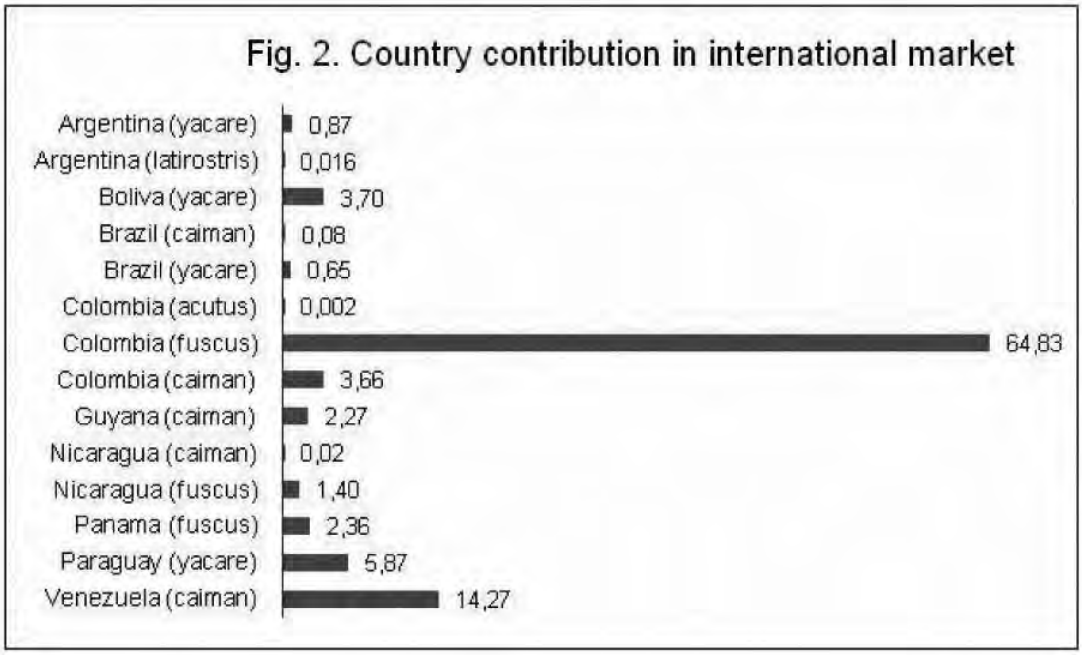


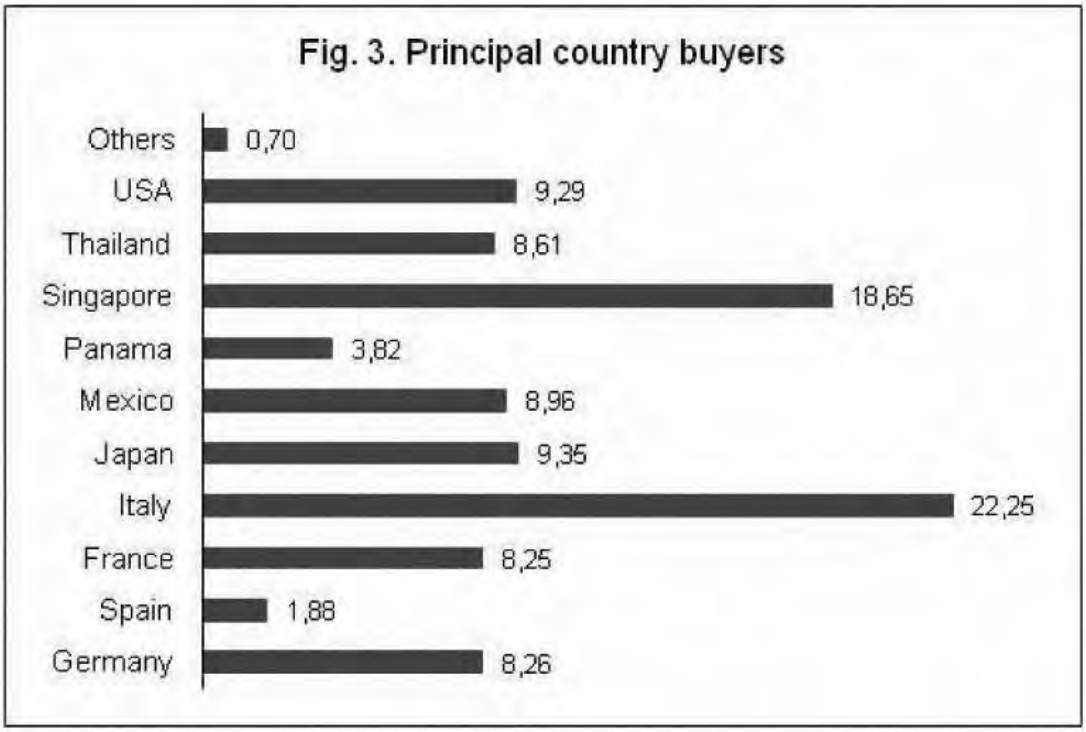

The tendency is to increase and shows three periods. The first between 1976 to 1983 where all programs began after the creation the Convention for International Trade of Endangered Species of Wild Fauna and Flora (CITES) and finalizing when Venezuela open the wild harvest program. The second period (1983 to 1993) the skin production is reduced had ban implemented in Paraguay and Panama y stop Colombia production. The principal countries that contributed are Guyana and Venezuela. The last period (1994 to 2006) the increase is must the captive breeding production in Colombia with *Caiman crocodilus fuscus* and lest percent Venezuela and reopen all program in the region, Paraguay with *Caiman yacare*, Panama with *C. c. fuscus*, Nicaragua with *C. crocodilus* and Brazil with *C. yacare*.

The figure 2 shows the country contribution into the international skins market in all time.

Colombia is the principal country in Latin America in skin production and international market with 68.48% of total (*C. c. fuscus* 64.83% and *C. crocodilus* 3.66%). Venezuela occupied the 2nd place with 14.27% and so far a small group integrating by Paraguay (5.87%), Bolivia (3.70%) and Guyana (2.27%) all skins coming from wild harvest.

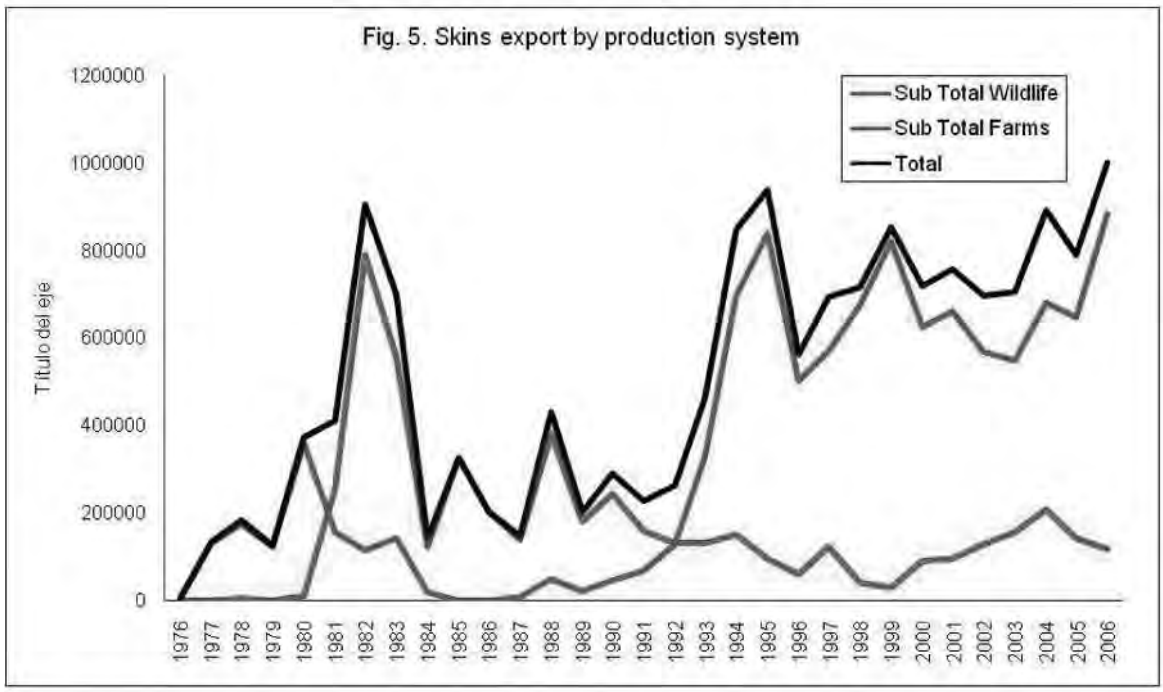
The country buyers is more diverse (figure 3), where Italy buy the 22.25% of Latin America production, followed by Singapore with 18.65%, where 98.67% coming from Colombia; and so far we see other country group integrate by Japan (9.35%), USA (9,29%), Mexico (8.96%), Thailand (8.61%), Germany (8.26%) and France (8.25%). Of this group Thailand and Mexico buy more skins from Colombia, the other is a mixes of all Latin America countries. The rest of production to international market is integrating 19 countries around the world.




The most important observation in this comment is that Asian market is dominated by Colombia skins, and the Europe market is it a mixture where Colombia has around the 50% of the skins sales.

The figure 5 shows the Latin America skins production by type: farms that include captive breeding and ranching systems versus wild harvest.




Three phases we observe in this figure; the first between 1976 to 1980 where all skins coming from Colombia and we estimate is a captive breeding production because in WCMC data base do not specify the origin. The second phase is dominated by wild harvest from 1981 to 1992, principally from Venezuela and Guyana, and some skins from Colombia of both sources (Captive and wild harvest). The last phase is clear dominated by Colombia captive breeding production. The wild harvest reduction is this phase is a consequence of different factors: Venezuela, Guyana and Nicaragua reduce its production and Paraguay moratorium.

In 30 year the skins market produce for Latin America 15,678,466 skins, where farms system is 10,607,435 (67.66%) and wild harvest is 5,071,032 (32.34%). This analysis does not include the skins used for domestic market.

The author tries to estimate the total incomes from the international market. But it is necessarily clarify how we do this estimation. In the case of wild harvest skins, we were based in an annual Venezuela price. Normally for the other countries with wild harvest their price is little low, between 2 to 4 US$ less. In relation of farms skins cases, we used the Colombia prices and applied to all countries.

The figure 6 shows the total net incomes and for production system. farms systems produce more money because put into the market more skins that wild harvest. In 30 year the skins market produce for Latin America 712,890,809 US$, where farms is 429,014,901 US$ (60.18%) and wild harvest is 283,875,908 US$ (39.82%). This analysis does not include the benefits for domestic market. In 2006 the net incomes was around 1,000, 442 US$, where 883,833 US$ (88.34%) from farms system and 116,609 US$ (11.66%) from wild harvest. When Argentina, Brazil and Mexico began to introduce more skins this situation would be change more drastically, because their production coming from ranching in Argentina and Brazil and captive breeding in Mexico.

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To all countries that prepare and send the Annual Cites reports. To WCMC for permit to use the trade date.

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# Toward the sustainable use of the *Crocodylus acutus* for local communities in the swamps of the Cispata bay, Caribean of Colombia

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**ABSTRACT:** the present Project sought, to formulate a pilot conservation strategy for the *Crocodylus acutus*, in the swamps of the Bay of Cispatá, old Mouth of the Río Sinú, in the Department of Córdoba-Caribbean of Colombia. With the combination of investigation activities, tracking down and monitoring, in their initial stages and with the formulation of a management plan for their later implementation, it is considered that it will be possible to cooperate to the conservation of the species, also in the local and the national level. The Program has the leadership of the Regional Autonomous Corporation of the Valleys of the Sinú and San Jorge, (CVS), and in the beginning by the "Proyecto Manglares" of the Ministry of Environment Housing and Territorial Development. Later on it has had the support of the Institute Alexander von Humboldt, FONADE, Conservation International Colombia (C.I.), Fundación Natura, Agrosoledad S.A., Zoben S.A. and Gaber S.A. with the help of these institutions and inside the activities developed between 1999 and 2008, it has stood out in the regional, national and international environment, the conformation of an association (ASOCAIMAN), integrated by a group of 18 old well-known hunters as "caimaneros" that have become the helpers in the conservation of the caiman of the Magdalena (*Crocodylus acutus*), of the Bay of Cispatá. The goal that is projected, after several investigation phases, will be based on the sustainable management of this resource, on the part of members of the local community, contemplating some of the conservation components suggested by several specialist for the crocodilians, like: (1) censuses, (2) recovery programs, (3) monitoring (4) biological Studies (5) mechanisms of caution (6) local benefits (7) agreements (8) control of the traffic, and (9) economic benefits.

**RESUMEN:** Con el presente Proyecto se pretende, formular una estrategia piloto de conservación para el *Crocodylus acutus*, en los manglares de la Bahía de Cispatá, antigua Boca del Río Sinú, en el Departamento de Córdoba-Caribe de Colombia. Con la combinación de actividades de investigación, seguimiento y monitoreo, en sus etapas iniciales y con la formulación de un plan de manejo para su implementación posterior, se considera que se podrá coadyuvar a la conservación de la especie, tanto en el ámbito local como nacional. El Programa ha sido liderado por la Corporación Autónoma Regional de los Valles del Sinú y San Jorge, (CVS), y en la parte inicial por el Proyecto Manglares del Ministerio de Ambiente Vivienda y Desarrollo Territorial. Posteriormente ha contado con el apoyo del Instituto Alexander von Humboldt, FONADE, Conservación Internacional Colombia (C.I.), Fundación Natura, Agrosoledad S.A., Zoben S.A. y Gaber S.A. Con ayuda de estas instituciones y dentro de las actividades desarrolladas entre 1999 y 2008, se ha destacado

en el ámbito regional, nacional e internacional, la conformación de una asociación (ASOCAIMAN), integrada por un grupo de 18 antiguos cazadores conocidos como ¨caimaneros¨ que se han convertido en los coadyuvadores en la conservación del caimán del magdalena o caimán aguja (*Crocodylus acutus*), de la Bahía de Cispatá. La meta que se proyecta, después de varias fases de investigación, se basará en el manejo sostenible de este recurso, por parte de miembros de la comunidad local, contemplando algunos de los componentes de conservación sugeridos por varios especialista para los crocodílideos, como son: (1) Censos, (2) Programas de recuperación, (3) Monitoreo (4) Estudios biológicos (5) Mecanismos de precaución (6) Beneficios locales (7) Acuerdos (8) Control del trafico, y (9) Beneficios económicos.

# Action Plans for Conservation of Babilla (*Caiman crocodilus fuscus*) in the marshland complex B15, municipalities of Achi, Altos del Rosario, Barranco de Loba, Pinillos, San Martín de Loba and Tiquisio, in the jurisdiction of the Autonomous Corporation of South of Bolivar (CSB), Colombia

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**ABSTRACT:** The Colombian Environmental Ministry (MAVDT) through Resolution 1660 of 2005, mandates that the Regional Autonomous Corporation (CRA) implement Conservation Actions Plans for the Babilla (*Caiman crocodilus fuscus*).
In the jurisdiction of the South Bolivar Corporation (CSB) the most important marshland complex of the country, the "Momposina Depression", represented by 6,036 km2, wetlands divided into 11 complexes.
From November of 2007 in complex B15 the conservation plan for the species was initiated; according to the directives of the National Plan for Crocodiles Conservation in Colombia. Initially three areas were selected out of 36 identified for the zone; in April 2008 another three will be chosen. In these six regions babilla populations and habitat will be monitored so that by the end of this year we will obtain results of the crocodiles census and an estimate of the population.
Also we will execute programs of Environmental Education and Institutional Building, with a view to creating and sensitizing the communities, and to generate attitude changes. The financing of the plan is from collaboration between the environmental authority and private crocodile farms. The MAVDT will contribute 74% of the resources and the remaining 26% from money collected from farmers for quotas for repopulation.

# Sustainable Use Program for *Caiman yacare* in La Estrella Swamp, Formosa Province, Argentina.

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**ABSTRACT:** Monitoring studies have indicated that the *Caiman yacare* population within La Estrella Swamp is abundant (>200 caimans.km$^{-1}$ in some places). Although there is currently no management plan in place for the species, consideration is being given to the wild harvesting of adults to produce leather and meat, and add extra value to the ecosystem. Such a program would encourage the use of the resource in a sustainable way, and provide additional income for local inhabitants. Survey methodologies for this program have been designed based on recommendations of IUCN-SSC CSG for management and use of wild populations, including night and day surveys, and questionnaires to local people. Harvesting quotas (15% of Class IV animals) will be based on monitoring. Each year, before the hunting season, the area will be survey to estimate the numbers of caimans in each size. The harvest season will be timed to occur before the reproductive season, but once the water lever is reduced (September to October). The program will allow harvesting of caimans 200 cm or longer in length, but will accept up to 20% of the quota being animals 180 and 199 cm. Where meat is to be used for human consumption, hunters must provide the program supervisors the animals they harvested that night at a specific point. Animals will be then be transported in refrigerated trucks to a processing facility.

# Integral use of *Caiman yacare*: driving to sustainable use in Bolivia.

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**ABSTRACT:** Since its creation, the Program for Conservation and Sustainable Use of *Caiman yacare* in Bolivia has focused in the use of leather. From 1999 to 2007 the use of 301937 individuals was authorized, from which were commercialized: 583453 flanks, 257772 tails, 17126 kg of meat and only 400 skulls. Conscious about that the lack of use of by-products that could have interesting markets, and aware about that the Management Plans are developing strategies to increase value and to obtain some by-products, we made an analysis about certain key factors, that could avoid the development of these processes: influence of market, difficulties of gathering and transportation of by-products, character of hunting (in a organizational approach), economical incentives, capabilities of negotiation, transparency in the organizations and other related to the use of illegal means. The new policies stimulate to that the benefits generated by natural resources should include a just and equitable distribution; if not, without an integral utilization local participation would be restricted, and the benefits only would be focused to a small number of actors creating gaps of gender, social status and above all, opportunities of conservation for this resource.

**RESUMEN:** Desde su creación, el Programa de Conservación y Aprovechamiento Sostenible de lagarto en Bolivia, se ha centrado el aprovechamiento del cuero. Se ha autorizado el aprovechamiento de 301937 lagartos, de los cuales se comercializaron 583453 flancos, 257772 colas, 17126 Kg de carne y sólo 400 cráneos. Concientes del desperdicio de toneladas de subproductos que podrían contar con mercados interesantes y que la ejecución de Planes de Manejo contemplan estrategias para agregar valor y obtener ciertos derivados, se realizó un análisis de los factores determinantes que podrían obstaculizar el desarrollo de estos procesos: la influencia de mercado, las posibilidades de transporte y acopio de subproductos, características de la cacería en cuanto a su organización, incentivos económicos, capacidades de negociación, de la transparencia en las organizaciones y otros relacionados a las vías ilegales de aprovechamiento. Las nuevas políticas impulsan a que los beneficios generados por los recursos naturales incluya una distribución justa y equitativa, al no existir un aprovechamiento integral, se restringe la participación local y se deja los beneficios a un número reducido de actores que forman parte de la cadena de valor creando brechas de género, de condición social y sobre todo de oportunidades de conservación para este recurso.

# Conservation Program, sustainable use & management of some wetlands in the State Of Atlántico, Phase II. Corporación Autónoma Regional Del Atlántico (CRA)

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**ABSTRACT:** Since 2004 the *Corporación Regional del Atlántico* (CRA), develops the conservation program sustainable use and management of its wetlands. Its principal strategy is the reintroduction of thousands of babilla individuals (*Caiman crocodilus fuscus*), respecting the structure of the natural populations. The program covered 8 wetlands of the state (c.a 24.000 ha) and involved 13 communities and according to the results it has adjusted, consolidating some communities, and proposing the integration of other species of the wetland to the management proposed by the CRA. The private enterprise (captive breeding) suministrated the hatchlings (13819) and subadults (5289). The first were delivered to 138 women of the communities, who took care of them for a year, receiving 0.30 US per each animal kept in good conditions; the second were released in the wetlands after following the appropriate protocols. The monitoring of the released specimens are carried out by the fishermen, the initial results determine a good adaptation of the individuals, established by its physical condition, growing, and rising of the nesting rates where before were scarce. Socially, the communities have appropriated of program, strengthening their sense of belonging and acceptation, of a specie which previously was unimportant or undesirable. In the future we look forward for the proper use of some of the specimens born from the previously released ones.

**RESUMEN:** Desde el 2004 la Corporación Autónoma Regional del Atlántico (CRA), desarrolla el programa de conservación manejo y uso sostenible de sus humedales. Su principal estrategia es la reintroducción de miles de individuos de babilla (*Caiman crocodilus fuscus*), respetando la estructura de las poblaciones naturales. El programa cubrió 8 humedales del departamento (c.a. 24.000 ha) e involucró a 13 comunidades y de acuerdo con los resultados se ha ajustado, consolidando algunas comunidades, y proponiendo la integración de otras especies del humedal al manejo propuesto por la CRA. La empresa privada (Zoocría) suministró los neonatos (13.819) y subadultos (5.289). Los primeros se entregaron a 138 mujeres de las comunidades quienes los cuidaron durante un año, recibiendo US 0.30 mes/animal mantenido en buenas condiciones; los segundos fueron liberados en los humedales luego de cumplir los protocolos apropiados. Los monitoreos de los ejemplares liberados son realizados por los pescadores, los resultados iniciales determinan una buena adaptación de los individuos, establecida por su condición física, crecimiento, y aumento de anidaciones donde antes eran escasas. Socialmente las comunidades se han apropiado del programa, afianzando su sentido de pertenencia y aceptación, a una especie que anteriormente les era indiferente o indeseable. En el futuro se proyecta, el aprovechamiento de algunos de los individuos nacidos de los ejemplares liberados.

## INTRODUCTION

Since the year 2004 the Corporación Autónoma Regional del Atlántico (CRA), develops the conservation, sustainable use and management program in its wetlands. Its main strategy is the reinsertion of thousands of babilla individuals (*Caiman crocodilus fuscus*), coming from the repopulation quota established in the Colombian law which corresponds to the 5% of the annual production of each farm. In practice, not all of the individuals obtained in the repopulation are released, many of them were negotiated with the entrepreneurs as a previous agreement with the environmental authorities. There are several reasons for this, but they mainly correspond to the high costs of the release logistics and the characteristics of the animals in matters of the morphometrics, sex and sanity. Many of the farm animals are only males because the farms program their incubators to obtain only this sex looking forward to greater and faster growth.

The general objective of the project has been: to establish a conservation, sustainable use and management program of some wetlands in the state of Atlántico through activities that allow the organizing of the environmental offer of the system, to improve the quality of life of the communities that depend on them.

Initially there are 6 municipalities involved in the state and the activities are carried out with 13 communities in 8 wetlands.

**Table 1.** Municipalities, communities and wetlands where the conservation program initiated. The presence of the communities and the reached areas have a coverage wider than the 60% of the state wetlands.

| MUNICIPIOS | COMUNIDADES | HUMEDALES |
|---|---|---|
| Repelón | Rotinet | Embalse del Guájaro |
| Sabanalarga | Repelón | Ciénaga de Tocagua |
| Sabanagrande | Aguada de Pablo | Cienaga de Luruaco |
| Santo Tomás | La Peña | Ciénaga Convento |
| Luruaco | Villa Rosa | Ciénaga de Santo Tomás |
| Ponedera | San Juan de Tocagua | Cienaga Luisa |
| | Luruaco | Ciénaga Uvero |
| | Malambo | |
| | Sabanagrande | |
| | Santo Tomás | |
| | Palmar de Varela | |
| | Ponedera | |
| | Santa Lucia | |

Until now there have been 14,306 individuals, 5,254 sub-adults and adults in sizes comprised between 90 and 120 cm long aiming to do a following and study the reflected adaptation in the reproductive events, and the corporal state. Additionally there were 9,052 hatchlings, in sizes comprised between 25 to 40 cm, delivered to 138 women in the community so they could rear them in concrete pens installed in the backyards of their houses. After 12 to 18

months of management the individuals were in a range between 60 to 85 cm. They were evaluated, selected and released after accomplishing the appropriated protocols. The women received US 0.30 month/animal kept in proper conditions, which provided them up to 30 US per month.

## METHODOLOGY

The whole program was elaborated designing 4 main strategies which complemented each other so there could be a proposed management of the resources by means of the community.

### 1. Strategy for the recuperation and habilitation of the wetland.

a) Definition of the water bodies.
b) Identification of the main causes of the deterioration and loss of biodiversity through the application and analysis of surveys.
c) Minimization of the polluting loads poured directly to the water bodies and control of their sources, with the purpose of guaranteeing the proper quality of the hydric resources.
d) Reforestation of the tributary micro-basins and the water bodies and its cushion zones
e) Arrange a conservation plan for the cynegetic species of the area of influence of the water bodies.

### 2. Strategy of action- participation

a) Reforestation of the tributary micro-basins of the water bodies and its cushion zones.
b) Cleaning, dismantling and recovery of the access canals in the water bodies and the contention walls.
c) Breeding of hatchlings. To develop this task several housewives from the communities were trained and organized, this is how in the backyard of every house a concrete[1] mobile pen was installed with the capacity to house 100 individuals for ten moths.
d) Verification of the nesting areas: fishermen were trained in the recollection, transport of nests and *ex situ* management.
e) Construction and management of the incubator: this activity has not been developed yet.
f) Monitoring and following of the released individuals. Interested members of the community were trained and interviewed so they could identify, capture and measure the individuals in night journeys. The payment was established as working days.

### 3. Strategy to identify the factors that cause damaging and loss of biodiversity of the studied wetlands.

a) Swamp characterization and its terrestrial areas.
b) Study of the species' population status.

[1]Design developed and approved by the experimental Project in Gambote, Bolívar. Fundación BIODIVERSA.

**4. Strategy of repopulation and/or reprovision.**

a) Selection of the people in charge of the hatchling breeding.
b) Construction of the needed infrastructure for the hatchling management.
c) Determination of the ecosystem's carrying capacity.
d) Training in the individual management.
e) Morphometric and population following of the managed individuals in confining.
f) Establishment of a census and preliminary structure of the resident babilla population.
g) Establishment of the geographical origin of the individuals to release.
h) Obtaining the individuals to release, sex identification and size establishment.
i) Physical and sanitary analysis of the individuals to release.
j) Establishment of the quarantine and complementary measurements if necessary.
k) Marking of the individuals and training of the community in this activity.
l) Marking through scale cut.
m) Release and following of the individuals.

## RESULTS

### Animal selection criteria

The selection and marking process of the animals was done by choosing healthy individuals, those who did not present conjunctivitis, skin diseases, respiratory infections, malnutrition and malformations.

The delivered sub-adults have a size comprised in the range of 90 to 120 cm long in total and the selected hatchlings oscillated in sizes comprised between 25 and 40 cm long.

### Introduction of invasive or alienigen species.

One of the mistaken alimentary politics in many countries shows in the promotion and introduction of foreign species hoping to obtain great amounts of bio-mass that help reduce hunger in its regions, using as arguments the high reproductive or growing rates of certain fish species. This is how species like the trout, salmon (Salmo spp, Onorhynchus spp), tilapias (Oreochromis spp, *Sarotherodon spp*, and *Tilapia*) perches (*Perca spp*) amongst other species arrived to tropical countries, species which once installed in their new habitats dedicate to compete without opponent for food, devouring the native species and becoming potential plagues that diminish the natural biodiversity in the areas, and in many occasions they cause the extinction of valuable species. In our case, we find the transplant of an endemic fish from the Orinoquía and Amazoía called cachama (*Colossoma spp*) which develops well in the water bodies where it's released, because it easily replaces fruits and seeds that obtains in the flooding forest and that are its natural diet for an omnivore one. Nevertheless in the Caribbean lakes these flooding processes do not take place and the species has to feed from anything it finds which can replace its natural nourishment. In the studied wetlands we have analyzed the stomachs of some of these fish and found that their main content corresponds to the apple snail (*Pomacea spp)* which is an important feeding *item* of the babilla during its whole life, but mainly during the first months. Until now we can't relate the presence of the cachama with the diminishing of the snail population but we can evidence their fierce appetite for this prey. The studies will establish the potential risks

of the introduction of this specie, not only for the babilla populations, but also to the Snail Kite (*Rostrhamus sociabilis*) and in general for the stability and well functioning of the wetland because the diminishing of the snail populations would ease the expansion of the aquatic vegetation (*Eichornia spp*) from which the *Pomacea* feed, accelerating the eutrophication of the wetlands.

**Released individuals**

The sex relation, temporally established amongst the released individuals, was of 1.6 males per female. During the next months the proportions to the ones found in nature, 1/1 male-female, will be adjusted.

The following information comes from the swamp San Juan de Tocagua where the greatest amount of information was found and the best understanding from the community was acquired. The captured individuals presented a range between 0 and 4.2 cm, after 74 and 183 days released (n=35), amongst this data, the males had an average growth rate of 1.76 cm and the females 0.69 cm and the total for the sample was of 0.87 cm. In general there were observed healthy animals, together with others adapted to the environment.

In relationship to the size of the nesting's, there could be established that from a total of 20 nests collected, 16 have between 17 to 20 eggs. Which coincide with the size of the females and their age, because most of them correspond to young females, just as the number of fertile eggs per nest (n= 20), with a range of 14 to 23 fertile eggs per nest, allows to assume a good relation of sexes, because it complements with the fact that 15 nests (n=20) did not present sterile eggs.

**Nests record in San Juan de Tocagua**

1. In the year 2006 in June there was a nest reported in the swamp of Tocagua.
2. In the year 2007 and subsequently to the releases of the individuals of the conservation program, a nest was reported in June, 10 nests in July and 9 in August.
3. In the year 2008 20 nests were reported in June without counting August, September, and October to end the season, which presents us a favourable outlook to the middle-term development of a management plan of the specie by the community.

**Survival of the hatchlings delivered to the protector mothers for their care.**

In the municipality of Santa Lucía the total survival rate was of 23.8%, this can be attributed to the lack of experience of the community in the handling of the animals, situation that was solved in the other programs in Palmar de Varela and San Juan de Tocagua where the survival rate were of 80% and 89.2%, respectively.

**Variation in the number of beneficiaries of the project.**

The beginnings of the program were as ambitious as expected, however in the rural realities of the underdevelopment in which they struggle, with equal magnitude, traditions, unsatisfied basic needs, money in the hands of the landowners, political interests, mistakes in the law and norm expeditions, together with the indifference of the government make the development of a program that combines the theoric expectations with the practical expected results almost impossible to accomplish. This is why in Colombia, with counted exceptions, wetlands are seen as more than potential areas to agriculture that areas destined to the preservation and the sustainable use of the biodiversity that lay there. Just as in many other regions of the planet, the wetlands are transformed into agricultural lands and then into livestocking areas through a process of dryings and sedimentations done artificially by the members of the same communities that, encouraged by the landowners after offering a generally bad paid job, accomplish their objectives. The constant changes in the political environmental interests of the administrative entities of the biodiversity turn administration priorities to forgotten processes when changes appear in the directors of those entities, unless several of these variables get together with the same interests to accomplish this objective. In the case of the Corporación Regional del Atlántico (CRA), the orientation of the wetland conservation program has kept the same with a few changes since the year 2004 thanks to the firm conviction of the private enterprise (captive breeding farms) and the directors of the corporation, who have seen in the sustainable development a valuable element to achieve the welfare of the communities and improve the quality of life by the intelligent use of the biodiversity. This also resumes in an efficient social control that can become an important political capital in the take of decisions and in the continuity of the regional environmental politics.

The program initiated in the year 2004 directly benefiting 351 members of the community, in the year 2007 this number decreased to 193, and nowadays there are 150 people, from two communities being directly benefited. Some of the reasons for this decrease were mentioned above, even when the vocation of the communities also counts in this process, the closer they are to the big urban centres, the harder it is to keep the interest for the sustainable management of a biodiversity that they don't know any longer and with the one they relate in a distant way. As a matter of fact, this evaluation was part of the job, and its initial results allowed us to redesign and cut the program in some areas and communities looking forward to strengthening others which allow, in middle-term, to serve as a convincing example to resume the processes with the more difficult communities.

One of the main conclusions of this program is the importance of the strength and the interest of the private enterprise in the accomplishment of the objectives; this can be, without doubt, the most important element of the conservation. When the government does not act, the interest of the private enterprise is decisive to achieve an alliance that can be translated in norms and fulfilment. Later the social control and other influencing elements in the conservation will appear.

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# Scientific and administrative progress of the Captive Breeding Programs of *Caiman crocodilus* and *Crocodylus acutus* in Colombia.

**Dirección de Ecosistemas**
**Ministerio de Ambiente, Vivienda y Desarrollo Territorial**

**ABSTRACT:** The importance of biodiversity, its conservation, sustainable use and benefit-sharing, are guiding principles of the environmental policies of Colombia. Within this framework, it is recognize that the sustainable use of biodiversity constitutes an important strategy for the conservation of the natural heritage and for the sustainable development of local communities.

In the last two decades, a number of productive systems in *Caiman crocodilus* and *Crocodylus acutus*, have consolidated in Colombia, heightening the importance of this productive sector in the country. Taking into account the significance of the captive breeding programs of *Caiman crocodilus* and *Crocodylus acutus*, the Ministry of Environment, Housing and Territorial Development and its Research Institutes, have undertaken actions towards the improvement of the programs ensuring the sustainability of the wild populations, the generation of economic and social benefits and as well the compliance with national and international regulations regarding the production and trade of goods derived from these species.

Thus, we present the implementation progress of administrative and legal aspects that regulate the captive breeding programs of *Caiman crocodilus* and *Crocodylus acutus* in Colombia, as a joint effort of the public and private sector in Colombia.

## Progress of the National Programme for Conservation and Sustainable Use of Crocodylia in Colombia

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**ABSTRACT:** In recent years, the directions and guidelines for environmental policy in Colombia, have sought to incorporate sustainable use of components of biodiversity, recognizing that the rational use of them is a key part of a strategy of conservation and a driver of regional economies, which brings benefits to local communities. In this context, the Programme for the Conservation and Sustainable Use of Crocodylia in Colombia, provides a general framework that defines the main action lines to be undertaken by the national environmental authorities, the scientific community, the private sector, local communities and other actors committed with the sustainable use and conservation of crocodilians in Colombia, in particular with *Caiman crocodilus* and *Crocodylus acutus*. The main action lines of the Programme will be presented, covering scientific, technical, environmental, social, economic and legal aspects, among others.

# Management of caiman in the Brazilian Amazon: A case study of the Mamirauá Reserve.

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**ABSTRACT:** The recent history of use of crocodilians in the Brazilian Amazon includes a period of widespread overexploitation for skins in the 1950s and 1960s, followed by recovery of caiman populations after the prohibition of all commercial hunting in 1967. With the increasing abundance of spectacled and black caiman in many parts of the Amazon a new phase of commercial exploitation began, this time for sale of the meat which in some areas has become an important source of income for *riberinho* communities. Nevertheless, hunting of caiman and the sale of their meat remains illegal in Brazil. As a first step towards the development of a legally, managed caiman hunting program, in 2004, the government of the state of Amazonas implemented an experimental harvests of caiman in the Mamirauá Sustainable Development Reserve (MSDR). The MSDR was chosen for trial management efforts because it was known to contain a large population of caiman and had been the site of basic research on caiman population biology by Da Silveira and colleagues since the mid-1990s. Now, four years since the beginning of the experimental management program, we review and evaluate this initiative both in terms of caiman management and as a potential legal economic alternative for local communities.

# Caiman Nest Monitoring by Local Communities in the Piagaçu-Purus Sustainable Development Reserve, Amazonas, Brazil.

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**ABSTRACT:** The lower Purus River in Brazil, the site of the Piagaçu-Purus Sustainable Development Reserve (PPSDR) is likely the largest source of wild-harvested crocodilian meat in the world. We estimate that >100 tons of dried-salted caiman meat originates from this region annually. While all hunting is currently illegal, the establishment of a managed caiman harvesting program for the PPSDR is being studied by the Instituto Piagaçu and local authorities. A prerequisite for the initiation of harvest is the implementation of a monitoring program that provides useable indices of population trends. In addition to direct measures of abundance and size-class structure from night-light surveys, in 2007 we implemented a community-based program to monitor the numbers of nests in specific areas of the PPSDR. Between 26 and 29 September 2007 we trained residents of communities in the PPSDR and two other Sustainable Development Reserves to collect data on location of nests, using hand-held GPS the people to collect relevant information on caiman nests. During a subsequent ten days period, four residents of the SDR-PP searched for caiman nests in different sectors of reserve. A total of 301 nests (19% *Melanosuchus niger* the rest *Caiman crocodilus*) were encountered adjacent to 51 water bodies. In addition to nest location, nest monitors recorded information on habitat, nest size, and the presence of eggs and attending females. In addition to being a powerful monitoring tool, information on the spatial distribution of nests in the PPSDR will be important for the proposing areas of zoned usage, particularly the establishment of no- hunting zones in important nesting regions. The data gathered will be used for the elaboration of a management plan for caiman population in the PPSDR.

# Conservation status of the "Broad-snouted Caiman" (*Caiman latirostris*): A Management Plan for Conservation in Tarija - Bolivia

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**ABSTRACT:** The "Broad-snouted Caiman" was categorized in Bolivia as critical (CR A1cd) and commercially extinct. We elaborated a Management Plan for *Caiman latirostris*, through an evaluation of the status of their wild populations during the dry seasons of 2004 and 2005. Also we conducted structured interviews to local socioeconomic actors, relating economic activities, perception and attitudes toward this species. We adjust their distribution area in the country. The average abundance was 6.17 individuals/ km of side and marginal species population structure or overexploited, confirming their threat status. The meetings carried out with the governmental and socioeconomic institutions of Villamontes, Yacuiba, and Bermejo municipalities showed that the major conflict between alligator and human populations' are related to accessibility and availability of water sources, due to human population's growth and has water unavailability in this arid ecosystem. Capacity building programs, for control and monitoring are presented in each municipality for this species conservation. However, this effort may not be achieved without political and economic support of departmental and municipal management, of this wildlife resource.

## INTRODUCTION

The wildlife in Bolivia had and currently has a great economic value, although the real contribution of this resource to the economy is underestimated by conventional valuation systems that already lacks commercial fitness, with some exceptions as a species whose pelts are worth commercial (Ojasti 2000, Townsend 2002). The skins economic value of these species led to their indiscriminate hunting. An activity that began intensively in 30s of the twentieth century, with the hunting of alligators, as black caiman (*Caiman "Melanosuchus" niger*), subsequently extended to yacares (*Caiman latirostris* and *Caiman yacare*), londras *(Lontra longicaudis enudris*), cats (*Leopardus, Oncifelis, Puma* and *Panthera*) and peccaries (*Pecari tajacu tajacu* y *Tayassu pecari albirostris*) (Aparicio and Rios 2004). This process continued before General and Permanent Veda declaration in 1990 (MDSPVMARNDF-DGB 2002).

The first important reference of Bolivian caimans and yacares, comes from Medem (1983) after that, King and Videz-Roca (1989), perform the second national alligator distribution inventory of and its population structure in some localities. Ergueta and Pacheco (1990), presents a diagnosis of 5 species of crocodiles with data on their distribution and ecology.

Information on *Caiman latirostris* is restricted to general studies on the alligators in Bolivia. According Medem (1983) they were distributed as far as Pando Department, while King and

Videz-Roca (1989) reported them only in Pilcomayo River area, considering this species commercially extinct in the country. The only previous work specific reference of this species in Bolivia, is the study of their Chaco region conservation status made by Pacheco and Llobet (1998). The authors confirm their endangered status, however they consider a possible recovery in a natural way, if the anthropic pressure situation decline, and for that they proposed an Action Plan, that never been implemented. Neither generated specific process for species conservation, most on the contrary has increased the pollution of major river tributaries of the area, as Pilcomayo, Bermejo and Grande de Tarija rivers. In the area have continued but in reduced levels the trade in meat and skins into neighboring countries, generating an unquantifiable poaching.

At international level CITES, believes that this specie qualifies to be included in "Appendix I". In our country Pacheco and Aparicio (1996) categorized this species in "In Danger" category. Aparicio (2003) considered this is the most threatened species of reptile in the country and categorized as in critical condition (CR A1cd).

## STUDY AREA

The study area is located at SE Bolivia, in Tarija department, of where they have assessed water bodies in three provinces.

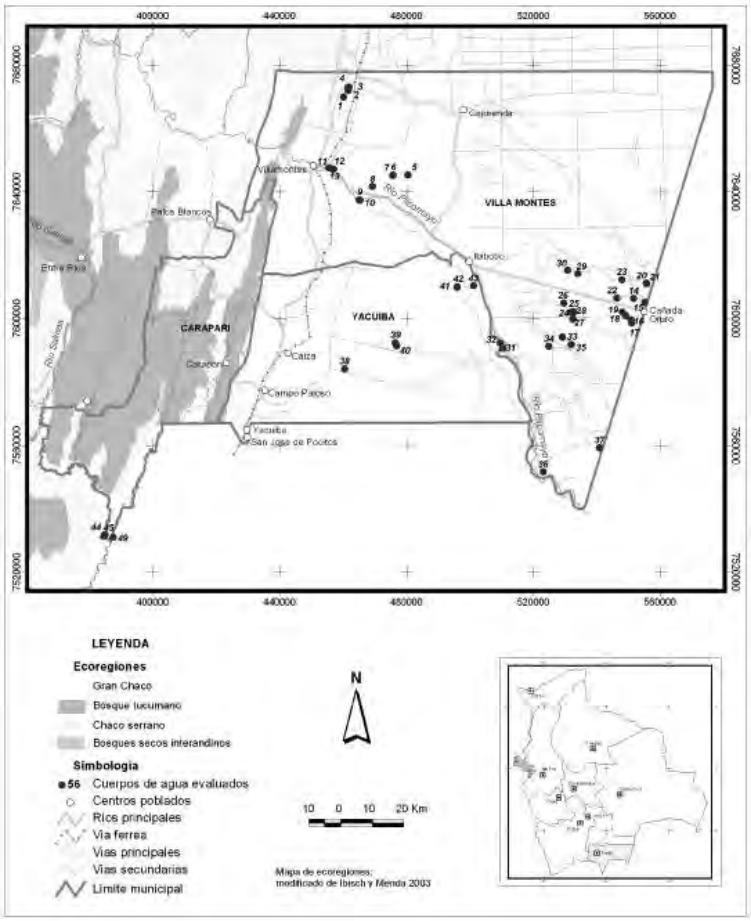



**Figure 1.** Localities of evaluation in Gran Chaco province, Tarija - Bolivia

Gran Chaco Province, in 2004, has an area of 17,428 km$^2$ and altitudes range of 200 to 600 m.o.s.l. (Figure 1). This zone is included in Gran Chaco ecoregion Presents warm steppe

climate, with very dry and hot winters, the temperatures are between 43 º C and -7 ° C. The summer rains (December to March) are torrential, reaching about 780 mm per year (Ibisch and Merida 2003, Montes de Oca 2005).

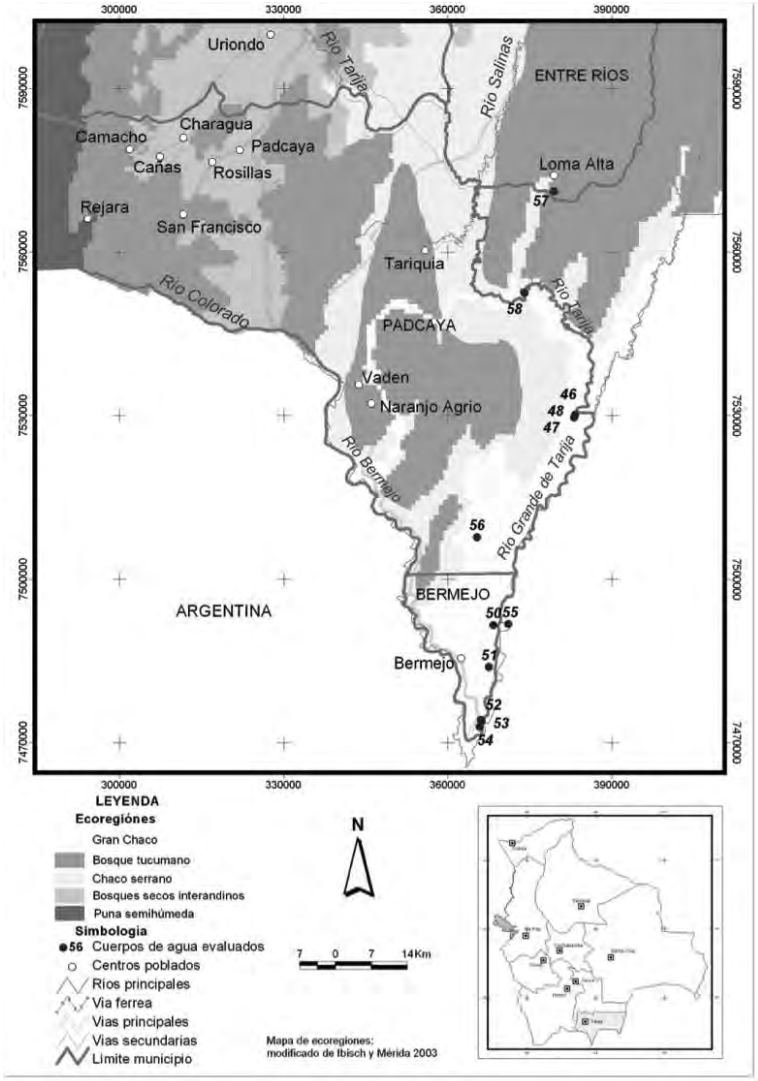



**Figure 2.** Evaluated Localities in Arce and O'Connor provinces, Tarija - Bolivia

Arce and O'Connor Provinces, in 2005, with altitudinal range between 180 to 550 m.o.s.l.(Figure 2). Most water bodies assessed in this area, correspond to Chaco Serrano ecoregion and only two to Gran Chaco ecoregion. This area has mesothermic hot and dry climate in winter, with annual temperatures between 20 and 24 ° C. The annual rainfall range of 1000 to 2000 mm, with approximately 6 to 7 arid months per year. (Ibisch and Merida 2003, Montes de Oca 2005).

## AIM

### General objective

To know, the *Caiman latirostris* population status, in natural distribution areas in Tarija-Bolivia.

**Specific objectives**

- To determine, population abundance and population structure by size class of this specie in Tarija.
- To identify, species natural distribution areas in Tarija
- To determine, the frequency and type of use of "yacare" by local people, as well as relevant specie information (time and nesting areas, hatching time, number of eggs per clutch, etc.) through structured interviews.
- To establish, the potential distribution area of Caiman del Chaco (*Caiman latirostris*) as tool for identifying priority areas for their populations conservation and monitoring
- To develop, a management plan for population recovery and conservation

## METHODOLOGY

Due to extensive area of study, fieldwork was conducted during dry seasons in 2004 and 2005. The first field campaign was conducted from June 10 to July 11 in 2004 in Gran Chaco province of Tarija and the second from August 24 to September 5, 2005 in Arce and O'Connor provinces of the same department.

### Abundance estimation of the population

Direct counts were conducted at night, during dry season, taking advantage of animal populations concentration in water bodies, caused by low water level (Aparicio 1997, Ojeda Leon et al. 1997, Pacheco 1993, Llobet and Aparicio 1999, Rios 2004).

Under this methodology, we walked around all water bodies perimeter, preferably on moonless nights. When this was not possible, we waited dark hours in moon nights. We counted, in each water body using a rubber boat or wooden barge; both boats were driven to rowing with constant speed. With help of Maglight flashlight of 6 V we registered all found individuals along route (Godshalk 1994, Velasco and Ayarzagueña 1995, MARNR - Profauna - UCV 1996, MARNR - Profauna - UCV 1998, Llobet and Aparicio 1999, Rios 2004).

In water bodies, of where it was not possible to sail, walks were held parallel to the shore, trying to cover entire perimeter. The distance in this case depended basically on accessibility, but at least we try to cover 1Km of perimeter (Llobet Aparicio and 1999, Rios 2004).

### Calculating area and perimeter of the evaluated water bodies

The water bodies were georeferenced during day, with help of Geo Positional Satellite (GPS) GARMIN 12 and its perimeter was delimited in canoe or walking around them, taking a point of georeferencing (UTM) approximately every 50 meters (Llobet and Aparicio 1999, Rios 2004). Based on this georeferencing we calculate the area of each water body (ha. of water) and its perimeter (km. from shore), using the processor maps WINDOWS Map Maker PRO VS 2.3. Efforts were made, trying to distance of georeferenced points to real shore, in the different water bodies, never ever assessed 50 cm. Not to make any correction at the time of calculating areas and perimeters.

**Population Structure**

To determine the size of population structure by “yacare” size, in each sighting sought greatest possible approach to estimate the total length (TL) of each individual (from the tip of the snout to the tip of the tail). If the animal's body was not completely visible, TL was estimated by the size of his head, which is part of the body that usually is visible in the water.

With these data “yacares” were classified into one of four size categories considered for this species (A. Larriera 2004, Yacaré Project, Santa Fe - Argentina, pers.com.): Class I, up to 50 cm; Class II, 51cm to 139 cm; Class III, 140cm to 179 cm; Class IV, more than 180 cm.

When TL was not possible to estimate, the animal was registered as eyes only (EO) (Godashalk 1994, Velasco and Ayarzagueña 1995, Llobet Aparicio and 1999, Rios 2003).

**Others data included**

1) Environmental data: average temperature at 1 m above the body of water surface at 10cm of deep, wind speed, cloud cover and moon phase. Both the environment temperature as the water was recorded with a thermometer Oakton Temp 5 Acorn Series. The wind speed was measured with an electronic anemometer Kestrel 1000
2) Physical data of water bodies: depth, macrophytes coverage and “palizada” (remnants of trunks and branches of trees).

**Interviews**

The populations *Caiman yacare* conservation, depends of changes in attitudes and perceptions of local residents. To obtain information over practices in *Caiman latirostris* use and hunting customs of local people, structured interviews were conducted (Bernard 1994). The interview was applied only once every interviewed and notifying in advance the goal of the interview (Fillion 1987).

Were selected key informants in each community, usually authorities or recognized persons and respected within the community, knowledgeable of the place and environment.

The interviews guide, was developed to aboard the following topics: Relative abundance and habitat use of the species; perception and attitude towards the species; biological knowledge; and resource use.

**Modeling the potential distribution of the chaco yacare**

To model the potential distribution area of chaco yacare (*Caiman latirostris*) we used MAXENT software (Phillips et al. 2004, 2006). We used goereferenced points of counts of sites (N = 58), except those in which there was not any individual. The data were cleaned based on criteria of altitude and political limits, for which data over the 500 m above sea level and about the limits of Bolivia with Argentina and Paraguay were eliminated (n = 6).

We used layers of information with a resolution of 1 km. We worked with seven layers of environmental data that were generated in the GIS software DIVA 5.4 (BIOCLIM 1, BIOCLIM 2, BIOCLIM 3, BIOCLIM 4, BIOCLIM 12, BIOCLIM 14, and BIOCLIM 15). Other layers of information used to model the range of chaco yacare were the map of vegetation from Navarro (2007), DEM, exposure, dominant soil, herbaceous coverage, and coverage of bare soil generated in the Arc View 3.2 software.

**Data analysis**

Both the abundance and the population structure were calculated excluding individuals of first year (Class I), because at this stage of life the survival rate is about more than 20% of individuals who born, many authors do not consider it appropriate to include this class to determine the characteristics of population because the strong monthly changes that suffers (Micucci and Waller 1995, Velasco and Ayarzagueña 1995).

For the analysis of population structure we used the comparison of histograms obtained with the work of Velasco and Ayarzagueña (1995) for *Caiman crocodilu*s in Venezuelan plains. Despite the limitations of this study, because it is another species of alligator, Barahona et al. (1996) and Prado (2005) used to describe population structures of *C. latirostris* and other species of alligators.

The abundance and population structure were analyzed by hydrographic sub-basin, comparing populations of yacaré in the sub-basin Pilcomayo River with populations of the sub-basin Bermejo River. Applied U of Mann-Whitney test (Siegel and Castellan 1995).

We do Spearman correlation test between data of environmental parameters considered and the abundance recorded. In the case of environmental discrete variables (moon phase and clouds) we used the Krukal-Wallis test ($a$ = 0.05)

For interviews analysis a numeric code was applied to each question separately, identifying the answers in all surveys and assigning a number that varied according to how much the kind of responses, but always used the "0" when the respondent said he does not know the answer to. This was able to obtain the percentages and frequencies of responses to different questions.

## RESULTS AND DISCUSSION

We evaluated a total of 54 water bodies of 42 belonging to the sub-basin of the Pilcomayo River, 12 in the sub-basin of the Bermejo River.

### Sub – basin of Pilcomayo River

**Abundance**

In the sub-basin of Pilcomayo River we recorded a total of 289 individuals at 29.21 km from shore travelled, of which 137 belonged to Class I (47.40%). The total abundance of yacaré for this sub basin was 5.2 ind. / km from shore by the abundance body of water varies from 0.00 to 46.81 ind. / ind. Km travelled shore.

During the evaluation was clear preference of these reptiles for calm waters, as reported by other authors (1983 Medem, Micucci and Waller 1995), because the highest abundance values were recorded in ponds, “atajados” (dike) and artificial ponds, while that four counts in Pilcomayo River had an abundance of 0.00 ind. / km from shore.

Waller and Micucci (1993), and Prado (2000), reported the same trend in evaluations conducted in Chaco provinces and Corrientes in Argentina. These authors believe that habitat structure is responsible for this trend, since yacaré prefer to inhabit temporary water bodies, with little depth and abundant vegetation cover.

Pacheco and Llobet (1998) reported an abundance of 3.3 ind / km from shore in water bodies at the same sub-basin, a less value that found in this study. However, the validity of this comparison should be considered relative and not conclusive, that only three of 22 water bodies tested by these authors, were repeated in this study. Likewise, the covered area during field work and time of year's count was different in these two studies.

Despite the distribution areas of this species in Bolivia, would be considered as marginal, the abundance of *C. latirostris* found in Pilcomayo River sub-basin in Bolivia, is not very different from reported rates in regions closer to the center of its range, except for abundance recorded by Waller and Micucci (2000) (in Prado 2005) of the 30.5 ind / km from shore in the system Iberá-Argentina. However, it is necessary to mention a major difference in terms of availability and quality of habitat for Yacaré between the Argentina region and Bolivia Chaco region

**Population Structure**

A total of 154 animals (53.29%) could not be assigned to any class size, so were registered as having eyes only (EO). This high percentage is because many of the counts were done on foot because the water was not deep enough and did not allow navigation; this made it impossible to estimate the size of various animals.

Therefore, the population structure of *C. latirostris* for this sub basin was calculated on the basis of 135 individuals, 64 belonged to the Class I (47.41%). The histogram shows a population that is dominated by young individuals (Figure 3).

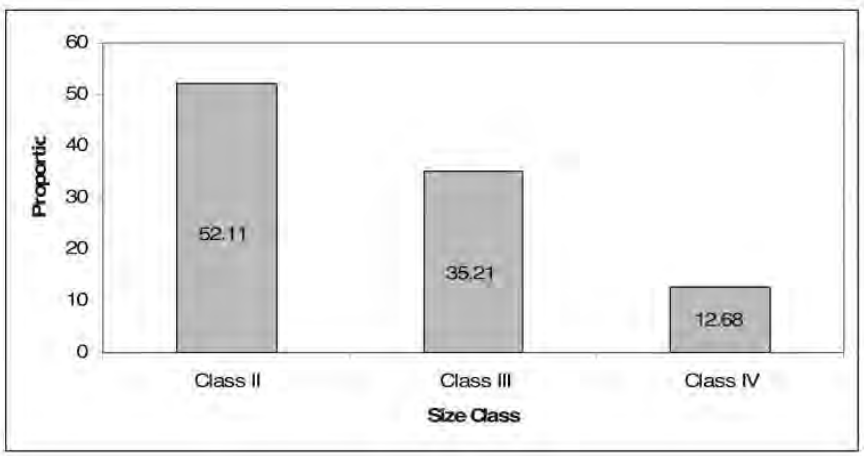



**Figure 3.** Histogram of the population structure of *Caiman latirostris* in Pilcomayo River sub basin in Tarija (n = 71).

This structure shows a distribution form of type "stair", with a proportion of Class IV below expectations (<15%), according to Velasco and Ayarzagueña (1995) caused by selective hunting of adult individuals, typical of stocks subject to sustainable harvests for several years. This result is reinforced by the study of Pacheco and Llobet (1998), which also reported a population structure in the form of a "stair" with histogram that presents fall in the form of negative exponential curve, typical of populations over exploited. Attributed primarily to strong pressure from hunting on such until the 1980s (Prado 2005).

However, Bolivian Chaco would be an area near the boundary of natural distribution of this species, is likely to be marginal and population in this case the natural form of distribution histogram would be kind of "stair" without the cause of it is the removal of individuals (Gorzula 1989, Rios 2004).

**Environmental data**

In general environmental variables as average temperature (ETEMP), water temperature (WTEMP) and wind speed (WSPEED) during evaluations in this sub-basin were fairly stable (Table 1).

**Table 1.** Spearman correlation coefficients and test results for the Kruskal-Wallis analysis of the relationship between environmental variables and abundance in the sub-basin of the Pilcomayo River

| Variable | ETEMP | WTEMP | WSPEED | Vegetation coverage | Cloudiness | Moon phase |
|---|---|---|---|---|---|---|
| **Abundance** | R=0.188<br>P=0.259 | R=0.006<br>P=0.971 | R=0.335(*)<br>P=0.046 | R=0.211<br>P=0.175 | $X^2$ = 6.52<br>gl = 5<br>P = 0.26 | $X^2$ = 0.33<br>gl = 2<br>P = 0.85 |

* Significative correlation

We found negative correlation between abundance of *C. latirostris* and WSPEED. Prado (2005) notes that this correlation could be explained from the fact that the movement of air masses generates waves with amplitude proportional to wind intensity and the water mirror presence of these. This fact would decrease the length of stay of yacares on surface; however this not affect the environmental variable population counts.

**Interviews**

We were conducted 24 structured interviews, of which 91.6% were males and 8.3% women in Yacuiba and Villamontes municipalities of Gran Chaco province, with ages ranging between 26 and 55 years. Most of them were farmer.

More than 33% of those interviewed mentioned that they had last seen *C. latirostris* 1 week ago or less (Figure 4). Furthermore, according to their perception of yacares abundance variation in the past 10 years, most noted that currently there would be more or equal amount of yacaré making 1, 5 and 10 years ago.

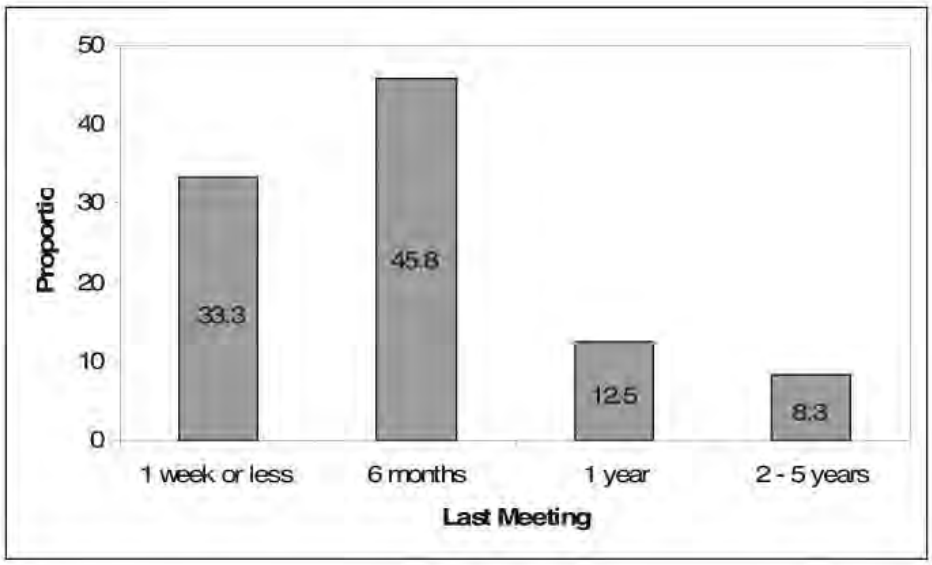



**Figure 4.** Time elapsed since the last meeting of the interviewees of the Gran Chaco province with *C. latirostris*, sub-basin of the Pilcomayo River (n = 24).

Perception and attitude towards the species

Most of our interviewees (45.8%) gave to this question double response, saying that Yacaré is harmful because attacks the small livestock (goats, pigs and cows' offspring), but felt some sympathy for the species (Figure 5). However, 92% indicated that they hunted this animal to avoid the danger of an attack, especially if found in watering – places for cattle near home.

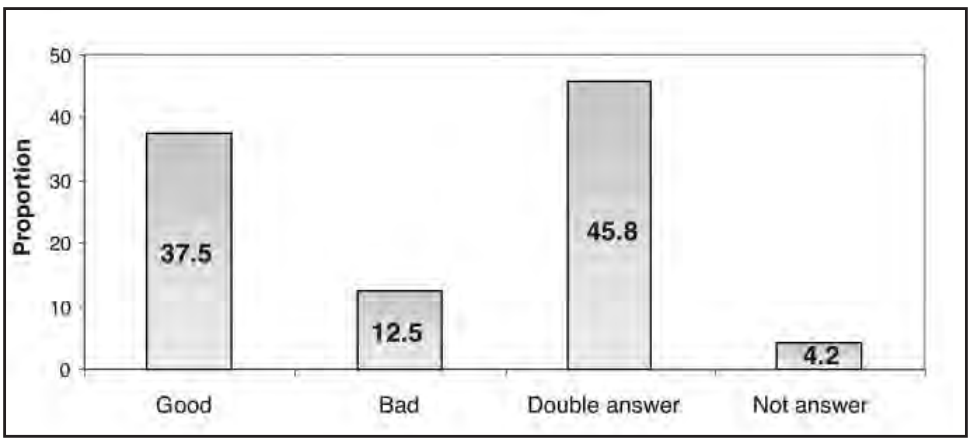



**Figure 5.** Yacare people perception in Pilcomayo River sub-basin of "Gran Chaco Province" (n = 24).

They also noted that if it was youth or newborns they left because the infants did not represent a real risk to them or their livestock, but if it was an adult (> 140 cm) the animal elimination was the unique option.

**Biological knowledge**

According the interviewees yacare is founded in ponds and in artificial ponds preferably, but some said that these reptiles were equally founded in any water body (Figure 6). Coinciding with partial results obtained in field evaluations and with results by Prado (2005), Scott et al. (1990) and Waller and Micucci (1993) in studies of habitat preference in Argentina.

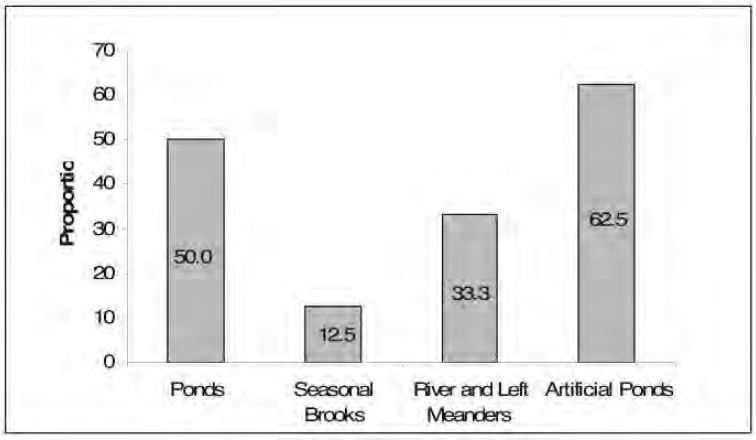



**Figure 6.** Type of *Caiman latirostris* favorites water bodies, according Gran Chaco Province informants, in Pilcomayo River sub-basin, Tarija (n = 24). The proportions are not adjusted to 100% because the respondents chose more than one option.

A total of 18 respondents (75%) were able to give some reference when asked about the time of egg-laying and hatching of them. But the answers are very diverse and even contradictory, so we do not consider reliable data provided (Table 2).

**Table 2.** Months of emergence of young yacarés (*C. latirostris*) according interviews in sub-basin of the Pilcomayo River in Gran Chaco province, Tarija department.

| Month | January | July | August | Octuber | November | December |
|---|---|---|---|---|---|---|
| Proporción de entrevistados | 22.2 | 11.1 | 16.7 | 5.6 | 27.8 | 16.7 |

These results show that local knowledge on reproductive aspects of *Caiman latirostris* is quite poor. Of the 18 respondents, just two referred that they had found yacare nest sometimes

**Resource using**

The most widely used part of yacaré, according to those interviewed, is fat for medicinal purposes (Figure 7), while the meat is used more as an input for the preparation of food for dogs. And the skin to draw up articles as leather crafts.

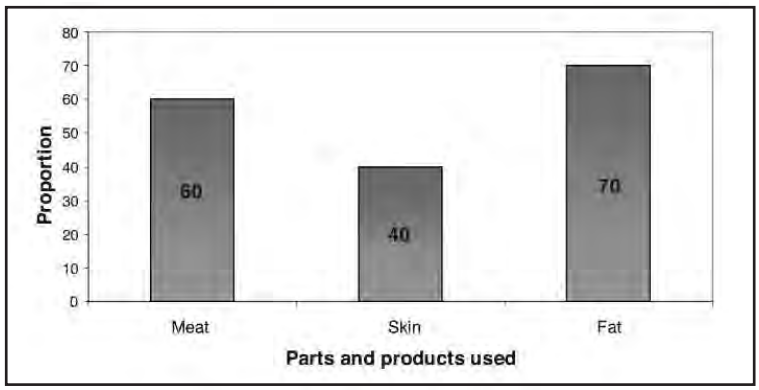



**Figure 7.** Parts of yacaré used by people of the Gran Chaco province, sub-basin of the Pilcomayo River in Tarija (n = 24). The proportions are not adjusted to 100% because the respondents chose more than one option.

**Bermejo River Sub- basin**

No records of yacare presence were prior to this study in Bermejo River sub – basin in Chaco, so the presented study data extend the range of this species in this Bolivia. sub-basin

**Abundance**

During this sub-basin evaluations were recorded 52 individuals in 6.44 km from shore, 6 of whom belonged to Class I (11.54%). The total abundance for this sub- basin is 7.14 ind. / km from shore, with an abundance of water body of 0.00 ind. / km to 16.36 ind. / km from shore.

The habitat availability in Bermejo River basin area. is much lower than Pilcomayo River sub-basin, mainly due to strong intervention by anthropogenic effect of clearing large tracts of land for planting sugarcane and the strong contamination of water bodies as result of sugar mill activity, which removes their waste into the Rio Grande product of sugar and alcohol production.

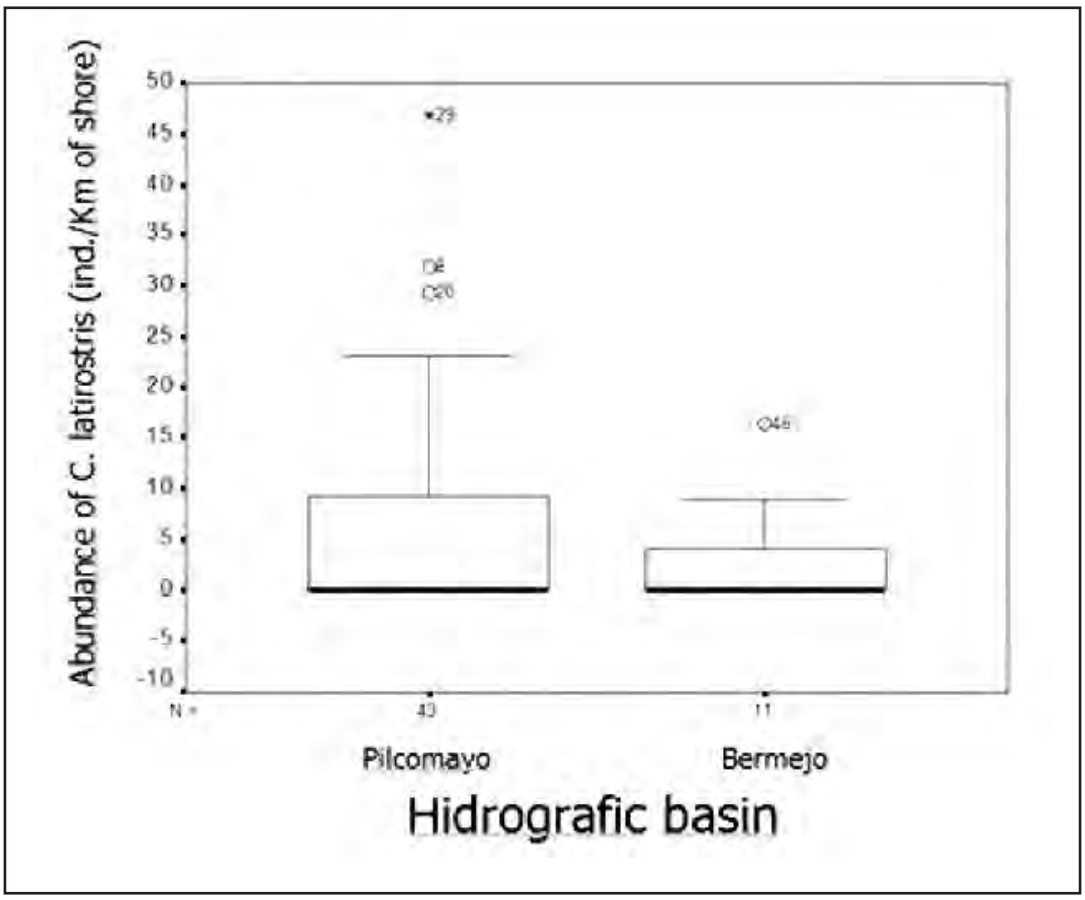



**Figure 8.** Variation in the abundance of Chaco yacare in Bermejo River basin and Pilcomayo in Tarija department.

When comparing the total abundance of yacaré between sub Pilcomayo and Bermejo, through a test of U Mann-Withney have not found a statistically significant difference (U= 185; P = 0.22; *a*=0.05), however the variation is more important in the Pilcomayo (Figure 8).

**Population Structure**

A total of 35 alligators (67.31%) could not be assigned to any size classes, so they were registered as EO. This percentage is even higher than achieved in Pilcomayo River sub-basin and is primarily due to same reason: all counts were conducted on foot because the water was not deep and did not allow navigation.

The population structure of *Caiman latirotris* in this sub- basi was calculated on the basis of 17 individuals, of which 2 (11.76%) were individuals in Class I. The structure of this population sizes shows a strong dominance of juvenile individuals Class II (n = 9; 60.00%) (Figure 9).

According to Velasco and Ayarzgueña (1995), this structure shows a form of distribution of type "stair", with a drop of negative exponential rate, caused by resource overexploitation. This structure differs of found in Pilcomayo River sub-basin that presents a linear-type fall. However, despite considering it is a marginal area of distribution of this reptile, the cause of the low proportion of adult individuals (Class III and IV) is probably result of strong negative impact of pollution and destruction of their habitat.

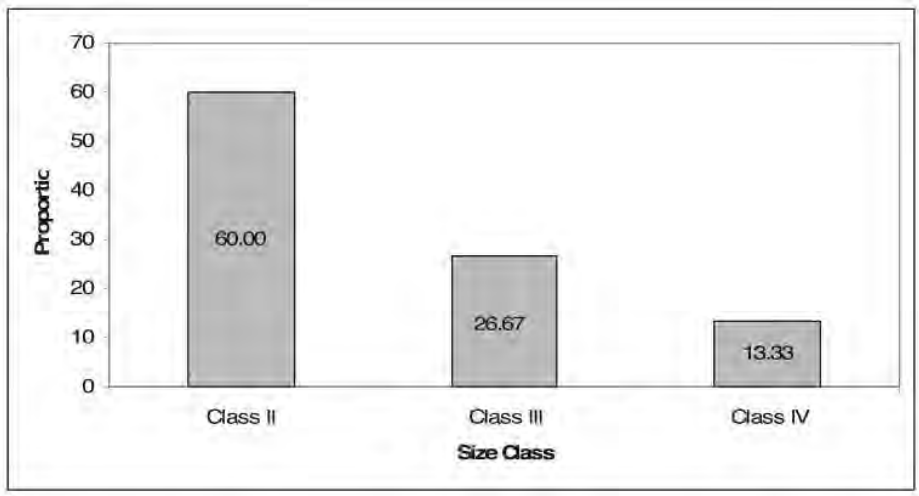



**Figure 9.** Histogram of *Caiman latirostris* population structure in water bodies in Bermejo River sub-basin in Tarija (n = 15).

Moreover, the Class I low proportion, joint with nests absence reports, the strong intervention, water pollution, and the low number of individual recorded during the counts suggest that this population is probably not reproductively active in the area, becoming a sink of wetlands and estuaries bordering Argentina, where habitats are available for the species, less intervened and polluted.

**Environmental data**

In general, the environmental variables as average temperature (ETEMP), and water temperature (WTEMP) during population evaluations conducted on waters bodies of this sub-basin, were fairly stable, except the wind speed (WSPEDD) with strong variations. We found a high positive correlation between WTEMP and WSPEDD (R = 0,804 (**), P = 0.005). However none of the environmental variables considered in the study showed a correlation with abundance in this area (Table 3).

**Table 3.** Spearman correlation coefficients and test results for the Kruskal-Wallis analysis of the relationship between environmental variables and abundance in Bermejo River sub-basin.

| Variable | WTEMP | ETEMP | WSPEED | Vegetation coverage | Moon phase |
|---|---|---|---|---|---|
| **Abundancia** | R=-0.037<br>P=0.919 | R-.098<br>P=.774 | R=-0. 219<br>P=0. 518 | R=0.576<br>P=0.064 | $X^2$ 1.592<br>gl = 1<br>P = 0.21 |

The cloudiness variable was excluded from this analysis because all evaluations in this study area were conducted on nights completely cleared.

**Interviews**

Eight structured interviews were conducted in Bermejo (n = 6) and Padcaya (n = 2) municipalities in Tarija department. All those interviewed were males whose ages ranged between 15 and 55 years old. Attempts to interview women, but these refused to be interviewed saying they did not know this animal or that they had never seen.

The 50% of respondents were engaged in parallel with agriculture and cattle rising. The others were “subprefectura” or municipal authorities.

Unlike the results of interviews in Pilcomayo River sub-basin, only 50% saw them in week or less (Figure 10), with encounters with this species less frequent. Likewise when asked about their perception about the variation of *C. latirostris* abundance in the past 10 years, replied that there are now fewer yacaré individuals than 1, 5 and 10 years.

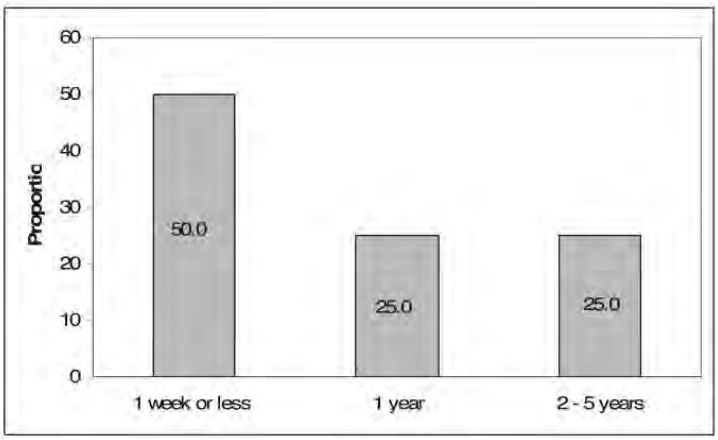



**Figure 10.** Time elapsed since last meeting with *C. latirostris* of the interviewees from Bermejo River sub-basin in Arce province, of the (n = 8).

**Perception and attitude towards the species**

In this region, apparently *Caiman latirostris* perception would be good, since 75% said it was an animal that pleased them and they believed it was an important element of occupied habitats, probably because the human population of this area is more urban (Figure 11). However, when respondents were asked to wonder if they hunted this animal or knew someone who did that in the area, 87.5% replied that people hunt for fear of these animals and to prevent family members or pets from aggressions.

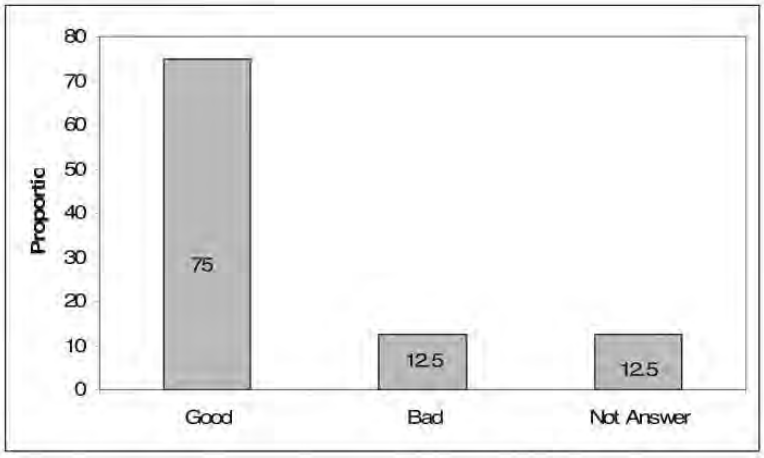



**Figure 11.** Perception of residents in the province Arce, Bermejo River sub-basin the (n = 8).

**Biological knowledge**

As in Pilcomayo River sub-basin, those interviewed pointed out that this species was more frequently and in greater quantity in ponds (Figure 12).

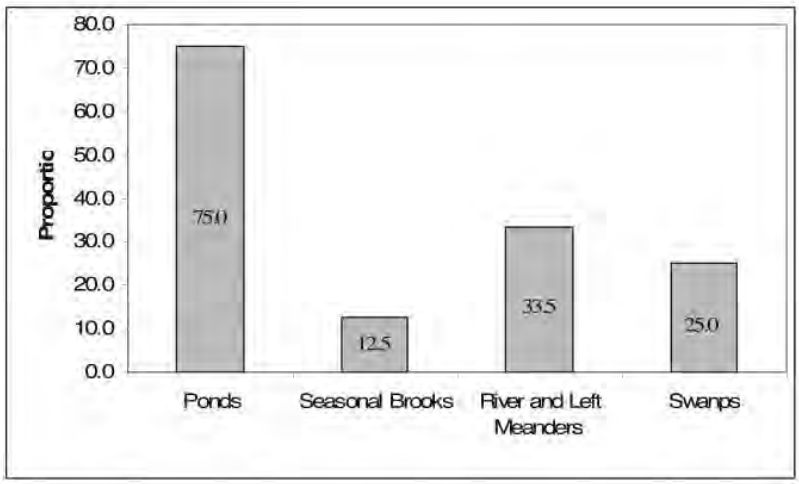



**Figure 12.** Type of *Caiman latirostris* favorites water bodies, according to informants in Bermejo River sub-basin in Arce province, of Tarija department (n = 8). The proportions are not adjusted to 100% because the respondents chose more than one option.

The marshes were one of the lowest frequencies, contradicting reported by Prado (2005), Scot et al. (1990) and Waller and Micucci (1993). However, it is possible that accessibility and visibility falls sharply in the perception of habitat preference expressed by local residents and the abundance of these reptiles underestimates in marshes or water bodies with abundant vegetation cover.

The information on aspects of the natural history of this species was quite low, 5 of 8 respondents (63.0%) said not knowing anything related to yacare reproduction. Of 3 respondents who answered this question, 2 (67.0%) mentioned that in April offspring's appeared in water bodies, but were contradicted by 1 interviewed (33.0%) stated that offspring's appeared in November. None of those interviewed in this region reported the presence of nests in the area.

**Resource use**
Villagers interviewed reported only skin use (50.0%) and meat (50.0%).

**Potential range of chaco yacare**
The range of Chaco yacare in Bolivian territory has been reduced compared to the range estimated by Medem (1983) and potentially be found only in Plata Basin (Rios and Aparicio in prep.) (Figure 13).

The distribution of Chaco Yacare is restricted to Pilcomayo and Bermejo rivers, in south of Bolivia. Remain a priority to implementation of ensure population conservation programs in Pilcomayo River in Chaco region, since in this region will find the most representative range within national territory and the most abundant populations.

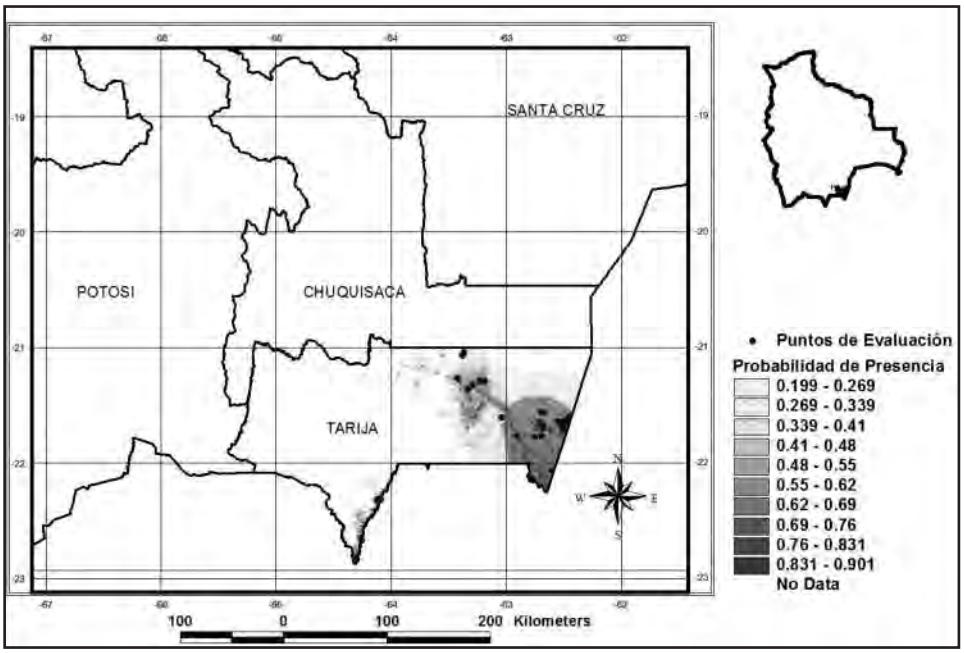



**Figure 13.** Map of the Potential *Caiman latirostris* distribution in Bolivia.

## MANAGEMENT PLAN FOR *Caiman latirostris* POPULATION RECOVERY AND CONSERVATION IN TARIJA DEPARTMENT

The proposed Management Plan for population recovery and yacaré (*Caiman latirostris*) conservation will be developed in natural species distribution areas in Villamontes, Yacuiba and Carapari in the Gran Chaco province, the municipalities of Bermejo and Padcaya in Arce province and Entre Rios in O'Connor province, from Department of Tarija. Is essential social and political institutions support, while during the management plan execution in the region and economic contribution of Prefecture of Tarija.

**Objectives of the Management Plan:**
The overall objective of Management Plan, is to achieve recovery of Chaco yacaré wild populations (*Caiman latirostris*), with the greatest environmental, economical and social benefit.

The specific objectives to be achieved are:

- To establish, program of population recovery and to promote the species.
- Strengthen local institutions capacity for environmental conservation lie.
- To promote, understanding and appreciation of this wildlife resource at the rural and urban populations in this region through their participation in the management plan and awareness.

**Programs**

The management plan proposed, presents five programs and five sub programs with activities that should be implemented and developed in three years (2006 - 2009). The following is a summary description of these:

**1. Management Program**

This program implies that species will be subject to drivers decisions and manipulations, in terms to achieve this must combine research and conservation.

**1.1. Sub evaluation program of "Yacaré" populations**

For implement the program to protect and conserve during the first year (2008), the information will be used on the population abundance obtained from the different water bodies during the 2004 and 2005 prospecting. But for the next steps, should be performed their respective population assessment in 3 provinces involved in population recovery and conservation program.

The objectives of this Sub Programme are:

- To know regularly the behavior of yacares populations in relation to their structure and abundance in each municipality natural areas.
- To know regularly if in each municipality yacaré populations is increasing or decreasing.

**1.2. Sub Development Program of the species**

According to assessment of wild populations of this species made by the authors in 2004 and 2005, we can ensure that we can not carry out program for using without causing the disappearance of this species in the country, unless actions are undertaken to enable the increase of animals to replace those individuals who might be exploited in the future. These management measures must be implemented taking into account the environmental and socio - economic actors involved.

The objectives of this Sub Programme are:

- To increase *C. latirostris* population abundance in species natural distribution areas, in 6 municipalities involved in the program.
- To reduce mortality and increase the chances of *C. latirostris*. offspring's survival

**1.3. Sub program of the species population recovery**

The damaged population restoration is often the first stage of management for this eventual use. The population recovery is function of time, demands hard work and continued funding, especially in long-cycle species (Ojasti 2000), such as Chaco yacaré (*Caiman latirostris*).

This sub program is one of the most delicate program component and is really necessary scientific information about biological and ecological species characteristics in the area,

will start just the second year of implementation of this plan.

The objectives of this sub program are:

- To increase the species natural range in each municipality territory.
- To allow the recovery of Chaco yacaré populations in ecologically functional densities.

**2. Protection and Control Program**

The management of wildlife does not guarantee their availability in long term, given the economic and social conditions prevailing in the country, requiring combine actions directly related to management of species with measures to monitor the proper implementation of guidelines in this process.

The objectives of this program are:

- To ensure, compliance with the guidelines set forth in this Management Plan.
- To allow, the normal development and implementation of recovery programs, and promoting environmental awareness.
- Monitor the effect of human activity on yacare habitats and populations in each municipality.
- To control illegal hunting, illegal trade of the skins and sub products in each municipality.

**3. Program for scientific research**

Studies the biology of Chaco Yacare (*Caiman latirostris*) in Bolivia, not even been started. This aspect is so dramatic, in this assessment local people mentioned that this species reproduces twice a year, once every two years and even some local people mentioned that the specie reproduces all year long and there is no scientific efforts that allows give the correct answer to this question. With regard to the ecology of the species, we only have information on their distribution and abundance.

The objectives of this program are:

- To generate knowledge about biology and ecology of *Caiman latirostris* in Bolivia.
- To get scientific and technical bases to permit species conservation.

**4. Monitoring Program**

The goal of monitoring is to observe the progress of work according to plan and guide as the experience you get on the way.

This program, will record changes in *Caiman latirostris* populations subject to management over time, through systematized file on information provided by research projects and the respective technical reports from various sub programs and programs of Management Plan.

The objectives of this program are:

- Monitoring and evaluation of habitat extent and quality, where there is presence of the yacaré.
- Monitoring and evaluation of populations density and distribution in each municipality.
- Monitoring and evaluation of programs and sub programs proposed in this plan of conservation and population recovery.
- Have technical and scientific information - necessary to make the most effective measures for species conservation and management.

**5. Training Program, teaching and environmental education**

The future of species under management depends on the attitude that society and individuals involved have on the process of sustainable development. Without the participation and support of local communities, any conservation project is doomed to failure or at least is very complicated in its implementation, for this reason the training, environmental education programs are essential to achieve this support and with the other programs all of them are the instrument required to pursue the objective of the management plan.

**5.1. Sub Training Program**

Seeking to avoid mistakes that could commit actors related to yacares conservation in Tarija, we intend to give the necessary information, so that residents of municipalities involved may be direct participants in conservation and repopulation of *Caiman latirostris* process, strengthen their capacities to carry out management of species, which in future will enable them to make sustainable use of this resource with greatest environmental, economical and community benefit.

The objective of the Sub Program:

- To train different actors involved in Plan of Conservation and repopulation of Chaco yacaré, in technical - scientific, legal and organizational aspects, to ensure its proper development.

**5.2. Sub education programs and Environmental Education**

The implementation of this sub program must ensure a continuous process of transmitting information between various actors involved in preservation and promotion of Caiman latirostris in the region, allowing really count and timely information, to facilitate the authorities to make decisions and willingness of local residents.

The objectives of the Sub Program:

- Local residents are known to be involved directly or indirectly with the Management Plan for the Conservation and population recovery of Chaco yacaré.
- Achieve changes in local people attitude, not only with the species subject to management, but with wildlife in general, through the understanding of the importance of conservation and its usefulness in achieving sustainable development.
- To disseminate the objectives, actions and results of activities carried out within management of this species to communities of the municipalities involved in this plan directly and indirectly throughout the Department of Tarija.

**CONCLUSIONS**

**a)** It has expanded the range of yacaré (*Caiman latirostris*) in Bolivia, previously reported only for Gran Chaco province (sub-basin Pilcomayo) in Tarija department of to the provinces Arce and O'Connor in the same department (sub-basin Bermejo).

**b)** The abundance of population for sub Pilcomayo River is 5.2 ind. / km from shore, and for the sub- basin of the Bermejo River is 7.14 ind. / km from shore. Taking an overall average of 6.17 for the department about 6.17 ind. / km from shore traveled.

**c)** We did not find a variation statistically significant of the abundance of populations of

the yacaré between the two sub-basins evaluated in the department of Tarija, which it's probably because both areas are within an area of marginal range of the species. However the difference in terms of the number of individuals founded, greater product availability and quality of habitat in the basin of the Pilcomayo, suggest a difference biologically significant.

**d)** In both populations were found a population structure dominated by young individuals (Class II) and low proportions of adults in the Class IV, which it's probably because it is marginal populations. But in the case of Bermejo, we found a strong negative impact of anthropogenic activities on the habitat of this species.

**e)** Based on the abundance and population structure, found in these two areas of the department of Tarija, we note that the conservation of both yacare´s populations are seriously threatened.

**f)** The growth and expansion of human populations are causing conflicts between Chaco yacares and local villagers, especially use of aquatic habitats.

**g)** None of the climatic factors considered in this study appears to affect negatively the results of the counts.

**h)** The implementation of plan to population recovery and the conservation of *Caiman latirostris*, constitutes the only way to allow this species will not disappear from the national territory and in the future can make a rational use.

**i)** According to majority of respondents perception, the abundance of *Caiman latirostris* in Pilcomayo River region have increased over the past 10 years, whereas in Bermejo River region have declined.

**j)** In general, here is a good perception in both areas of study. However in Pilcomayo River region has seen a strong adults hunting activity as use practice (in some cases), control and prevention, which could adversely affect populations of this species.

**k)** Local knowledge on aspects of the natural history of this species is weak or nonexistent (in the case of Bermejo). That's probably because much of population of these two regions is migrant from other departments in the country, and others have long ago moved to urban centers losing contact with wild species.

**l)** There was not registered the presence of *Caiman yacare* in both of the sub- river basin evaluated in the department of Tarija, it is probably that in this region this species was founded in allopatry because of their greater plasticity on habitat requirements

**m)** The potential distribution area, of *Caiman latirostris*, in Bolivian “Chaco” are restricted to sub – basin of Pilcomayo and Bermejo rivers.

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# Conservation status of the habitats of dwarf caiman, *Paleosuchus palpebrosus*, in the region surrounding Pantanal.

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**ABSTRACT:** Research on the ecology of the dwarf caiman, *Paleosuchus palpebrosus*, were initiated in the 1990's in the area surrounding western Pantanal, while reproductive aspects have been studied in the Central Amazon since 2001. The main goals of the study are to evaluate the quality and problems regarding the conservation of the natural habitat in the areas surrounding the Pantanal. I found young and adult dwarf caiman and analyzed the impacts of habitat alterations on the heads of the six rivers e small rivers. The habitats visited were found to be in alarming condition, due to deforestation along the riverbanks, silting, industrial pollution, mining activities, urban sewage, human habitation, fishing and caimans poaching. Total destruction of the vegetation is being caused by agriculture, mainly soybeans and sugar cane to supply the ethanol factories. The dwarf caimans apparently resist to the pressures of habitat destruction as well as the enormous pressure of predatory hunting in these rivers in the surrounding Pantanal. However this may be due to the existence of pockets of pristine areas which act as source of individuals. The dwarf caiman could effectively act as flagship species for the conservation of the habitats and fauna to rivers surrounding the Pantanal.

# Contingent Actions with Crocodilians of Chiapas, México.

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**ABSTRACT:** Chiapas is one of the two states of Mexico that has the three species in the country (*Caiman crocodilus fuscus, Crocodylus moreletii* y *C. acutus)*; however, into their distribution rage cohabit with the human and it unchains different situations of negative interaction between both species. In a regular way, as part of a specific program of Crocodile Museum, there is a following and attention to such questions, using a strategy of immediate in collaboration with federal environmental instances, including a methodology of concientization, environmental education and rescue of crocodilians. All that has promoted in lots of regions a better aware to solve these kind of events, where regularly and thanks to current reports we can attend an approximate of 80 to 100 contingent situations per year, with a success of 90% of the cases; the majority of the situations are focused to the presence of big size specimens, or occasions where human populations invades crocodilians territory and begin to appear specimens in urban areas where the community get scared, that's why it is worth to know these kind of successful actions of conservations.

**RESUMEN:** Chiapas es uno de los dos estados de México que cuenta con las 3 especies del país (*Caiman crocodilus fuscus, Crocodylus moreletii* y *C. acutus*), sin embargo, dentro de sus rangos de distribución cohabita con el humano y de ello se desencadenan diversas situaciones de interacción negativa entre ambas especies. De manera regular, como parte de un Programa específico del Museo Cocodrilo se da seguimiento y atención a dichos asuntos, mediante una estrategia de atención inmediata en colaboración con las instancias ambientales Federales, incluyendo una metodología de concientización, educación ambiental y rescate de cocodrilianos. Todo ello, ha promovido en muchas regiones una mejor conciencia para resolver este tipo de eventos, donde regularmente y gracias a los reportes actuales se logran atender en un promedio de 80 a 100 situaciones contingentes al año, con un éxito de hasta el 90% de los casos; en la mayor parte de estas situaciones se enfocan a presencia de ejemplares de gran tamaño, o situaciones donde la población humana ha invadido el territorio de los Cocodrilianos y comienzan a aparecer ejemplares en áreas urbanas donde asustan a las comunidades, por lo que vale la pena dar a conocer este tipo de acciones de conservación exitosas.

# The reintroduction of the Cuban crocodile in Lanier Swamp (Isle of Youth, Cuba): asserts, failures and lessons.

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**ABSTRACT:** There are only two locations reported for the Cuban crocodile (*Crocodylus rhombifer*) in historic times: Zapata Swamp, in Cuba mainland and Lanier Swamp, in the Isle of Youth. Being virtually extirpated from the second location during the first half of the XX Century, the National Enterprise for the Conservation of Flora and Fauna (Ministry of Agriculture) started a restocking program in 1986, being the first step, the construction of a crocodile breeding facility: Cayo Potrero Farm, situated in the middle of Lanier Swamp. A first group of 600 individuals was released in 1994, being followed by successive introductions in the following years. Successful breeding, nesting, hatching, individual growth and dispersal among the reintroduced population have been documented by periodical monitoring carried out since 1996 up to the present. But also several negative human-originated impacts, such as poaching, have put in jeopardy the outcome of the whole operation. In this presentation we analyze, from the point of view of our experience, the importance of a correct, multi-approach planning of restocking operations, that takes into account not only biological or ecological, but also economic, educational and cultural factors of the local communities, and their possible involvement in the restocking project, their education and direct participation in the efforts and possible benefits.

# The Marsh to Market Story: "How Sustainable Use of Crocodilians Benefits Commerce, Conservation and Communities"

**Don Ashley**

World trade in crocodilians has increased to more than 600,000 classic skins annually since 2004. This is 20% above the historical peak of half a million classic hides a year reached in the early 1960's. Caiman skins lag below the historical ratio of 3 to 1 classic hides, but are nearing a million hides annually. With new management initiatives in Brazil, Bolivia and other Latin American countries at least 1.2 million caiman hides could be sustained annually by 2010.

The alligator continues to produce about 60% of the classic hide world supply, producing more than 350,000 skins a year. Louisiana now estimates the raw value of the alligator to exceed $60 million annually, with most of that value going to coastal Louisiana communities. As important, private landowners now earn more than 50% of their surface revenue (all income minus minerals, oil and gas) from the alligator, providing unprecedented value from a renewable natural resource of the marsh.

About 2000 Louisiana trappers participate in the fall harvest of 32,000 wild alligators and 55 alligator farms collect more than 350,000 eggs every summer. The economic benefit to coastal communities and economic incentives to conserve wetland habitat binds the alligator to the community culture. In turn, a community constituency respects the alligator, conserves the marsh and depends upon the annual income.

A conservative estimate puts the annual world classic raw hide value at $100 million. Tannery leather values would double this to $200 million and manufacturing of value added finished products is estimated to be at least $400 million. The retail value of finished classic products is at least $1 billion and when the market power of leading fashion brands like Hermes, Gucci Louis Vuitton, Chanel, and others are considered, the value is probably more.

The benefits to commerce through income, jobs and re-investment are relatively easy to document. The emerging benefits to wetland protection and recognition by private landowners of renewable resource values are just beginning to be realized. The greatest challenge may be to engage the manufacturing, retail and fashion house sector to fully accept their role in the sustainable use success story, particularly the opportunity to help economically sustain the trade and to further multiply community benefits --which should include educational enhancements to rural communities.

While a $100 million a year classic raw value is a significant milestone, that value remains just 10% of a conservative $1 billion retail value. For the Marsh to Market story to successfully continue and ensure the trade remains legal, sustainable and verifiable, another chapter must follow -- certifiable trade, which will require the combined efforts of all market sectors—from the Marsh to the Market.

# Economics Benefits of the Commercial Harvest Program of Spectacled Caiman (*Caiman crocodilus*) in Venezuela (1983-2007)

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**ABSTRACT:** The Program of Commercial Use of Spectacled caiman in Venezuela is developed for 24 years, and they are characterized to present great annual variations in the harvest, the percentage of benefit on the capital inverted and in the cost of the fiscal rates. 1.393.803 caimans have been harvested and 1.354.990 has been exported. Four participant sectors in the Program are identified: Governmental, Producers, Hunters and Tanners and Traders. The Governmental sector has obtained benefits by collection to the National Treasure of 807,652 US$ and the Ministry of the Popular Power for the Environment of 11.231.524 US$. The producer sector has invested the amount of 18.034.710 US$, its income are 59.931.676 US$, which represents a 232% of the conducted investment. The tanners and traders have invested 58.404.724 US$, with income of 95.084.040 US$, which means a benefit of 63% of the investment. The hunters benefit has been of 11.979.172 US$. The registered total investment in the Program during 17 years (producer, tanners and traders) are 70.221.161 US$ and the income have been 132.714.434 US$, which means a percentage of 89% of benefit on the investment. The number of tanners and traders has varied per year in 6 to 10, the average of producers is of 329/year. The hunters we considered between 600 and 1,000 people/year. In this analysis the big importance is shown that the skins prices have by external factors to the national life, especially the wars, economic crises and the fashions. The Program of wild harvest is by its characteristics typically male, nevertheless, when new forms of use are developed, especially with the meat, the female participation will be preponderant.

**RESUMEN:** El Programa de Aprovechamiento Comercial de la baba en Venezuela se desarrolla desde hace 24 años, y se caracteriza por presentar grandes variaciones anuales en la cosecha, en los porcentajes de beneficio sobre el capital invertido y en el costo de las tasas fiscales. Se han cosechado 1.393.803 babas y se han exportado 1.354.990. Se identifican 4 sectores participantes en el Programa: Gubernamental, Productor, Cazador e Industriales y Comercializadores. El sector Gubernamental ha obtenido beneficios por recaudación al Tesoro Nacional de 807.652 US$ y el Ministerio del Poder Popular para el Ambiente de 11.231.524 US$. El sector productor ha invertido la cantidad de 18.034.710 US$, sus ingresos son de 59.931.676 US$, lo que representa un 232% de la inversión efectuada. Los industriales y comercializadores han invertido 58.404.724 US$, con unos ingresos de 95.084.040 US$, lo que significa un beneficio del 63% de la inversión. El beneficio de los cazadores ha sido de 11.979.172 US$. La inversión total registrada en el Programa durante 17 años (productores, industriales y comercializadores) es de 70.221.161 US$ y los ingresos han sido 132.714.434 US$, lo que significa un porcentaje del 89% de beneficio sobre la inversión. El número de industriales y comercializadores ha variado por año entre 6 y 10, mientras que el promedio de productores es de 329/año. Los cazadores los estimamos entre 600 y 1.000 personas/año. En este análisis se pone de manifiesto la enorme importancia que tienen sobre los precios de las pieles factores externos a la vida nacional, especialmente las guerras, las crisis económicas y las modas. El Programa de cosecha silvestre es por sus características típicamente masculino, sin embargo, cuando se desarrollan nuevas formas de aprovechamiento, especialmente con la carne, el empleo femenino pasa a ser preponderante.

# Endogenous conservation of wildlife faced to the trade of crocodile organs in traditional medicine purposes in Benin (West Africa)

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**ABSTRACT:** The study seeks to understand the current distribution of crocodile species, the endogenous methods of their conservation and the trade of crocodile organs used in traditional medicine in Benin. 141 water-holes invading by crocodiles were visited. The 3 african crocodiles species exist in Benin: *Crocodylus niloticus*, *Mecistops cataphractus* and *Osteolaemus tetraspis*. *Crocodylus niloticus* was widely distributed (98 %). Depending on the localities, crocodiles were protected because they represent a divinity for certain some people and a totem for others. Such beliefs or customs are the grassroots of «endogenous conservation» and represent the most important method of crocodile conservation. This pact creates a relationship between crocodiles and people based on reciprocity and mutual respect.

Investigations on 33 local markets in Benin, Nigeria and Niger Republics shown that 17 organs and products of crocodile were sold: skin, muzzle, legs, bone, fat, eggs, egg's shell, anus, dropping, teeth, bile, liver, lungs, heart, penis, stones contained in crocodile stomach and alive animal. Crocodile organs cost twice more expensive in Nigeria and Niger ($P<0;05$). This study shows that when wildlife becomes marketable without control local customs, beliefs could be weakened and endogenous conservation jeopardized.

**Key words:** Crocodile, endogenous conservation, trade, traditional medicine, Benin.

# Sustainable Harvest of Wild Adult American alligators (*Alligator mississippiensis*) in Florida

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**ABSTRACT:** Crocodilians have long generation times, high fecundity, and low egg and juvenile survival rates. Consequently, adult females have a high expected value to the population. Harvest of wild adult crocodilians has, therefore, been discouraged by conservation and wildlife trade organizations, such as the CSG and CITES. Ranching has been encouraged as the preferred low-risk option for commercial consumptive harvest of wild crocodilians. Many country and state harvest programs have embarked on capital-intensive ranching programs that have had difficulty maintaining profitability. In Florida, three American alligator (*Alligator mississippiensis*) management programs allow the harvest of larger alligators; nuisance alligators, private lands alligators, and alligators on public waters. We present harvest results and population trend data for harvests on public waters, which are open to the general public. During 1988-2007, adult alligators on 47 alligator management units (AMU) were intensively harvested at a target harvest rate of 6% per year. Harvest quotas were based on population estimates from 1-2 night spotlight surveys conducted each year. Actual mean estimated harvest rate for all AMUs was 5.4% of the adult population. Of 47 areas that were harvested, adult alligator populations increased on 24, remained stable on 17, and declined on 6 (Fig. 1). One area was dropped from the harvest program because it could not sustain harvests. A mean of approximately 23% of the harvest was comprised of adult-sized females during 2000-2006. Simultaneous 50% egg harvests were conducted on 24 AMUs, which also had adult harvests. Of these areas, 23 indicating stable to increasing populations of non-hatchling alligators. Public waters harvests have shifted from commercial harvests to primarily recreational harvests over the years, with each hunter now limited to a quota of two alligators. In 2007, approximately 4,300 licensed hunters harvested approximately 6,500 alligators and paid $1.3 million in fees for that privilege. These revenues are used to support alligator management and conservation in Florida. Harvest programs of large wild crocodilians have lower potential production levels than harvests for ranching, but they also have lower capitalization and operational costs, and are usually profitable if done on a commercial basis. This study, indicate that harvests of adult alligators can be sustainable. However, population monitoring needs to be conducted and regulations enforced to ensure that harvest levels are maintained within target ranges.

# Effects of Hurricanes Katrina and Rita and Severe Drought Conditions on Alligator Nesting in Coastal Louisiana

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**ABSTRACT:** The American alligator (*Alligator mississippiensis*) occurs in a variety of habitats, with the majority of Louisiana's population inhabiting coastal marshes. In 2005, Louisiana's coastal alligator population generated its fourth highest nesting output. However, this high nest productivity would be followed by a tandem of devastating environmental events including: two major hurricanes and a severe drought. Due to the state's unique topography, the residual effects of these regional storms and drought conditions caused dramatic alterations in both habitat quality and animal physiology. During summer 2006, LDWF program staff estimated by aerial survey that only 20,387 alligator nests were present in coastal marsh habitat, a dramatic decrease from the previous year's estimate of 41,392 nests. As our alligator management program's philosophy of sustainable utilization encompasses the inter-relationship between habitat quality, nesting output and wild harvest, program staff temporally reduced harvest quotas in several parishes which demonstrated both significant habitat damage and reduced nesting. In 2007, marked improvements in habitat quality (i.e., increased rainfall, marsh vegetation regeneration) were observed. These improved environmental conditions paired with a highly resilient alligator population led to the third best year for alligator nesting on record, with some 42,150 nests being produced in Louisiana's coastal habitat.

# Effect of Hurricane Rita and a Severe Drought on Alligators in Southwest Louisiana

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**ABSTRACT:** Coastal Louisiana was impacted by two major hurricanes in 2005, with Hurricane Katrina coming ashore in August closely followed by Hurricane Rita making landfall in September. The American alligator (*Alligator mississippiensis*) occurs in a variety of habitats statewide, with the most dense population occurring in the coastal marshes. Alligators do exist in brackish marshes, but poorly tolerate highly saline conditions and do not nest in salt marshes. We collected blood samples from a large series of alligators, to determine how the hurricane storm surge and inundation of coastal marshes with salt water affected them. We measured plasma osmolality, corticosterone (stress hormone), and electrolytes (Na, K, and Cl). The first set of blood samples were obtained within a month of Hurricane Rita and were from 11 alligators (size range 167.6 – 256.5 cm) collected at Holly Beach, Louisiana. From 8 February – 9 August 2006, another 201 alligators (size range 61.0 – 243.8 cm) were sampled on Rockefeller Wildlife Refuge. During this study, habitat conditions in Louisiana were adversely affected by the worst drought in 111 years of recorded weather data. Plasma corticosterone from the eleven alligators sampled at Holly Beach within a month of Hurricane Rita were very high, averaging 10.34 ng/ml. Plasma sodium and plasma corticosterone were strongly positively correlated with plasma osmolality in these samples. By February when we were first able to sample alligators at Rockefeller Refuge, plasma corticosterone levels were normal (0.40 ng/ml, n = 33). By early spring drought conditions were intensifying, and plasma corticosterone levels began to rise, with average levels of 3.12 ng/ml in April and 5.92 ng/ml in May. The highest corticosterone level we have ever measured (36.21 ng/ml) was recorded in an alligator caught in June, at the peak intensity of the drought; this animal had a baseline corticosterone level of 0.41 ng/ml when caught in July 2001. The severe drought had a profound negative impact on alligator nesting in coastal Louisiana in 2006, with an estimated production of only 20,387 nests as compared to 41,392 nests in 2005. Late summer rainfall in 2006 led to some dilution of high salinities (9 August salinity values 5.7 – 9.3 ppt as compared to 8.8 – 10 .4 ppt on Feb 8, and 7.4 – 12.5 ppt on April 6) and alligator corticosterone levels decreased to 1.78 ng/ml; essentially back to normal levels for wild alligators. Adequate rainfall in winter 2007 led to marked improvement in habitat conditions, and 2007 was the third best year for alligator nesting on record, with some 42,315 nests being produced in the coastal zone of Louisiana. This resilient species rapidly recovered from severe environmental impacts but long term monitoring will continue.

# Dr. Tirtha Man Maskey Wildlife Centre

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**ABSTRACT:** Dr. Tirtha Maskey is a well known name in Nepalese Conservation especially for his significant contribution to Gharial Conservation. We lost him after an unfortunate Chopper crash on 23rd September 2006.

Dr. Maskey has left behind a huge resource for present and future wildlife managers. To leave this knowledge, WWG established Dr. Maskey Wildlife Centre including a library on its office. To complete these resources, WWG has published two books which one presenting his PHD Thesis entitled: "Gharial Conservation in Nepal". This book is a scientific research entitled: "Movement and Survival of Captive Reared Gharial in the Narayani River, Nepal ". It describes technique and conservation effort to breed gharials before release it to their natural habitat and so bring this specie back from the extinction. Moreover it includes the latest presentation of Dr. Maskey in June 2006 (18th CSG meeting) entitled as "Gharial Conservation in Nepal: Results of a Population Reinforcement Program". The main objective behind publishing this book is to provide Dr. Maskey's latest information about the Gharial conservation in Nepal.

Lastly, we believe that Dr. Maskey's works will also be a motivation for others to carry out similar studies to save endangered species from extinction.

# The Importance of Systematics in Conservation: The Nile Crocodile (*Crocodylus niloticus*) As A Case Study

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**ABSTRACT:** As our understanding of evolution, and its impact on the ecology and population dynamics within any given species, become more refined systematic questions become more relevant to conservation decision making. How can we conserve a species we do not yet recognize as a valid taxon? Similarly, how do we set conservation priorities if we do not understand the evolutionary trajectories of, and relationships between, different populations within a known species? Starting with its formal description in 1768, the Nile crocodile has been a plague of taxonomic and systematic confusion. Laurenti (1768) incorrectly described *Crocodylus niloticus* from illustrations of a dwarf caiman (*Paleosuchus spp*.) (Magnusson 1992) with a text description so vague it could equally be applied to *C. palustris* and *C. porosus* (Anderson 1899). Geoffrey (1807) and Cuvier (1812) both examined actual specimens from throughout Africa and described as many as five separate species from different regions throughout the continent (Anderson 1899). Fuchs (1974) proposed 7 distinct subspecies based on morphological characters from skins in the commercial trade. Two recent studies, Schmitz *et al*. (2003) and Hekkala (2004), have reignited this controversy with molecular systematic evidence. This preliminary molecular data suggests that Nile crocodile populations in West Africa represent a unique lineage older than modern *C. niloticus*, creating not an issue of splitting taxa but recognizing two equally valid, cryptic taxa. Here we present a review of Nile crocodile systematics, including new molecular data, with a discussion on the conservation implications.

# The phylogeography of the yacare caiman, *Caiman yacare*, of central South America

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**ABSTRACT:** The yacare caiman, *Caiman yacare*, occupies wetland habitat in portions of two major river basins of central South America. The species occurs in the Amazon river basin of Bolivia and the Paraguay-Paraná river basin of Brazil, Paraguay and Argentina. It is one of the most abundant crocodylian species of the world and represents a high percentage of the world hide trade.
In this study, DNA was extracted from 211 samples taken over the entire range (Amazon, n=143; Paraguay-Paraná, n=68) and examined. Molecular analyses were performed using cytochrome b mitochondrial markers. Twenty-five haplotypes were discovered, each occurring in only one river basin (Amazon, n=16; Paraguay-Paraná, n=9). Distinct molecular compositions were found in drainages within the two basins. Results from this study may be valuable for wildlife management of sustainable harvest planning and the conservation of the species.

**RESUMEN:** El lagarto, *Caiman yacare*, habita humedales en porciones de las dos cuencas mayores en centro Sud América. La especie ocurre dentro la cuenca del río Amazonas boliviano y la cuenca de los ríos Paraguay-Paraná en Brasil, Paraguay y Argentina. Es una de las especies de cocodrilianos más abundantes del mundo y representa un alto porcentaje del comercio mundial de cueros.
En este estudio, se extrajo ADN de 211 muestras tomadas de la distribución entera (Amazonas, n=143' Paraguay-Paraná, n=68) y fueron examinadas. Se realizó analisis moleculares utilizando los marcadores mitocondrial citacromo b. Se descubrió 25 haplotipos, de los cuales ocurre unicamente en una sola cuenca (Amazonas, n=16; Paraguay-Paraná, n=9). Drenajes adentro de las dos cuencas mayores tambien tienen composiciones moleculares distintas. Los resultados de este estudio pueden ser útiles en el manejo de los lagartos dentro la planificación de la cosecha sostenible y la conservación de la especie.

## INTRODUCTION

The yacare caiman has been subjected to large-scale commercial hunting during most of the last 50 years. Much of the exploitation in the range states of Bolivia, Brazil, Paraguay and Argentina occurred with government authorization. Little effective control existed in spite of legislation specifying limits for export quotas, minimum size, and hunting seasons. Although Brazil made all commercial exploitation of wild populations illegal in 1967, porous frontiers in all of the range states allowed free movement of wildlife products. Government officials and military personnel were frequently involved in the exploitation, complicating control efforts. CITES has imposed trade sanctions, and both Bolivia and Paraguay, the largest exporters of yacare hides, have independently suspended exports at different points in time.

All four range states have sustainable use programs in development, although none have firm biological bases for determining the harvest regions. In Bolivia, where the only functioning wild harvest currently occurs, size-class abundance data from limited surveys is the only factor employed. While adequate as a minimum measure when sufficient surveys are undertaken, additional population data would provide for more robust management. The Venezuela program placed a priority on obtaining biological data for a decade before harvest, and a self-financing program developed incorporating user fees. The under-funded program in Bolivia continues to suffer from inadequate survey personnel, lack of control of areas harvested and annual over-harvesting. Wildlife management programs are often non-existent or severely under-funded. Hides are reported to be collected clandestinely throughout the year (J. Aparicio, pers. comm.).

The following study presents the first molecular data for *Caiman yacare* populations. Information on the phylogeny, haplotype partitioning, gene flow and cryptic populations will be valuable for wildlife managers to incorporate into sustainable use planning. Detailed laboratory procedures have been omitted for simplicity.

**Sample preparation**

The fresh samples prepared for this study consisted mainly of blood or muscle. Blood samples were taken from the cervical sinus using sterile 2 cc syringes and needles (Olson *et al.* 1975) and a sample of about 1 ml was drawn and introduced into blood lysis buffer (modified from White and Densmore 1992). All of the Paraguayan samples (n=21) originated from the osteological collections in the Florida Museum of Natural History (FLMNH) at the University of Florida. All bone sample preparations were performed in a laminar flow hood to reduce the chance of cross-contamination. A Dremel MultiPro™ variable speed rotary tool was used to perforate the bone. Replicate sample tubes were made for each specimen.

All samples were obtained with proper documentation. The Bolivian authorities issued CITES export permit No. 00470 and US entry was under CITES import permit No. 816827. Argentine samples were exported with CITES permit No. 023752 and imported under US CITES permit No. 03US714329/9. Venezuelan material was obtained by FLMNH from the Venezuelan Government Wildlife Service.

DNA isolations were usually performed with a phenol-chloroform isoamyl alcohol (PCl) isolation protocol modified from Hillis *et al.* (1996) with 95% ethanol precipitation. Bone samples proved the most problematic, requiring the most time invested and generally resulting in low amounts and poor quality of the DNA extracted. Bone samples require special preparation to eliminate PCR inhibitors that may be co-isolated during extraction. A successful protocol, modified from Ye *et al*. (2004), overcame these problems. Bone samples were processed with the Qiaquick™ PCR Purification Kit with very good results. The PCR inhibitors were successfully removed with the kit.

**Sequence data**

The cytochrome (cyt) *b* gene was selected for intraspecific relationship analyses. The *Caiman* cyt *b* gene is 1150 bp long, from position 14,461 to 15,610. This is a highly variable region and of great value for phylogenetic research (Avise 2000, 2004).

The entire mitochondrial (mt) genome for a closely related *Caiman crocodilus* (Janke et al.2001, GenBank accession number NC_002744.2) from a Venezuelan specimen has been published. This facilitated direct comparison with my PCR amplification product sequences and in the design of new primers. The *Caiman* mtDNA genome is 17,900 bp long and all further position references are relative to the Caiman mitochondrial genome of Janke et al. (2001) unless otherwise noted.

Amplifications using primers modified from Glenn *et al*. (1998) successfully produced 668 bp Cyb Fragment 1. A new set of cyt *b* primers were designed to amplify a partially overlapping fragment, Cyb Fragment 2. This new fragment is 649 bp long, with a 117 bp overlap for sequence concatenation. The reconstructed fragment was 1200 bp long, corresponding from positions 14,461 to 15,660 and represents the entire cyt *b* gene. Other primers were designed as needed to complete the amplifications for intransigent samples. Resulting sequences had an average of 1143 bp.

The preliminary PCR amplifications of cyt *b* fragments were run on an Applied Biosystems, Inc. (ABI) 377 and 3700 automated sequencers as well as Amersham MegaBACE™ 1000 96 capillary sequencers. For haplotype verification, new PCR amplification products were resequenced. Sequences were evaluated and concatenated using Sequencher 4.5. Files were then imported into PAUP 4.0b10 (Swofford 1998) for phylogenetic analyses and tree generation. Likelihood model selection was performed using MODELTEST v.3.06 (Posada and Crandall 1998). Tree support through Bayesian inference was accomplished using MrBayes v.3.1.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). TCS v. 1.21 software (Clement *et al*. 2000) was used to create haplotype genealogy networks by implementing the “statistical parsimony” algorithm described by Templeton *et al*. (1992). Arlequin v. 3.0 (Excoffier *et al*. 2005) was used for AMOVA analyses, genetic distance measurements, and Mantel tests.

**RESULTS**

Cytochrome *b* sequences for each of the 214 samples were evaluated using Sequencher 4.5. Some samples were re-amplified as necessary for error-free final sequences. The concatenated sequences, averaging 1143 bp, were compared and analyzed to determine haplotypes. New haplotypes were analyzed with maximum parsimony (MP), maximum likelihood (ML) and minimum evolution (ME) comparisons for associations with other haplotypes. A conservative approach was taken in establishing haplotypes and samples were reamplified and sequenced repeatedly.

The evolving group of haplotypes was consistently re-analyzed, especially the 12 ‘singleton’ haplotypes represented by only 1 individual. Haplotype trees were constructed and one step associations were carefully analyzed to verify differences. Upon final haplotype assignment, the group was assembled into a contig in Sequencher 4.5 and imported to Paup v.4.0b10. Additional analyses were performed with Arlequin v.3.0, as well as DNAsp v.4.10.4 (Rozas *et al*. 2003). As the haplotype group was assembled, the sample sequence data set was repeatedly compared and assigned as needed. Twenty-five cytochrome *b* haplotypes were finally determined.

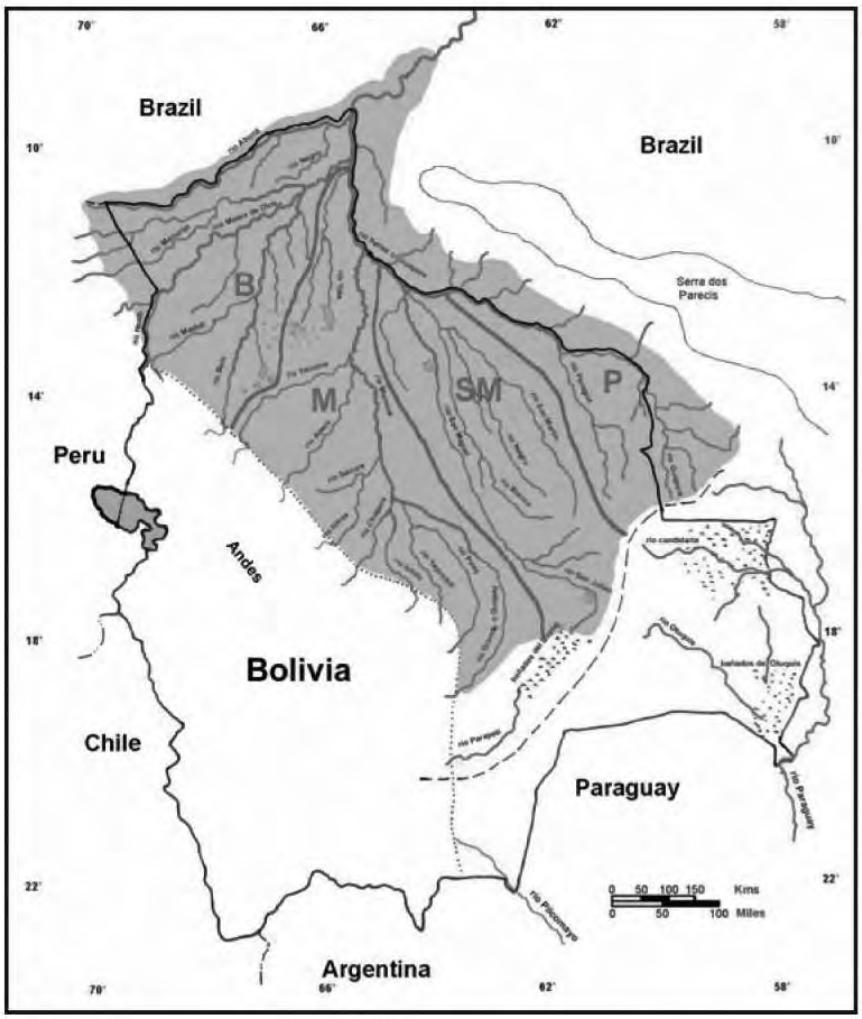



**Figure 1.** River drainage divisions used for segregating samples from the Amazon (northern) distribution of *Caiman yacare*. B = río Beni, M = río Mamoré, SM = río San Miguel, P = río Paraguá.

Analysis began by assessing the haplotypes as one group. The samples, and also the haplotypes as seen below, were split into two groups representing the two major distribution areas (north and south) (see Fig. 1). These were further divided into 'populations', taking into consideration the sampling localities and hydrogeographic topography. Although the yacare caiman is not principally a riverine crocodylian, they are always present even in the largest rivers. River systems are assumed to be an important conduit for migration and resulting gene flow and are used here to help define boundaries for the analyses. The Amazon distribution (northern) was divided into 4 main drainages: the río Beni, río Mamoré, río San Miguel and río Paraguá.

The Paraguay-Paraná (southern) distribution represents a very different, linear topology and was divided into two portions (See Fig. 2). It has been noted in molecular studies of fish that genetic diversity generally increases downstream, especially when taking into account additive functions of major tributaries. In the southern distribution of *Caiman yacare*, the río Pilcomayo is a major tributary with very large populations along its considerable length (~700 km). With this in mind, the arbitrary division was placed just upriver of the union the río Pilcomayo and the río Paraguay.




**Figure 2.** River drainage division used for segregating samples from the río Paraguay-Paraná (southern) distribution of *Caiman yacare*. Py = río Paraguay, Pa = río Paraná. Note length and location of western tributary río Pilcomayo.

After all sample haplotypes were determined, the samples were assigned and a drainage distribution table was constructed (see Table 1). Of the 214 samples, 16 haplotypes are distributed in the northern distribution and 9 in the southern distribution. There is a complete division of haplotypes with none shared in both distributions. A comparison of all haplotypes (1197 bp) revealed 23 polymorphic sites with 10 singleton variable sites and 12 parsimony informative sites. A total of 1063 invariable sites were detected and, as a result of different primer sets and inconsistent success in amplification, 112 sites had missing data.

The haplotypes trees were constructed using PAUP 4.10b with MP, ME and ML evolutionary models without resolving all haplotype associations. Haplotypes always segregated between the two distribution basins with none shared, but the internal topologies varied and many branches had poor bootstrap support (see Figure 3). Maximum parsimony (MP) analyses used heuristic searches starting with stepwise addition trees and replicated 100 times. Branch swapping was performed by the tree-bisection-reconnection (TBR) method. While 98 MP trees were constructed with the identical scores, they had high concordance on the main branch arrangements and differed mainly on tip associations, especially within the southern distribution (see Figure 3).

This becomes evident when a comparison is made between the 2 distributions with this 1161 base data set. The northern distribution (16 cyt *b* haplotypes, mean 1155 base length) has 7 variable parsimony uninformative sites with 7 parsimony informative sites. The mean

frequency base compositions are 30.3%A, 25.4%T, 34.2%C, and 10.1%G. The southern distribution (9 cyt *b* haplotypes, mean 1151 base length) has 9 variable parsimony uninformative sites with only 2 parsimony informative sites. The mean frequency base compositions are 30.2%A, 25.2%T, 34.3%C, and 10.3%G. These data show an under-representation of guanine as described for the mitochondrial genome by Zhang and Hewitt (1996).

**Table 1.** Distribution of 25 cytochrome *b* haplotypes for *Caiman yacare*. No haplotypes detected are shared among basins. Locality for original haplotype specimens: Bo=Bolivia, Pa=Paraguay.

| **Haplotype:** | **Beni** | **Mamore** | **San Miguel** | **Paragua** | **Paraguay** | **Parana** |
|---|---|---|---|---|---|---|
| **Amazon** | N=20 | N=29 | N=74 | N=20 | N=44 | N=22 |
| Cy01Bo | 2 | 0 | 0 | 0 | 0 | 0 |
| Cy03Bo | 16 | 18 | 39 | 0 | 0 | 0 |
| Cy05Bo | 2 | 7 | 7 | 0 | 0 | 0 |
| Cy02Bo | 0 | 1 | 0 | 0 | 0 | 0 |
| Cy04Bo | 0 | 1 | 15 | 18 | 0 | 0 |
| Cy29Bo | 0 | 1 | 1 | 0 | 0 | 0 |
| Cy30Bo | 0 | 1 | 2 | 0 | 0 | 0 |
| Cy07Bo | 0 | 0 | 1 | 0 | 0 | 0 |
| Cy12Bo | 0 | 0 | 1 | 0 | 0 | 0 |
| Cy14Bo | 0 | 0 | 1 | 0 | 0 | 0 |
| Cy21Bo | 0 | 0 | 2 | 0 | 0 | 0 |
| Cy22Bo | 0 | 0 | 1 | 0 | 0 | 0 |
| Cy23Bo | 0 | 0 | 1 | 0 | 0 | 0 |
| Cy28Bo | 0 | 0 | 3 | 0 | 0 | 0 |
| Cy08Bo | 0 | 0 | 0 | 1 | 0 | 0 |
| Cy27Bo | 0 | 0 | 0 | 1 | 0 | 0 |
| **Paraguay-Paraná** | | | | | | |
| Cy15Bo | 0 | 0 | 0 | 0 | 22 | 1 |
| Cy16Bo | 0 | 0 | 0 | 0 | 3 | 3 |
| Cy17Bo | 0 | 0 | 0 | 0 | 1 | 0 |
| Cy18Bo | 0 | 0 | 0 | 0 | 0 | 1 |
| Cy19Bo | 0 | 0 | 0 | 0 | 10 | 17 |
| Cy20Bo | 0 | 0 | 0 | 0 | 1 | 0 |
| Cy24Bo | 0 | 0 | 0 | 0 | 3 | 0 |
| Cy25Pa | 0 | 0 | 0 | 0 | 1 | 0 |
| Cy26Pa | 0 | 0 | 0 | 0 | 2 | 0 |

The Tamura and Nei model (TrN+I) of evolution was selected using Modeltest 3.06 for ML analyses. Excess transitions, unequal nucleotide frequencies and a variation of substitution rate among different sites are taken into account in this model. In addition, the proportion of invariable sites (+I) is used in the calculation. Employing the likelihood test ratio (LTR), a molecular clock model was compared and accepted, and a phylogram tree, rooted at the *C. latirostris* split at 24 mya, was constructed and measured. Results show that the haplotype divergence time between basins is relatively recent and is estimated at ~1.5 mya and subsequent within basin radiation of populations occurred in the last 500,000 years. This follows a similar pattern seen for *Caiman crocodilus* cytochrome *b* sequences, with the

divergence between the Amazon and Orinoco basins occurring at ~2.75 mya and within basin radiation occurring during the last million years (see Godshalk, this volume).

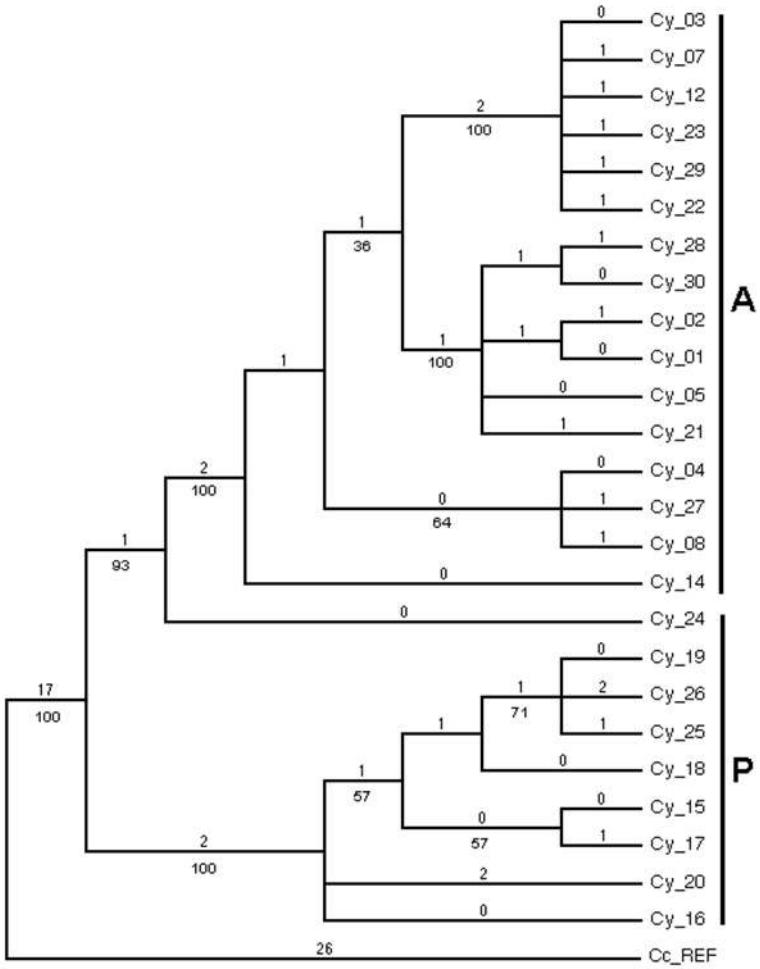



**Figure 3.** Maximum parsimony (MP) tree for 25 cytochrome b haplotypes of *Caiman yacare* rooted with *Caiman crocodilus*. Number above branch refers to mutational steps, number below refers to percentage consensus of 98 equally parsimonious trees. (length=158, CI=0.918). Bracket A = Amazon basin, northern distribution, P = Paraguay-Paraná basin, southern distribution. Haplotypes are unique to each distribution. Abbreviations: Cc_REF(*Caiman crocodilus* reference sequence), Cy_ (*C. yacare* plus haplotype number).

As many haplotypes differ with single mutational steps, haplotypes trees could not be easily resolved using bootstrap values. A haplotype network was constructed using TCS v.2.1.1 to better understand the phylogeny. A 15 single mutational step difference occurs between the 2 most distant haplotypes in the network. Within both North and South haplotype networks, a 7 mutational step difference occurs between the most distant haplotypes (see Fig. 4). While some haplotype reticulations remain unresolved, the overall pattern and distinction between basins are clear. The separation between basin clades is 2 or 3 mutational steps depending on the reticulation.

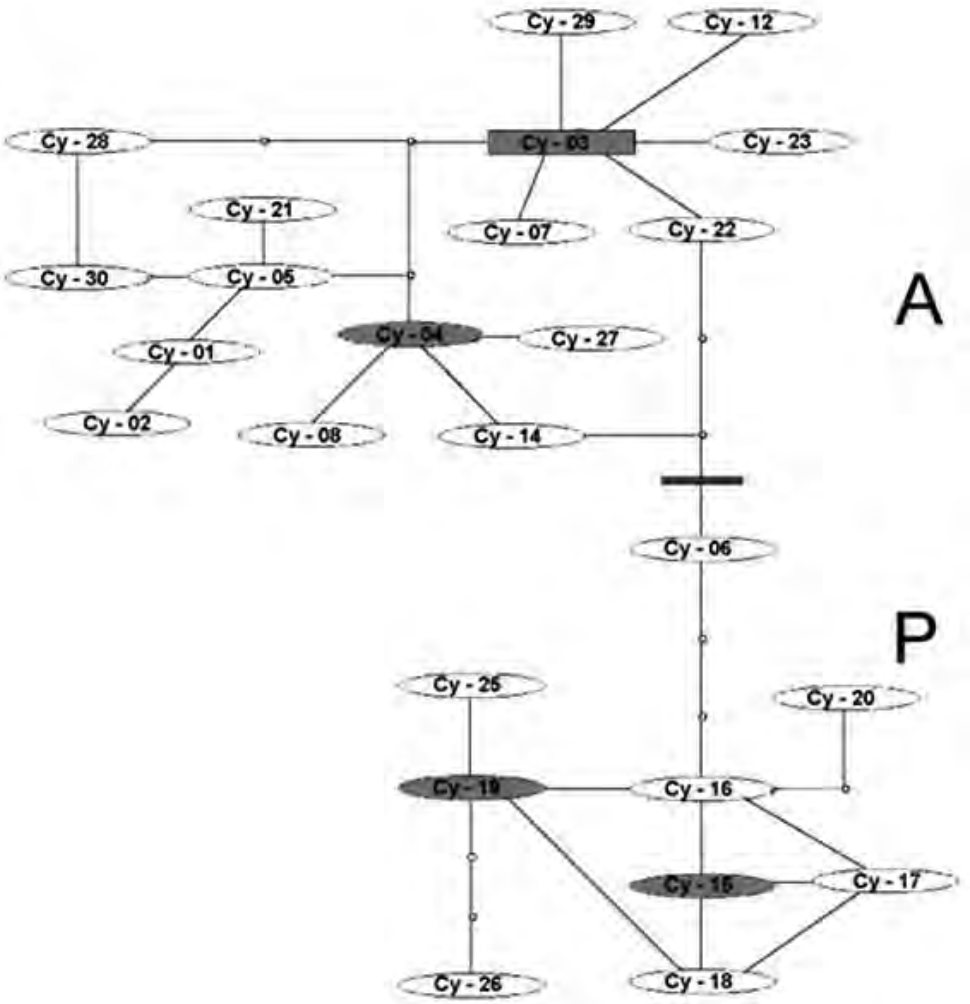



**Figure 4.** Genealogy network for 25cytochrome b haplotypes in *Caiman yacare*. Geographic division (blue line) occurs between river basins: A = Amazon, P = Paraguay-Paraná. Branch length between haplotypes and nodes equal one base change. Red symbols indicate the most common haplotypes in each distribution. Haplotype Cy-03 (rectangle) may represent the ancestral line.

Results from an Analysis of Molecular Variance (AMOVA) (Excoffier *et al*., 1992) implemented in Arlequin v3.0 (Excoffier *et al*. 2005) added information on the distribution and separation of haplotypes: 72.4% of the variation was attributed between the 2 basins, 7.7% of variation was attributed between populations within the basins, and 19.9% of the variation was attributed to within population differences. The pairwise distance method $F_{st}$ value between basins was 0.7576, significant at the $p < 0.001$ level.

Within the northern distribution, two common haplotypes account for 76.2% of the 143 samples from the basin (Cy_03=51%, Cy_04=25.2%). Private haplotypes (i.e. occurring in only one population) were detected in all 4 populations of the Amazon distribution. A similar situation exists in the southern distribution. Two common haplotypes account for 75.7% of the 66 samples from the basin (Cy_19=40.9%, Cy_15=34.8%). Private haplotypes were also detected in both populations of the southern distribution. An AMOVA analysis on the drainage populations provides additional information on within and between basin comparisons (see Table 2).

**Table 2.** Analysis of $F_{st}$ values for populations of *Caiman yacare*. Top set: Population pairwise distance $F_{st}$ values, Bottom set: Matrix of significant $F_{st}$ values, $p < 0.05$.

| | | Beni | Mamoré | San Miguel | Paraguá | Paraguay | Paraná |
|---|---|---|---|---|---|---|---|
| **North** | Beni | 0 | | | | | |
| | Mamoré | -0.00576 | 0 | | | | |
| | San Miguel | 0.04044 | 0.00761 | 0 | | | |
| | Paraguá | 0.76667 | 0.64328 | 0.46204 | 0 | | |
| **South** | | | | | | | |
| | Paraguay | 0.78787 | 0.76621 | 0.74353 | 0.81377 | 0 | |
| | Paraná | 0.89419 | 0.85349 | 0.79758 | 0.95018 | 0.22362 | 0 |

| | | Beni | Mamoré | San Miguel | Paraguá | Paraguay | Paraná |
|---|---|---|---|---|---|---|---|
| **North** | Beni | | - | - | + | + | + |
| | Mamoré | - | | - | + | + | + |
| | San Miguel | - | - | | + | + | + |
| | Paraguá | + | + | + | | + | + |
| **South** | | | | | | | |
| | Paraguay | + | + | + | + | | + |
| | Paraná | + | + | + | + | + | |

Pairwise distance comparisons were made within and between all populations and a matrix was constructed using Arlequin 3.0 (Excoffier *et al.* 2005). A corrected average pairwise difference was also computed (see Table 3).

**Table 3.** Average pairwise distances for populations of *Caiman yacare*. Above the diagonal: average number of pairwise differences between populations $(\pi_{XY})$. Diagonal elements: average number of pairwise differences within population $(\pi_X)$ . Below diagonal: corrected average pairwise difference $(\pi_{XY}-(\pi_X+\pi_Y)/2)$.

| | | Beni | Mamoré | San Miguel | Paraguá | Paraguay | Paraná |
|---|---|---|---|---|---|---|---|
| **North** | Beni | 1.20000 | 1.41034 | 1.66757 | 3.00000 | 7.55581 | 7.80909 |
| | Mamoré | -0.00493 | 1.63054 | 1.81081 | 2.82414 | 7.43865 | 7.70219 |
| | San Miguel | 0.08978 | 0.01775 | 1.95557 | 2.47838 | 7.41169 | 7.65479 |
| | Paraguá | 2.30000 | 1.90887 | 1.40059 | 0.20000 | 6.77442 | 7.00909 |
| **South** | Paraguay | 6.04662 | 5.71419 | 5.52471 | 5.76523 | 1.81838 | 1.56237 |
| | Paraná | 6.96667 | 6.64450 | 6.43458 | 6.66667 | 0.41075 | 0.48485 |

A Mantel test was implemented by Arlequin v.3.0 to test the correlation between genetic pairwise distance $F_{st}$ values and riverine distances between populations. A positive correlation was determined ($r=0.6962$) and was established through a permutation test (2000 permutations) and was significant at the $p<0.01$ level. Spatial autocorrelations are significant following the $F_{st}$ values given in Table 2. using the distance matrix constructed for the test (see Table 5)

## DISCUSSION

Populations of *Caiman yacare* are subject to strong pressures from legal harvest as well as low level poaching. Thousands of adult animals are culled annually from wild populations in Bolivia. The harvest program has been temporarily suspended in Paraguay but preparations are being made to resume. Yet surprisingly, little information has been accumulated about the biology of the yacare caiman. Much of what is known comes from the Brazilian Pantanal where conditions are unique and wild harvest is not permitted. However, wildlife managers must incorporate all relevant information in order to make informed decisions regarding sustainable use of this species.

Data generated from this study underscore the potential and the limitations of molecular information. These data compliment knowledge on the biology of the species. From the mtDNA sequence data, derived from matrilineal inheritance, a distinct split is seen between the North and South haplotype distributions (see Table 3, Figure 3). Results from the Analysis of Molecular Variance (AMOVA) attributes 72.4% of the variation between the two distributions.

Considering the sample localities and overall sample size, shared haplotypes would likely have been detected. Of the 214 animals sampled for this study, 22 caiman were from the Bolivian Pantanal. This is close to the Amazon basin boundary, an area of potential mixing between the North and South distributions. Additionally, 9 samples were from northern Paraguay along the main río Paraguay corridor, relatively close to another potential mixing area. These data suggest that the geographic separation of the río Amazon basin (Northern distribution) and the ríos Paraguay-Paraná basin may be a significant barrier for migration of females.

Large adult male caiman establish breeding areas which are defended against competing males. These dominant male patrol their area and aggressively exclude not only reproductive adult males, but also sub-adults (Thorbjarnarson 1991, 1993; Coutinho 2000). Studies on seasonal movements show that while caiman are displaced by the rigors of the intense wet and dry season regime, their net movement is generally not great. Adult females, however, show less movement and the adult males respond to this by establishing territories relatively close to the dry season refugia. In this manner, sub adult males are forced further away from the dry season habitat (Thorjarnarson 1991, 1993).

The genealogy network graphically displays the division between haplotype groups of the two major distributions (see Figure 4). A minimum of 5 evolutionary steps separate the two haplotype groups. Most relationships of within group haplotypes are separated by single evolutionary step. Although some relationships between haplotypes cannot be resolved by transition-transversion analyses and lead to multiple pathways, these reticulations do not disrupt the overall structure of the 2 group networks.

The effect of the large basin haplotype division is emphasized by the relative within group uniformity. The $F_{st}$ values for cytochrome b haplotypes indicate that while some geographic structuring is evident, the río Beni, the río Mamoré and río San Miguel populations are not significantly different from each other (see Table 4). This is understandable given the topography and environments resulting from periodic wet season flooding. Annual flooding

events often negate drainage boundaries and large-scale pooling, particularly acute approaching river junctions, frequently extend hundreds of $km^2$. This region is covered with isolated lakes and ponds of various sizes that may serve as 'stepping stones' for gene flow between river drainages that provide much shorter distances than linear riverine mileage would imply.

Ephemeral streams crossing the open savannas may also serve as genetic conduits. These conditions of seasonally coalescing waterbodies, permanent lakes and intermittent streams are common but found to a lesser degree in the eastern region of río San Miguel. For this reason, $F_{st}$ values are slightly higher than Beni or Mamoré values but still not significant at the $p < 0.05$ level. I collected 74 samples from the río San Miguel population and similarities detected in samples from both the río Beni (N=20) and río Mamoré (N=29) populations reflect the fact that they are largely subsets of the San Miguel population.

This is not appear to be the case with the río Paraguá population (N=20). The geography of the area has isolated the river to a greater degree than the other three populations mentioned above. The Fst values indicate the distinction although this is primarily due to the presence of 2 unique haplotypes.

In a comparison of $F_{st}$ values and average pairwise distances, it can be observed that the greatest similarity for populations within the Northern distribution is with the nearest geographic neighbor (see Tables 4 and 5).

For the Southern distribution, the río Paraguay population is significantly different from the Paraná population at $p < 0.05$ level (see Tables 4 and 5). In the linear hydro-geographic setting of this distribution, there are no consistent short-cut conduits for gene flow outside of the main river corridors as seen on the flooded savannas of Bolivia. In this sense, riverine and straightline distances are essentially the same.

Results of a Mantel test also confirms structuring according to river geography. There is a positive correlation of isolation by distance. These data indicate important processes of haplotype distribution among the populations. Movements by female caiman are sufficient to distribute common mtDNA haplotypes across large areas, but restricted enough for the evolution and establishment of geographically isolated haplotypes.

It has been shown that phylogeographic patterns that are shared by sympatric or co-distributed species may shed light on a common historic biogeography (Avise 2000). In this manner, comparative molecular analyses for the sympatric crocodylian species may help the understanding of the development of the Amazon. Numerous vertebrate species are common to both the Amazon and Paraguay river basins but no comparative studies have yet been undertaken. Further investigation into the molecular patterns exhibited by these species may assist in reconstructing the geographic histories that until now have proved elusive.

**Conservation Genetics** – Genetic information is serving an increasing role in conservation biology. New techniques have been developed that produce more data, more accurately in less time and for less money than previously imagined. The utility of the resulting data depends on the unique situation of species in question. For example, genetic profiling is crucial for work with endangered species when considering captive breeding, re-introduction programs or in population viability modeling. This, however, is not the case with *Caiman*

*yacare*. The yacare caiman, along with the common caiman, *C. crocodilus*, are perhaps the two most numerous of crocodylian species. Conservation genetics have a much different role to play in the management of abundant populations. Data generated from the analyses in this study as documented above will have impact in the following areas:

**Taxonomic clarification** – Conservation efforts can become focused when taxonomic uncertainties are resolved. Daugherty *et al*. (1990) observed that accurate taxonomies "are not irrelevant abstractions, but the essential foundations of conservation practice". *Caiman yacare* is now regarded by some researchers as a subspecies of the common caiman. This unwarranted convention has been adopted by some authors since Werner (1933) first referred to the yacare caiman as *Caiman crocodilus yacare*. Apparently, this is based only on superficial similarity rather than biological objectivity and is counter to the rules of the International Commission of Zoological Nomenclature. The data presented above emphasizes the position of *C. yacare* as a species. This presents no legal ramifications as CITES, the US Fish and Wildlife Service, the European Union and all the range states (Argentina, Bolivia, Brazil, and Paraguay) have previously regarded *C. yacare* as a species in their laws and regulations.

The unexpected results regarding the genetic distance as described with the *Caiman crocodilus fuscus* haplotype (see Godshalk, this volume) must be resolved through further study. Review of the biogeographic history suggests comparable reproductive separation from the Orinoco basin populations of *Caiman crocodilus*. This may lead to a new species designation for the Central American populations, now considered *C. c. chiapasius*. Resolving the identity for the Pacific drainage populations from Colombia, also considered *C. c. chiapasius*, is also necessary but very difficult given the political situation there.

There remains the uncertain identity of populations from the Caribbean drainages of northern Colombia and western Venezuela. Genetic analyses of these *C. c. fuscus* (*sensu stricto*) populations would help complete the genetic mosaic of this wide ranging group. This could be undertaken with the collaboration of Colombian and Venezuelan colleagues. A taxonomic change for these latter populations might entail legal repercussions, as Colombian hide exports from farming operations in the region are very large (>500,000/yr, Velasco and de Sola 2005) and the regulations have been in place for over 15 years.

Data presented in Godshalk (this volume) show significant genetic distance between the Orinoco and Amazon populations of *C. crocodilus*. Reproductive isolation is complete owing to geographic separation and the processes toward separate speciation continue. Further investigations are required to reveal the extent of separation and clarify the taxonomic status.

**Genetic diversity** – Wildlife species are typically characterized according to the genetic diversity detected within and between the various subpopulations. Appropriate species management will seek to quantify and maintain a broad level of natural hetrozygosity. Relative heterozygosity levels should be maintained to avoid inbreeding or bottlenecks. The results of this study show that the populations of *C. yacare* show a relatively high degree of genetic diversity compared to non-piscine vertebrates. Large population numbers remaining in the range states have maintained high numbers of haplotypes for cytochrome *b*. No indications of inbreeding depression or reduction in heterozygosity due to population bottlenecks were detected as measured by $F_{is}$ values (data not shown).

As this species is subject to commercial exploitation that selectively eliminates large adult males, wildlife managers will need to periodically monitor the genetic diversity, drift or reduction in heterozygosity. Controversy exists over this type of harvest strategy, especially in a species where dominant males represent a disproportionate segment of the breeding population. Continual removal of the largest members, and potentially a large segment of the breeding alpha male population, may ultimately cause an unfavorable genetic drift.

**Characterizing movements** – Effective wildlife management requires knowledge of species movements. Biological investigations can yield direct information on movements at a certain scale. Genetic data can give insight to movement on a much different scales. As described above, the cytochrome b data reveal that female yacare caiman do not appear to cross the barrier between the Amazon and Paraguay-Paraná river basins with enough regularity to establish haplotypes that are shared between basins. This result was unanticipated, although movements by females are generally less than males. This barrier may not impede migration of males, however, resulting in the lack of distinct segregation of microsatellite alleles (data not shown). This movement would be very difficult to quantify by other methods. Further studies will be necessary if wildlife managers want more fine scale movement data.

While traditional F-statistics and other genetic metrics produce migration indices, often expressed as Nm values, wildlife managers must be aware of the inherent limitations. These data are directly related to, and limited by, the quality and quantity of the source data. Information on the actual migration numbers per generation, and implications to the study populations, are usually not clear. For example, the genetic contribution of 10 migrants per generation from the río Paraguá to the Pantanal area, with local effective populations ($N_e$) in the millions, would likely be insignificant. Introduced alleles and haplotypes would be subject to lineage sorting and genetic swamping by the sheer magnitude of numbers. The situation is very different for an endangered species where the Ne is low and the relative genetic contribution of each migrant is higher.

**Reserve design** – Molecular data can be incorporated in future reserve design and modification of existing protected areas. These wetlands must be interspersed over a large area to form buffer zones for general recuperation in the event of over harvesting. Especially important to keep in mind are areas such as the río Paraguá where unique haplotypes and alleles are found. The present study should be considered only preliminary as important areas, such as the western río Madre de Díos, southern Mamoré, eastern Iténez, eastern Pantanal and Pilcomayo were not surveyed at all.

Natural corridors must be maintained for continued gene flow. This does not pose a problem in the near future for Bolivia and Paraguay where the land use in the primary habitat is for extensive cattle ranching. Rapid development in Brazilian Pantanal and northern Argentina gives cause for concern. Conversion to large-scale agriculture is a growing problem with reduction of habitat and contamination with herbicides, fertilizers and pesticides. There is also a growing potential for large-scale habitat disruption if the proposed waterway (Hidrovía) from the Brazil to the Atlantic is approved and constructed.

A good example of a positive improvement to reserve design comes from Bolivia. Noel Kempff Mercado National Park is located on the Brazilian Shield along the río Paraguá. It includes important catchment basins and ecosystems on the east side of the river but the

previous park boundary basically paralleled the río Paraguá was but set back several kilometers. Recent legislation increased the Park holdings to include the main river corridor and protect the connectivity of affluents to the main river. This improvement may imply potential protection for unique *C. yacare* populations described in this study from the río Paraguá drainage.

**Evaluating translocations** – Given the large numbers in wild *Caiman yacare* populations, translocations do not appear necessary in the near future. However, unforeseen events leading to local extirpation through poaching or contamination might necessitate re-introductions in selected areas. Very low population levels may also lead to inbreeding depression.

Genetic profiling of the translocation stocks would be advised for maintenance of prior diversity. Fortunately, yacare hides are currently of relatively low value and hunting ceases at a certain "catch per unit effort" threshold. Typically, when this occurs a large population of sub-adults and juveniles remain. Due to young age and small size at sexual maturity, *Caiman yacare* and *C. crocodilus* populations rebound relatively quickly without outside assistance. Some are reproductive at about 1 m total length.

With many of the larger crocodylian species, both size and time to maturity work against them. In those species, many individuals attain a valuable size and are killed before they reach the reproductive age/size class. Quite often sympatric *Caiman* species move into the habitat previously occupied by the extirpated species, making additional hurdles to recovery. These are typically the species requiring translocations.

**Forensics** – The field of wildlife forensics has moved forward quickly with the advances in molecular techniques. Data from this study give authorities the ability to identify raw *Caiman yacare* hides from those of common caiman with certainty. This segregation by visual means requires comparison of whole flanks and is imprecise at best.

If future harvest zones are to be monitored, haplotype data could be used to give an indication for the area of origin. The border between Paraguay and Bolivia has been notoriously porous with wildlife products passing in both directions. The distinction of Bolivian Amazon versus the Paraguayan haplotypes is clear as shown in this study. The only zone of ambiguity would come from caiman originating in the Bolivian Pantanal.

The next step will be to develop molecular protocols for working with tanned hides and manufactured products. Unfortunately, the normal procedures for fresh tissue are rendered useless once the hide has been subjected to the chemical treatments of tanning. As many hides are processed to the chrome tan or "crust" stage before international shipment, sensitive molecular inspection is circumvented. Once these technical problems are overcome, many of the current difficulties of hide identification and product origin can be resolved. CITES regulations have greatly reduced the current volume of illegal crocodylian hides on the world market, but stricter controls resulting from new tools can further reduce that portion. Genetic information from studies such as this will be collected and implemented as new techniques are developed.

## CONCLUSIONS

1) *Caiman yacare* is a distinct Evolutionarily Significant Unit (ESU). It was originally described as a full species and has a distribution with nearly complete reproduction separation from the closely related common caiman, *Caiman crocodilus*. Comparison with Amazon *C. crocodilus* sequences shows genetic separation with no apparent influence from *C yacare* migation downstream.

2) *Caiman yacare* can be identified from every other crocodylian species, including *Caiman crocodilus* and subspecies, by comparison of cytochrome b or 16S sequences.

3) The Brazilian Shield, which forms most of the northern boundary and causes the río Madera constriction, is an effective barrier to upstream migration of *C. crocodilus* from the north. While hybridization is possible at the very limited areas of contact between the two species, there has been no detectable genetic introgression. Analyses from this study show distinct genetic separation and significant genetic distance between them.

4) The 25 *Caiman yacare* cytochrome *b* haplotypes are divided between the two main distribution areas, río Amazon and río Paraguay-Paraná, with no haplotypes shared between the two. This indicates that there is no effective migration of females between these two populations.

5) *Caiman yacare* populations are weakly structured in relation to the pattern of river drainages within their distribution. This is shown in analyses for both mtDNA sequences and microsatellite allele frequencies. While microsatellite allele frequencies differ according to geography, all loci are shared by all populations.

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# Did Schmidt just scratch the surface? Resolution on the taxonomy, phylogeography and population structure of the African dwarf crocodile

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**ABSTRACT:** The taxonomy of the African dwarf crocodile (genus *Osteolaemus*) has been disputed since a novel morphotype was discovered in the early 20th Century. Because this poorly-known reptile is widely hunted throughout the forests of Central and West Africa, resolving the existence and extent of taxonomic units has important management and conservation implications. A lack of molecular data from individuals of known origin and disagreement on diagnostic morphological characters have hindered attempts to settle one of the last remaining taxonomic questions in the Crocodylia. We have resolved this debate by sequencing three mitochondrial and two nuclear genes using a large sample of dwarf crocodiles from known localities across major drainage basins of forested Africa. Concordant results from Bayesian, maximum likelihood, maximum parsimony and population aggregation analytical methods (PAA) reveal three highly distinct clades with a Congo Basin form as basal to sister lineages from the Ogooué Basin and West Africa. Corrected genetic distances between clades ranged from 0.2-0.6% in nuclear fragments and as much as 16.2% in mitochondrial COI. Population aggregation, using fixed and alternate character (nucleotide) states to cluster or divide populations, recovered 232 such molecular characters in 4286 bp of sequence data and unambiguously aggregated populations into their respective geographic clade. Private haplotypes in all five gene fragments provide further support for the independent evolution of three dwarf crocodile lineages. Several morphological characters coincide with our phylogenetic analyses to distinguish crocodiles in the Congo Basin from Ogooué Basin and West African forms, but no fixed morphological differences have yet been documented between the latter two regions. This study highlights the importance of using widespread taxon sampling and a multiple evidence approach to diagnose species boundaries and reveal the existence of cryptic diversity.

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# The first genetic linkage map for the saltwater crocodile (*Crocodylus porosus*)

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**ABSTRACT:** Genetic maps currently exist for many commercially as well as evolutionary important species. In this study we presents the first genetic-linkage map for the Saltwater Crocodile (*Crocodylus porosus*), and indeed the first for the Class Reptilia. We constructed male, female and sex-averaged linkage maps for *C. porosus* using a total of 189 microsatellite DNA markers typed for between 83 and 482 individuals from between five and ten families obtained from Darwin Crocodile Farm, NT Australia. We identified 12 linkage groups (LG) with LG sizes ranging from two to 53 loci. The overall map consists of 161 loci, while 28 markers still remain unlinked. LG are currently being physically anchored to chromosomes (2n=34) using Fluourescent *In Situ* Hybridisation (FISH) methods. The current linkage map confirms that female crocodiles have extraordinarily higher rates of recombination than males, with overall map lengths of 1636.5 cM and 275.2 cM respectively. This uncommonly large difference in recombination rates is one of the largest reported for a vertebrate species. This first genetic map lays the framework for further mapping analyses, with QTL mapping of economically and evolutionarily important traits currently underway in the saltwater crocodile.

## INTRODUCTION

Genetic linkage maps currently exist for many commercially important species and are essential research tools for the mapping of economically, as well as evolutionary important traits. Dense genetic maps necessary for the accurate mapping of quantitative trait loci (QTL) require many polymorphic markers spaced evenly across the genome. Microsatellites are ideal markers for genetic mapping in many species, as they are relatively frequent throughout eukaryote genomes, co-dominant, hyper-variable, and are often highly informative within pedigrees. In a recent paper by Miles *et al*. (2008a), 253 novel polymorphic microsatellites were identified and evaluated for the saltwater crocodile (*Crocodylus porosus*). These markers were generated for the purpose of constructing a genetic linkage map. Evidence of genetic linkage between microsatellites was previously reported in crocodilians by Isberg *et al*. (2006). However, to our knowledge, no genetic map for a crocodilian, or any other member of the Class Reptilia, has thus far been reported. Herein we report the first microsatellite-based genetic linkage map for the saltwater crocodile (*Crocodylus porosus*),

and indeed for any other reptilian. Currently, linkage groups (LG) are being physically anchored to *C.porosus* chromosomes (2n=34) via fluorescent *in-situ* hybridization (FISH) methods, and we expect the final anchored genetic linkage map to be completed in late 2008. The existing linkage map confirms that female crocodiles have extraordinarily higher rates of recombination than males (Isberg *et al*. 2006), with a ratio of female recombination versus male recombination of 5.95, the second highest ratio reported for any vertebrate species. The recent generation of a crocodilian genetic linkage map has also made it possible to perform systematic searches for individual loci affecting quantitative traits of economic importance in farmed Australian saltwater crocodiles. Whole-genome QTL scans are currently underway at Darwin Crocodile Farm and the University of Sydney.

## METHODS

### The 'Porosus' mapping population

The Porosus resource is a two-generation pedigree originating from Darwin Crocodile Farm, NT, Australia. This commercial population consists of a total of 482 individuals from ten full-sib families, with clutches from 2005-2007. Family sizes ranged widely with numbers ranging from 13 to 89 individuals. This resource can be sub-divided into two separate resources: the *linkage mapping resource* and the *QTL mapping resource*. Microsatellite genotype data from each resource was analysed using the software Cervus 3.0 (Kalinowski *et al.* 2007) to confirm parentage and pedigree integrity. Parentage analysis was conducted using a typing error rate of 0.01 and a strict confidence interval of 95%.

#### *Linkage Mapping Resource*

The linkage mapping resource originally consisted of 96 individuals from six full-sib families, but was later culled to 83 individuals from five full-sib families due to the incorrect parentage assignment for one clutch. Family cohorts were selected from 2007 offspring only, and family sizes ranging from 14 to 18 individuals. This resource was typed for 189 loci with the ensuing data used to construct the framework linkage map.

#### *QTL Mapping Resource*

Additional genotype data arising from subsequent QTL scans was incorporated into the final linkage map construction to bolster statistical support for the map order. This QTL mapping resource consisted of 482 individuals from ten full-sib families (some of which overlapped with the linkage resource families), with clutches taken from 2005, 2006 and 2007, where possible. The additional individuals were genotyped for 82 microsatellites selected for their even distribution across the framework linkage map.

### DNA extraction

DNA was isolated from whole blood samples using a modified phenol-chloroform extraction protocol adapted from Sambrook *et al*. (1989). The DNA resource is available upon request for those who wish to contribute further to the crocodilian genetic linkage map.

**Microsatellite genotyping**

189 microsatellites were mapped in this study, and PCR conditions for these markers are described in Miles *et al*. (2008a). CAG-universal primers were labeled with either VIC, 6-FAM or NED fluorescent dyes. PCR amplicons for each of the respective panels were pooled (VIC, 6-Fam and NED) and analysed on an ABI 3130xl automated DNA sequencer. Raw genotype data was imported into Genemapper version 4.0 (Applied Biosystems) for genotype analysis. Genotypes for each microsatellite loci were scored, exported then compiled into a single data set via a custome BioPython script for linkage analyses.

**Linkage Map Construction**

Cri-Map v2.4 was used to perform linkage analyses and map construction for *C.porosus* (Green, 1990). Although many modern and more user friendly programs are available, none seem to have the same flexibility that enables Cri-Map to infer phase of (unsampled) parental generations (assuming we call the sampled generations F1 and F2) and enable linkage analysis with simple two-generation pedigrees. Genotype datasets for the two mapping resources were combined using the MERGE option in CRI-MAP. Markers were then sorted into LGs by TWOPOINT analysis with a threshold LOD ≥ 3.0, and later ordered within these groups using the BUILD multipoint analyses. LOD ≥ 2.0 was chosen as the minimum statistical support criterion for ascertaining locus order within LGs using the multipoint BUILD function. Loci that could not be confidently placed within the map order at the minimum LOD ≥ 2.0 were later added using the function ALL, which places loci in the most likely map position with the greatest statistical support. The function, FLIPS6, was later used to verify the final locus order of adjacent loci within LGs, ensuring the order with the greatest statistical support was retained. Centi-Morgan (cM) map distances were calculated using the Kosambi mapping function within CRI-MAP.

**Physical Mapping**

In conjunction with the construction of the linkage map, a bacterial artificial chromosome (BAC) library was established at Mississippi Genome Exploration Laboratory (MGEL), Mississippi State University, USA, with 2.8x coverage of the *C. porosus* genome. At the University of Sydney and South Eastern Area Laboratory Services, BAC filters were screened with $P^{32}$-labeled overgo probes designed from microsatellite loci already mapped to the terminal ends of LGs. BAC clones identified to contain mapped microsatellites will be fluorescently labeled with FITC and Spectrum Red (Vysis) fluorochromes using nick translation, and then hybridized onto *C.porosus* metaphase preparations via fluorescent *in-situ* hybridization (FISH) methods. The physical mapping of the terminal loci will enable the anchoring and orientation of LGs to *C.porosus* chromosomes.
Further refinement of the standard G-banded *Crocodylus porosus* karyotype was also carried out, including the production of a chromosome ideogram with band allocation providing a standardized reference for chromosomal locations.

## RESULTS

Of the 189 microsatellites incorporated in the analyses, 161 loci were assigned to 12 LG, with 28 loci remaining unassigned. These LG comprise from two to 53 loci. Additional markers and perhaps additional informative meioses may be required to incorporate these unassigned microsatellites into the map. Initial mapping attempts revealed that the total length of the sex-averaged recombination map was 800.4 cM, with the sex-specific maps for male and female being 275.2 cM and 1636.5 cM respectively. These results show that the difference in recombination fraction between sexes was uncommonly high, almost six times higher in the female than that of the male. Idiograms for the respective maps are not presented here. However, comprehensive map illustrations will be reported upon completion of the physically anchored map. Preliminary results for the FISH mapping are presented in figure 1, and are described in the Dalzell *et al*. (2008).

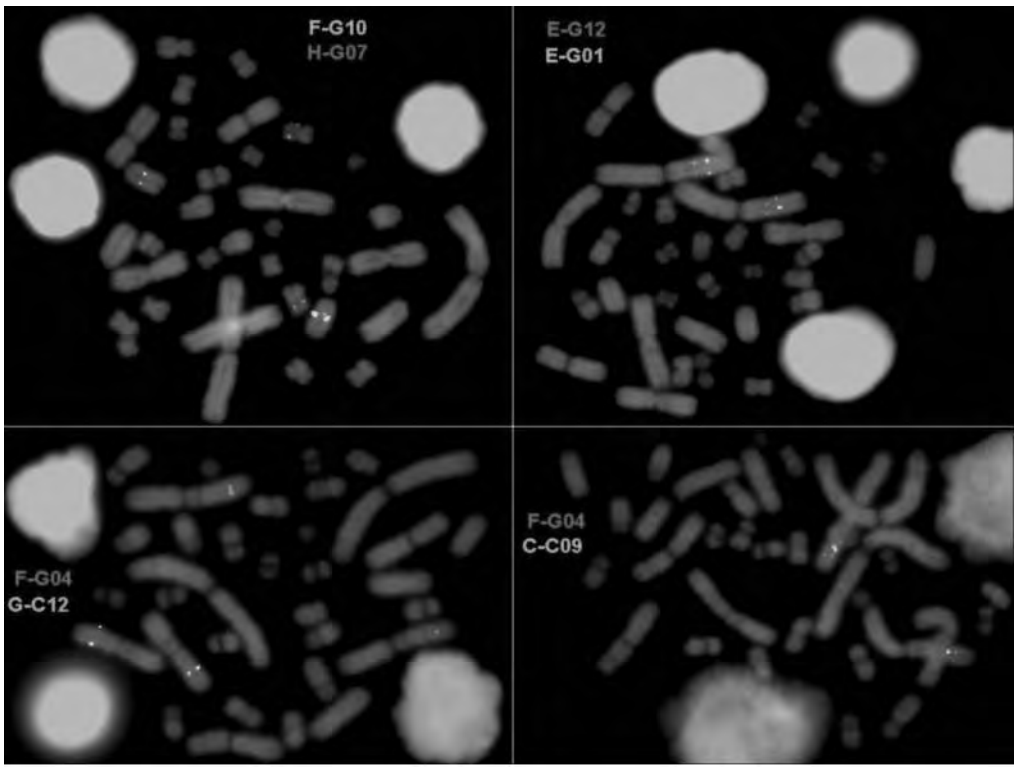



**Figure 1** Fluorescent in-situ hybridization (FISH) of fosmid clones to *C.porosus* chromosomes. Preliminary work towards the FISH mapping of BAC clones to anchor linkage map.

## DISCUSSION

This paper described the first genetic linkage map for a crocodilian, and indeed for any other reptilian species. The present crocodilian map revealed an uncommonly large difference in the recombination frequencies between sexes, and apart from maps reported for some fish species (Coimbra et al. 2003; Moen *et al*. 2004), this is the greatest disparity yet reported for a vertebrate species. This phenomenon of differential recombination frequency is not uncommon. However, as previously reported by Moen *et al*. (2004), the ratio between recombination rates usually lies between 1.0 and 2.0, as evident in some of the most comprehensive linkage maps for vertebrates (Gyapay et al. 1994; Archibald *et a*l. 1995; Dietrich *et al.* 1996). The reasons for these differences in recombination frequency are

currently unknown. Sex-specific recombination rates have typically invoked an involvement of the sex chromosomes, with the heterogametic sex having the least recombination (with exceptions). However, crocodilians exhibit temperature dependent sex (TDS) determination (Lang *et al.* 1994), thus the high degree of heterochiasmy presumably relates to other major differences in the processes of male and female meioses. This is an area for further investigation.

Although the current map does incorporate 161 microsatelites, a large number of markers remain unmapped due to their lack of linkage to other markers. These unlinked markers indicate that the true map length is likely to be larger than that covered by the current sex-specific and sex averaged maps, which are relatively small for a vertebrate. Genome coverage will be better assessed following the physically mapping of LG terminal loci, which will not only anchor the map, but also will reveal the extent of chromosomal coverage for each of the respective LG. Additional markers, and/or additional informative meioses, will likely be required for the incorporation of the currently unlinked microsatellites into the existing map. However, as reported by Shedlock *et al.* (2007), the low levels of repetitive sequence that exist in non-avian reptiles could limit the saturation of a crocodilian linkage map with microsatellites alone. For this reason, the identification and mapping of single nucleotide polymorphism (SNP) markers will likely be required for the generation of saturated genome-wide linkage map. The current microsatellite-based linkage map for *C.porosus* serves as a framework for the future mapping of SNPs and other markers in the effort to generate a dense genetic map for the crocodilian.

A high density genetic linkage map is the first step towards the identification of loci contributing to genetic variance in economically and evolutionary important traits in crocodilians. The current *C.porosus* map, whilst only of medium density, has already facilitated systematic searches for QTL in Australian farmed saltwater crocodiles. Preliminary results from the first QTL scans in a crocodilian were presented by Miles et al. (2008b), and we expect more extensive results to be reported shortly.

This crocodilian linkage map will also facilitate comparative mapping and evolutionary studies in crocodilians and other closely related reptiles. The microsatellites isolated and mapped may also serve as a bridge for comparative mapping efforts, as microsatellites exhibit high levels of cross-amplification success in many species, including crocodilians (Dever *et al*. 2002; Fitzsimmons et al. 2002; Zucoloto *et al.* 2006; Miles et al. 2008c). For this reason, 82 of the microsatellites mapped in this study were also evaluated for their amplification success in 18 other non-source species of crocodilian, the results of which are presented in a recent paper by Miles *et al.* (2008c). It is hoped that in addition to providing the first genetic map for a crocodilian, this marker resource will provide polymorphic markers for several crocodilian species previously lacking informative genetic markers.

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# Development of Microsatellite Markers for Siamese Crocodile (*Crocodylus siamensis*)

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**ABSTRACT:** New Microsatellite markers were developed for Siamese Crocodile (*Crocodylus siamensis*) by constructing a library for microsatellite DNA. Construction and characterization of the library are described in the present study. Twenty microsatellite markers were developed from a $(AC)_{15}$ enriched microsatellite DNA library. Among the twenty microsatellite loci, ten (50%) were polymorphic, where as the rest were monomorphic (with two to four alleles per locus). The microsatellite sequences obtained could be classified structurally into perfect repeats (80%) and imperfect repeats (20%). No incomplete repeat type was observed. These markers were tested in five individuals of the same species and these tests resulted in twenty new microsatellites markers for *C. siamensis*. Low number of alleles (1-4 alleles) with an average of 1.7 alleles per locus was observed. The average length of uninterrupted repeats from the selected clones was 12.3.

## INTRODUCTION

The Siamese crocodile *(Crocodylus siamensis*) is a critically endangered species of freshwater crocodiles. It was previously distributed throughout Southeast Asia. However, Thailand has extensive crocodile farms of *C. siamensis* and *C. porosus* breeds. Unintentional hybridization is often a more serious problem of conservation concern because it can occur undetected, particularly if hybrids do not have distinguishing morphological features. However, intentional hybridization could become a concern, if mixed species populations become the source for reintroduction efforts. In this case, hybridization may have dramatic effects for native endangered species e.g. Siamese crocodiles, if human-induce introductions arise from the population consisting a few or all hybrid offspring (Allendorf *et al*., 2001).

In addition, developing a plan for preventing such hybridization for the Siamese crocodiles is limited since clear observation is rarely. Clear observation is rarely possible because mating occurs in the water and often involves groups of males and females which are difficult to differentiate (Lang, 1989). Even if a female is observed to be mounted by more than one

male (Davis *et al*., 2000), it is unclear whether multiple males successfully copulate and inseminate her, resulting in fertilized eggs. Such observations have led to the supposition that female crocodiles may produce clutches of progenies by multiple males. Hybrids can be difficult to distinguish based on morphology, thus there is a need to develop molecular techniques to identify species status of individuals considered for release into the wild and to establish captive breeding programs for conservation.

Microsatellites have been the genetic tool of choice for DNA based parentage systems due to their highly polymorphic nature and have been employed for refined estimating of kinship and parentage (Bruford and Wayne, 1993) in many organisms including crocodiles (Glenn *et al*., 1998; Davis *et a*l., 2000). They are nuclear markers that consist of short tandem repeats, usually 1-5 bp in length, such as (AC)n or (ATT)n (Beckmann and Weber, 1992). They are found approximately every 10 kb in the eukaryotic genome and their repeat arrays are generally no longer than 300 bps (Stallings *et al*., 1991). Polymorphism arises through variation in the number of repeat units present, possibly owing to slipped-strand mispairing (Schlötterer and Tautz, 1992). Variation at microsatellite loci can be assayed by PCR amplification using primers complementary to unique sequences flanking specific repetitive arrays, followed by electrophoretic sizing of the PCR products (Tautz, 1989).

In the current study, microsatellite primers were developed to compare population genetic structure in crocodilian families (Glenn *et al*., 1996; FitzSimmons *et. al*., 2001). Several sets of primers have been designed for three other species of Crocodylus (including the Cuban crocodile, *C. rhombifer*, the salt-water crocodile, *C. porosus* and the Australian freshwater crocodile, *C. johnsoni*). However, within this genus, most tests of cross-species PCR amplification indicated the presence of homologous microsatellite loci that were variable (FitzSimmons *et al*., 2001). Herein, we describe the development of new microsatellite DNA primers for the *C. siamensis*. This is an important first step that should help to establish conservation strategies and contribute to an understanding of the structure of wild, remnant populations for this species

## MATERIALS AND METHODS

**Sample collection and DNA extraction:** Whole blood (5ml) was collected without injury to individual from an anterior dorsal sinus of a live caught wild Siamese Crocodile. The sample was kept on ice and sent to laboratory for DNA extraction and used for constructing a microsatellite library. In addition, five individuals of the same species were selected from the Sriracha breeding farm. Blood samples were collected as indicated above for the analysis of designed microsatellite primers.

Genomic DNA was digested with *Taq* I, in a final volume of 100 µl that composed of 10 µl of 10x *Taq* I buffer, 0.1 U/µl BSA, 0.25 U/µl of *Taq* I, and 1µ g/µl of genomic DNA. The mixture was incubated at 65$^{o}$C for at least five hours or overnight. Digested DNA was run on a 0.8% agarose gel with ethidium bromide and visualized under ultraviolet (UV). DNA fragments with an average size of 500 to 1000 bps were isolated from agarose gel and purified by using QIAquick spin column (QIAGEN) followed by ethanol precipitation. This isolated DNA fragments were ligated into the Cla I site pBluescript II KS+ (Takara) and transformed into fresh competent XL1-Blue supercompetent cells (Strategene) by heat shocking. Transformed cells were grown up overnight on LB agar plates containing 50 µg/ml

ampicillin with Xgal and IPTG. Recombinant colonies were transferred onto Hybond nylon membranes (Amersham, Sydney) and followed by hybridizing with synthetic oligonucleotide microsatellite probe d(AC)15. Prehybridization and hybridization were carried out at 42ºC in 6x SSC (from a 20x stock = 3M NaCl, 0.3 M sodium citrate), 5x denhardt reagent (from a 50x stock = 1% BSA fraction V, 1% Ficoll and 1% polyvinylpropylene) and 0.1% SDS. After hybridization, it was washed twice (30 min) in a 6x SSC, 0.1% SDS solution. Filters were screened for microsatellite repeated by using Gene-Images random primer labelling kit (Amersham Pharmacia Biotech) and exposed to X-ray film. After alignment to autoradiography images, positive colonies were selected and a recombinant plasmid was isolated by using alkaline preparation and screened for recombinants DNA by restriction enzyme. The potentially positive recombinants were sequenced automatically (Fluorescent dye method, Applied Biosystems).

**Primer design and genotyping:** Primer pairs were designed to amplify the flanking regions of selected microsatellites using the program Genetyx software (GENETYX software development Co. Ltd., Tokyo, Japan). Primers were about 18-24 bp in length, with calculated annealing temperatures of 50-65oC with a maximum 4oC difference between each pair, and no primer dimer of hairpin formation. In the genotyping, DNA samples from 5 Siamese Crocodiles were amplified in a 25 ul final volume of 1x PCR buffer (20 mM Tris-HCl, pH 8.4; 50 mM KCl), 1.5 mM MgCl2, 0.2 mM each dNTP, 0.4 mM of each primer, 0.02 U/µl. Taq DNA polymerase, and 25 ng of DNA. The amplified conditions are started with denaturing step of 94oC for 3 min and followed by 35 cycles of 94oC for 1 min, the appropriate annealing temperature for 1 min, and amplification at 72oC for 1 min. Products were stored at 4oC until ready to be analyzed and scored. The alleles of the microsatellite primers were detected in 6% denaturing polyacrylamide gel electrophoresis and their expected sizes were compared with standard size of ϕ x HinfI. The program Kodak 1D Digital Science V. 3.0.2: Scientific Imaging System (Eastman Kodak Company, New Haven, CT) was used for analyses of scientific images.

## RESULTS AND DISCUSSION

Total 215 clones of transformant were constructed and hybridized with d(AC)15 probe. It was found that 59 clones or 27.44% of total clones with tandem repeat nucleotide were recognized and selected. From these positive clones, 30 of them (50.7% of total $d(AC)_{15}$ positive clones) were selected for DNA sequencing. The sequencing result gave only 22 clones that can be selected to design primer. The other eight clones cannot be studied further due to inadequate lengths of flanking sequence. Thirty-two microsatellite primers were designed from 22 positive clones and only 20 of these provided reliable amplifications (Table 1). The DNA sequences of the cloned alleles were submitted to Genbank (accession numbers EF413033-EF413054). The microsatellite polymorphism in the farm population of Siamese Crocodiles has been analysed by SDS-PAGE. Low number of alleles (1-4 alleles) with an average of 1.7 alleles per locus was observed (Table 1). The average length of uninterrupted repeats from the selected clones was 12.3. Ten (50 % of total microsatellite primers) of the 20 microsatellite loci were polymorphic. Information of all primers designed, including repeat motif, expected size, observed sizes, PCR conditions, and other characteristics, is presented in Table 1 and the illustrated of loci markers *CS*-4, *CS*-5 and *CS*-21 are presented in Figure 1.

**Table 1** Primer parameter and observed values for *Crocodylus siamensis*

| Locus (GenBank accession no) | Primer: ( 5' ---> 3' ) | Lenght | %GC | TM | Repeats | Expected size (bp) | Observed allele size range (bp) | Alleles per locus | PCR condition | | | Polymorphism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Annealing (°C) | $Mg^{2+}$ (mM) | Cycle | |
| CS2 | F: GCAAACCTATCACCAGTTCAAGC | 23 | 47.82 | 58.76 | $(CA_8)$ | 230 | 230-250 | 2 | 68 | 1.5 | 40 | Yes |
| (EF4130039) | R: TCTCTGTCACCTGAACCTTCCTC | 23 | 52.17 | 58.80 | | | | | | | | |
| CS4 | F: CCATGCCCTACCACACAACTG | 21 | 57.14 | 58.79 | $(AC_{13})$ | 200 | 200-210 | 2 | 65 | 1.5 | 35 | Yes |
| (EF4130051) | R: CAAACACAAAGGCATTCAAAGATG | 24 | 37.50 | 58.69 | | | | | | | | |
| CS5 | F: TCTCTCCTTCTTTCTGCTTGTGC | 23 | 47.82 | 58.76 | $(AC_{16})$ | 200 | 190-210 | 2 | 65 | 1.5 | 35 | Yes |
| (EF4130052) | R: GTGCAAGGTCTGTACCTGTGTGA | 23 | 52.17 | 58.80 | | | | | | | | |
| CS10 | F: TGACAGTGGCTTTATTGAACAGG | 23 | 43.47 | 58.72 | $(GC_3AC_{17})$ | 240 | 240-250 | 2 | 65 | 1.5 | 30 | Yes |
| (EF4130033) | R: TGGACTCTCTCCTCCTGGACTTC | 23 | 56.52 | 58.85 | | | | | | | | |
| CS12 | F: GGACAGCAACAGAAAAGACAGG | 22 | 50.00 | 58.75 | $(AG_8)$ | 200 | 200 | 1 | 60 | 1.5 | 35 | No |
| (EF4130034) | R: ATAGGAAGCGTTCGTGCTGATG | 22 | 50.00 | 58.75 | | | | | | | | |
| CS14 | F: CCTTCATGTGGATTAGGAACAGG | 23 | 47.82 | 58.76 | $(AC_{10})$ | 270 | 270 | 1 | 60 | 1.5 | 35 | No |
| (EF4130035) | R: TAGCAGCTTGAAGTGGGTAGCAG | 23 | 52.17 | 58.80 | | | | | | | | |
| CS15 | F: TCACCTGCATATTTCTCCTTCCA | 23 | 43.47 | 58.72 | $(TC_6)$ | 220 | 210-230 | 4 | 65 | 1.5 | 35 | Yes |
| (EF4130036) | R: GACCGGGGCTCAGTAAAACAC | 21 | 57.14 | 58.79 | | | | | | | | |
| CS17 | F: GATCCCTCCTACACACACACG | 21 | 57.14 | 58.79 | $(GC_3AC_{12})$ | 180 | 170-200 | 2 | 60 | 1.5 | 35 | Yes |
| (EF4130037) | R: TATTAGGGCTGGACAGTCAAAGG | 23 | 47.82 | 58.76 | | | | | | | | |
| CS18 | F: GCAGAACCATAACACATGCACAG | 23 | 47.82 | 58.76 | $(A_{19})$ | 240 | 240 | 1 | 60 | 1.5 | 35 | No |
| (EF4130038) | R: GCATTCCATAGTCCCCTCCATAG | 23 | 52.17 | 58.80 | | | | | | | | |
| CS20 | F: CCTTCCGCCCAAAATAATTGC | 21 | 47.61 | 58.69 | $(C_{10}AC_5)$ | 249 | 249 | 1 | 60 | 1.5 | 35 | No |
| (EF4130040) | R: CCCTGCACTAACAAGGAGCAG | 21 | 42.85 | 58.79 | | | | | | | | |
| CS21 | F: ATTTCCACTCACAGCTTCAAACC | 23 | 43.47 | 58.72 | $(AC_5)$ | 260 | 260-270 | 2 | 60 | 1.5 | 35 | Yes |
| (EF4130041) | R: GGATGCTTGTAGGTCTTGTTTAGC | 24 | 45.83 | 58.77 | | | | | | | | |
| CS22 | F: CTGTAGGCTGTGACAAAATCCTG | 23 | 47.82 | 58.76 | $(AC_7)$ | 180 | 180 | 1 | 60 | 1.5 | 35 | No |
| (EF4130042) | R: CATAACCAACCAGAATGTGACTGC | 24 | 45.83 | 58.72 | | | | | | | | |
| CS24 | F: GAACCCAGGAGGCAGGAGAG | 20 | 65.00 | 58.83 | $(AC_{26})$ | 270 | 270 | 1 | 68 | 1.5 | 35 | No |
| (EF4130043) | R: CACATACACAGAACCCAGGTGTG | 23 | 52.17 | 58.80 | | | | | | | | |
| CS25 | F: ATCCTCAGTGTCATCTGCTCACC | 23 | 52.17 | 58.80 | $(AC_9)$ | 180 | 180 | 1 | 65 | 1.5 | 35 | No |
| (EF4130044) | R: TCTTCCCTGCTCACCTTTCTTTC | 23 | 47.82 | 58.76 | | | | | | | | |
| CS26 | F: GCCATGTGTACTAACTGGGAAGTC | 24 | 50.00 | 58.81 | $(AC_{17})$ | 240 | 240 | 1 | 65 | 1.5 | 35 | No |
| (EF4130045) | R: GCCATTTTTAGTCAGGTTGTTGC | 23 | 43.47 | 58.72 | | | | | | | | |
| CS28 | F: CAGTCTCCAGCACTTGGGGATAG | 23 | 56.52 | 58.85 | $(AC_{15})$ | 200 | 180-200 | 2 | 68 | 1.5 | 35 | Yes |
| (EF4130046) | R: TTGGCTACAAAGGACCAACTCAC | 23 | 47.82 | 58.76 | | | | | | | | |
| CS30 | F: ACTCACACTACCATCTCCAGCAC | 23 | 52.17 | 58.80 | $(AC_{15})$ | 210 | 210 | 1 | 68 | 1.5 | 35 | No |
| (EF4130047) | R: TGTGTGTCATGTGCGTGAACC | 21 | 52.38 | 58.74 | | | | | | | | |
| CS32 | F: GTACCAAGCCCCTTTAACACCTG | 23 | 52.17 | 58.80 | $(AC_{17})$ | 245 | 245 | 1 | 68 | 1.5 | 35 | No |
| (EF4130048) | R: GGGGAGAAGGAAACTAGGAGAGG | 23 | 56.52 | 58.85 | | | | | | | | |
| CS33 | F: ATCAACTTTCAGCCTGGGATAGG | 23 | 47.82 | 58.76 | $(AC_{12})$ | 200 | 200-210 | 2 | 68 | 1.5 | 40 | Yes |
| (EF4130049) | R: ATGTCTTATTGCTTCCCTTGCAC | 23 | 43.47 | 58.72 | | | | | | | | |
| CS35 | F: GTCTGAAAAGGGTGTTTGTG | 20 | 45.00 | 58.63 | $(AC_{17})$ | 230 | 230-240 | 2 | 60 | 1.5 | 35 | Yes |
| (EF4130050) | R: CCTAAACTGTTAGAAGCCAAG | 21 | 42.85 | 58.64 | | | | | | | | |

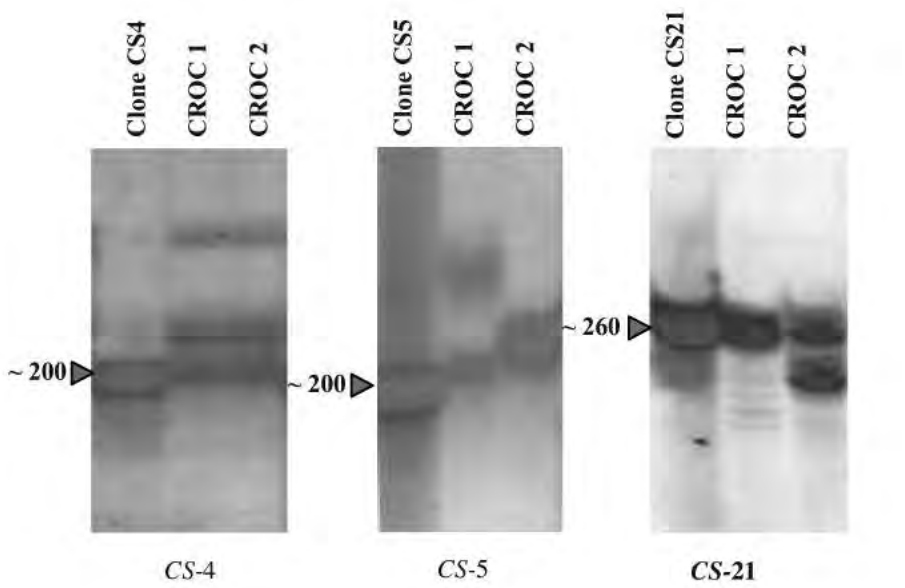



**Figure 1.** PCR product from locus *CS*-4, *CS*-5, and *CS*-21 showed polymorphic pattern.

A total of 20 microsatellite loci were structural analysis as indicated by Weber (1990) and 16 loci exhibited perfect microsatellite motifs without any interruption in the repeats, where as 4 loci contained compound microsatellite repeats with a run of CA or GT repeats. No imperfect repeat locus was observed in the study.

The enrichment procedure that we employed was successful. The number of microsatellite library obtained in our study was di-nucleotide repeats which are common in Crocodylidae and Alligatoridae as previously reported by Glenn *et al.* (1998) for *A. mississipiensis*, FitzSimmons *et al.* (2001) for *C. porosus* and *C. johnstoni*, and Zucoloto *et al.* (2002) for *Caiman latirostris*.

Regarding to the former, captive colonies could be more efficiently managed by establishing individual pedigrees that would help to keep inbreeding coefficient as low as possible. With respect to the latter, genetic studies of the behavioral ecology of remnant populations will allow assessment of mating systems and dispersal patterns of wild individuals, helping researchers to understand how the remnant populations use the landscape.

In future studies, we will further characterize the primers obtained by verifying segregation and heterozygosity. The development of these new microsatellite markers significantly increases our capability to assess the diversity of *C. siamensis* in Thailand. These new markers will improve exclusion power for maternity tests and the resolution of parentage identification among wild individuals in *C. siamensis*.

**ACKNOWLEDGEMENTS**

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# Using Microsatellites to Describe Hybridization Between *Crocodylus acutus* and *Crocodylus moreletii* in the Yucatan Peninsula

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**ABSTRACT:** We tested for hybridization among wild populations of American crocodile (*C. acutus*) and Morelet's crocodile (*C. moreletii*) in the Yucatan peninsula by using Bayesian assignment tests based on microsatellite data compared to mitochondrial and morphological assignments. Skin clips from 83 individuals were taken for genetic identification, and a total of 32 individuals (38.6 %) exhibited some evidence of ancestral admixture by combined morphological, mitochondrial and microsatellite analyses. The majority of hybrids were classified as F2 hybrids and backcrosses to *C. moreletii*. Most of the introgression occurs in two national biosphere reserves located on the northern and eastern coasts of the Yucatan Peninsula. Preliminary tests did not find a significant decrease in hybridity across three life stages, thus far indicating a low level of selection against hybrids. Model-based analyses on multilocus genotypes of pure individuals returned little geographic partitioning in both *C. acutus* and *C. moreletii*.

# Cryptic species as a frame on use and conservation of *Crocodylus acutus*.

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**ABSTRACT:** Resolving taxonomic incongruence and delimiting regional management units for the genus *Crocodylus* in the Neotropics is currently one of the most relevant conservation and management issues for crocodile specialists. The small sample size used to establish New World *Crocodylus* morphological and molecular systematics has limited the understanding of the evolutionary process and has left out many undescribed cryptic species. Using mtDNA sequences form cytochrome oxidase I (COI) and cytochrome *b* (Cyt b) and 7 microsatellites designed by Fitzsimmons we challenged the paradigm that *Crocodylus acutus* is a single wide ranging species. Our findings will have a significant impact on American crocodile conservation and management plan since we can recognize at least five Evolutionarily Significant Units that should be managed as fully independent entities in order to maintain genetic biodiversity that will high productivity in captive populations and long term survivor of the taxa.

**RESUMEN:** Resolver las incongruencias taxonómicas y delimitar las unidades regionales de manejo y conservación para las especies del género *Crocodylus* en el Neotrópico es actualmente uno de los aspectos más relevantes para los especialistas en cocodrilos. El pequeño numero de muestras utilizadas en los estudios de sistemática morfológica y molecular de los cocodrilos del Nuevo Mundo a limitado el entendimiento del proceso evolutivo del grupo y a impedido el reconocimiento de muchas especies crípticas. Usando un amplio numero de muestras y secuencias de dos generes mitocondriales, citocromo oxidasa I (COI) y citicromo *b* (Cyt b) y 7 microsatélites diseñados por Fitzsimmons, nosotros decidimos evaluar el paradigma que *Crocodylus acutus* es una sola especie de amplia distribución. Los resultados de esta investigación tendrán un impacto significativo en el plan de conservación y manejo de los cocodrilos americanos porque el estudio recupera por lo menos cinco diferentes Unidades Evolutivas Significativas que deberían ser manejadas como unidades totalmente independientes para mantener la diversidad genética que promueva una mejor y más alta producción en cautiverio y la sobrevivencia del grupo a largo plazo.

# Genetic characterization of Cuban populations of *Crocodylus* (Crocodylia: Crocodilidae): *C. rhombifer, C. acutus* and suspected hybrids using mitochondria and microsatellite markers.

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**ABSTRACT:** *Crocodylus rhombifer* has the smallest natural distribution of any species in the order Crocodylia (400-500 km$^2$) and is one of the world's most endangered crocodilians. In the Cuban Archipelago, this endemic species coexists with the American crocodile (*Crocodylus. acutus*). These two species readily hybridize both in the wild and in captivity. In this study, mitochondrial DNA sequences and microsatellites *loci* were evaluated as tools to characterize genetically wild-caught individuals. Seventy-three samples were taken from three locations on Cuban archipelago: two in Zapata Swamp for *C. rhombifer* and suspected hybrids and the remaining in Birama Swamp for *C. acutus*. Genetic diversity from the *C. acutus* population in terms of observed heterocigosity (Ho=0.59) was higher than other populations inside the genus. On the other hand, the comparisons per pair of samples revealed significant genetic differentiation based on $F_{ST}$ ($F_{ST}$=0.338, p=0.05). Sequences analysis revealed *C. rhombifer* and suspected hybrids had the same haplotype, suggesting that all suspected hybrid samples represented crosses between female *C. rhombifer* and male *C. acutus*. Finally, the Cuban *C. acutus* haplotype was more closely related to the Cuban *C. rhombifer* haplotype than to the Mesoamerican *C. acutus* haplotype, suggesting a possible status species specific different to the current considered.

# Development of Innate Immunity in Juvenile American Alligators

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**ABSTRACT:** Previous results in our laboratory indicated that hatchling alligators do not exhibit serum complement-mediated innate immune activity, but develop this activity during their first year. Thirty five alligators, from seven different wild clutches, were housed indoors (30$^{o}$C) and fed ad libitum for approximately 2 months. Half of the alligators were then moved to outdoor tanks, while the other half remained indoors, such that animals from each clutch were represented in both groups. Blood was collected from each alligator at various times during their first year. Early results have indicated that alligators kept outside develop immunity much earlier than those housed indoors. Furthermore, immunity developed during the winter months when activity levels were low and nutritional intake was nonexistent. These results indicate that factors other than optimal metabolic temperature and growth may have an influence on the development of immunity. In addition, we observed clutch effects. Alligators from clutch #6 housed indoors developed immunity earlier than other alligators housed under the same conditions. Likewise, alligators in clutch #6 exhibited the highest mean immune activity relative to other groups in the outdoor environment. These results suggest that captive breeding programs for crocodilians might be able to utilize immune parameters as a selectable trait.

# Experimental Lead Poisoning and Blood ALAD Enzyme Activity in Juvenile Alligators

**Valentine A. Lance, Lynnie Cua, Ruth M. Elsey and Phillip L. Trosclair III**

**ABSTRACT:** In a previous study we showed that captive adult alligators inadvertently fed lead pellets had extremely high lead concentrations in liver, kidney and bone, but had no obvious signs of pathology (Lance *et al*.2006). Conversely, similar incidental lead ingestion in captive juvenile alligators on a commercial farm led to some mortality (Camus *et al*. 1998). To investigate the effect of lead consumption on juvenile alligators we force-fed five groups of eight juvenile alligators (body weight 1.8 to 4.8 kg) lead pellets in gelatin capsules at doses of 0, 0.25, 0.50, 1.0, and 2.0 g/kg body weight. Blood samples were collected before treatment and at two, four and six weeks and analyzed for lead concentrations and ALAD (aminolevulinic acid dehydratase) activity. ALAD activity depression is used to monitor lead exposure in humans. Alligators were monitored for three months following treatment. We could detect no differences in growth rates among treatments during the three months. The lowest dose tested, 0.25 g/kg body weight, caused a significant depression of ALAD activity (~90%) in whole blood at two weeks post treatment. Blood lead concentrations remained elevated throughout the sampling period and showed a significant correlation with ALAD activity depression ($p < 0.01$).
Alligator blood ALAD appears to be as sensitive to lead poisoning as other vertebrates, but no effect of lead on appetite and growth could be detected.

Camus *et al*. (1998) J. World Aqucult. Soc. 29:370-376.
Lance *et al*. (2006) Comp. Biochem Physiol. C. 142:30-35.

# Differential Protein Expression of Alligator Leukocytes Induced by Injection of Bacterial Lipopolysaccharide

**Mark Merchant[1] and Clint Kinney**

**[1]Department of Chemistry, McNeese State University, Lake Charles, Louisiana**

**ABSTRACT:** Blood was collected from juvenile American alligators prior to, and 24 hours after intraperitoneal injection of bacterial lipopolysaccharide (LPS). The leukocytes were isolated from each sample, and the protein was extracted. The samples were analyzed by Two Dimensional Difference In-Gel Electrophoresis (2D-DIGE) to determine changes in protein expression upon LPS injection. The results indicated that the expression of several proteins was increased upon LPS treatment, while the expression of several others decreased. The quantitative increases and decreases were determined by digital densitometry. Fourteen proteins of interest were picked from the two dimensional gel and subjected to MALDI-MS/MS to acquire partial peptide sequence data. These peptide sequences were compared to those within the Entrez National Institutes of Health website protein sequence database to determine the identity of each protein. The identity and function of these alligator leukocyte proteins will be discussed.

# Characterizations of serum complement activity of Broad-Snouted Caiman (*Caiman latirostris*)

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**ABSTRACT:** Immunity to infection is mediated by two general systems: acquired (or adaptive) and innate (or natural). Such systems have evolved and diversified in response to many factors, principally to the environment in which organism lives. Crocodilians exhibit aggressive social behaviors, and frequently serious wounds, including loss of entire limbs, resulting from these conflicts. Despite these serious injuries, they usually show no signs of infection.

Several recent findings in the field of immunology have reinforced the importance of examining functional features of immune systems in a variety of organisms. These immune features, such as the serum complement system, have been identified in crocodilians. Those findings could be related in high resistance to infections. This study was conducted to detect and characterize the concentration-, temperature-, and time-dependent serum complement activity of *Caiman latirostris*.

# Chlamydial infection outbreak in the Northern Territory Crocodile Industry, June 2006, Australia

**Ian Jerrett - Berrimah Veterinary Laboratories, DPIFM, Darwin NT**
**Jane Oakey-Tropical and Aquatic Animal Health Laboratory, DPIF Qld**
**Damien Higgins and Tiziana Beninati -Faculty of Veterinary Science, University of Sydney**
**Presented by Vicki Simlesa Northern Territory Government Berrimah Farm, Australia**
**Berrimah Farm, GPO Box 3000, Darwin, Northern Territory, Australia 0801**

## INTRODUCTION

The Chlamydia outbreak occurred in the Darwin region of Australia between June and August 2006.

Two farms experienced high morbidity/mortality during the outbreak in 2-6 month old juveniles. One farm had 96% loss and the other 29% loss. Total mortality >3000 deaths. Two further farms experienced low morbidity disease in 1-3 year old animals. During the outbreak, farms observed signs of severe infections both in the eyes and throat of animals. Animals were unable to see, eat or swallow in severe infections.

Samples from the eyes and throat, tested positive for Chlamydia by PCR. Chlamydia not known cause but Chlamydia was detected in a high number of animals.

In total sixty animals were post mortem and gross lesions were observed in the form of Severe conjunctivitis, Fibrinous oropharyngitis and Occasional cloacitis.

Severe conjunctivitis consisted of fibrin and mycoid discharge young animals (Figure 1) and in older animals of 1-3 year age class, the third eyelid sealed induces blindness. (Figure 2)



**Figure 1**



**Figure 2**

Fibrinous oropharyngitis consisted of redness in the throat due to inflammation (Figure 3) and a mass of fibrin build up (Figure 4) which caused asphyxiation. In the early stages of infection the mass of fibrin build up prevented the animal from eating or swallowing. In older animals nodules were also seen. The nodules formed due to the aggregation of lymphocytes cells. (Figure 5)



**Figure 3**



**Figure 4**

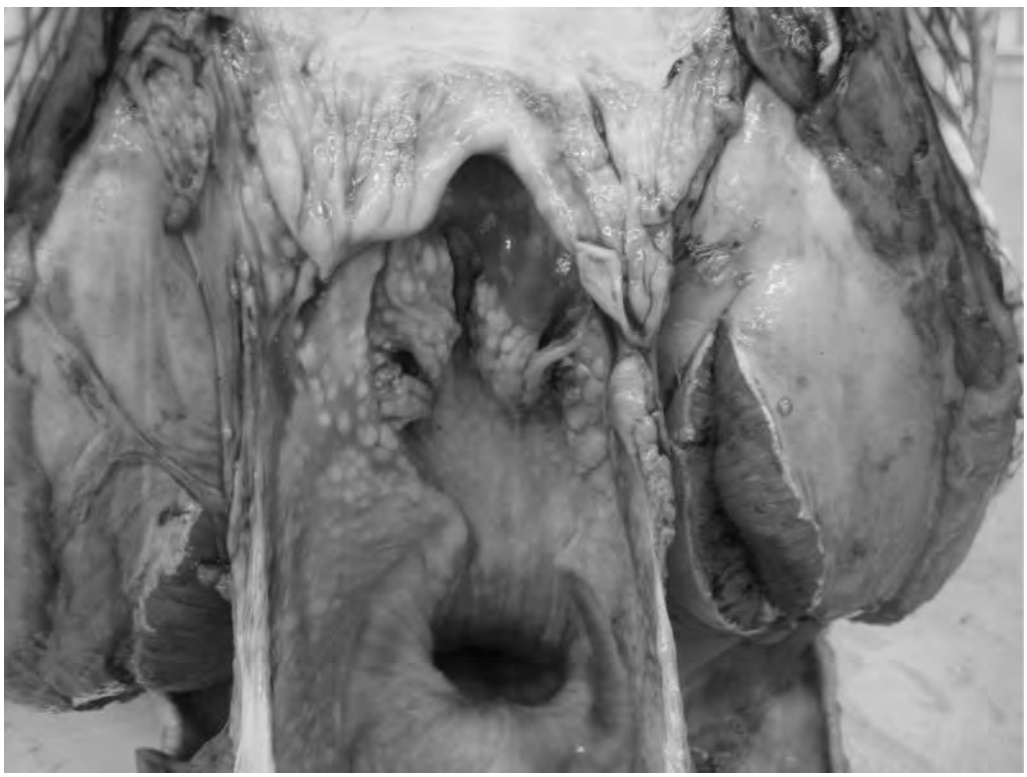


**Figure 5**

Occasional cloacitis consisted of fibrin discharge and nodules in older animals. (Figure 6)

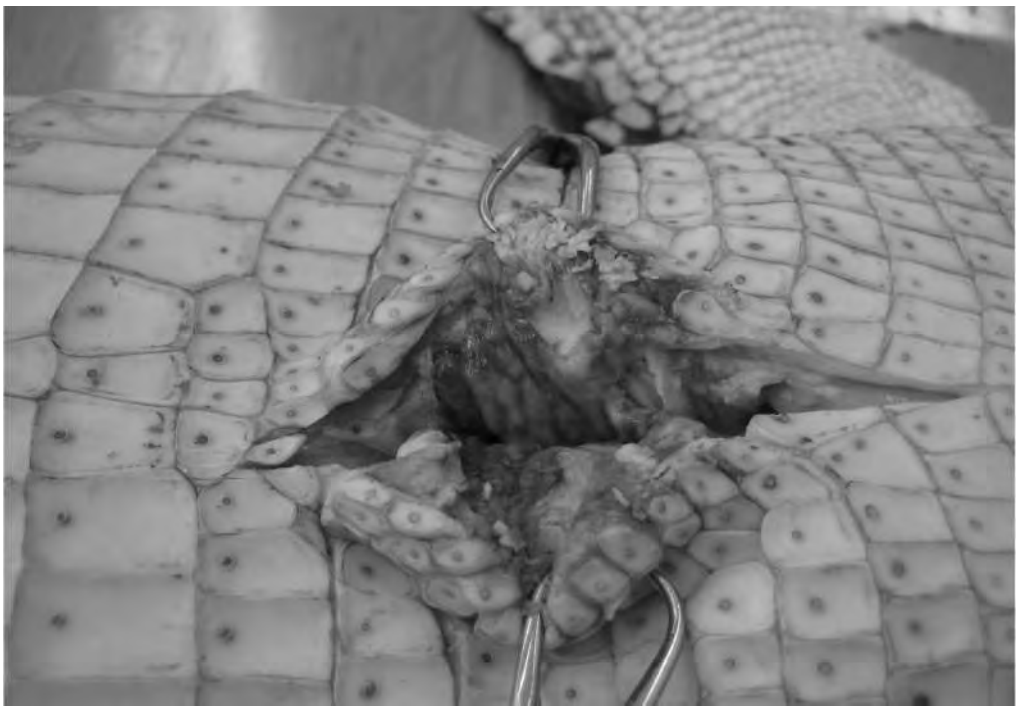


**Figure 6**

In histology the normal structure of columnar epithelial cells flattened out and therefore normal function of the cells was lost. (Figures 7-9)

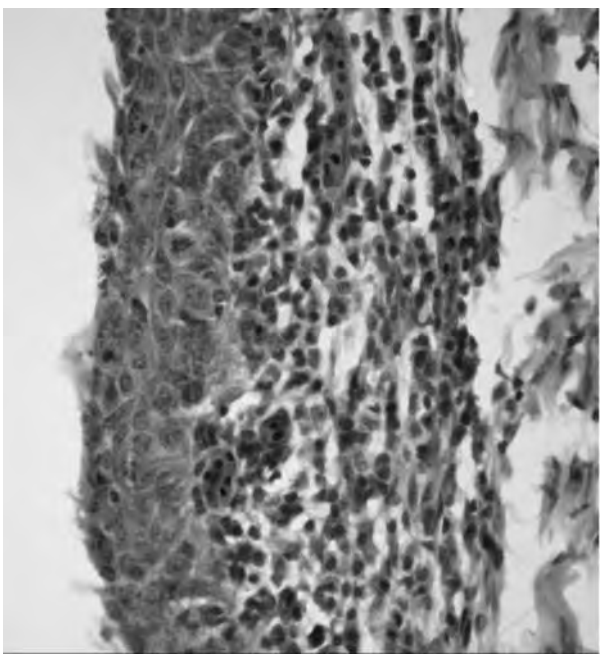


**Figure 7- Conjunctiva**

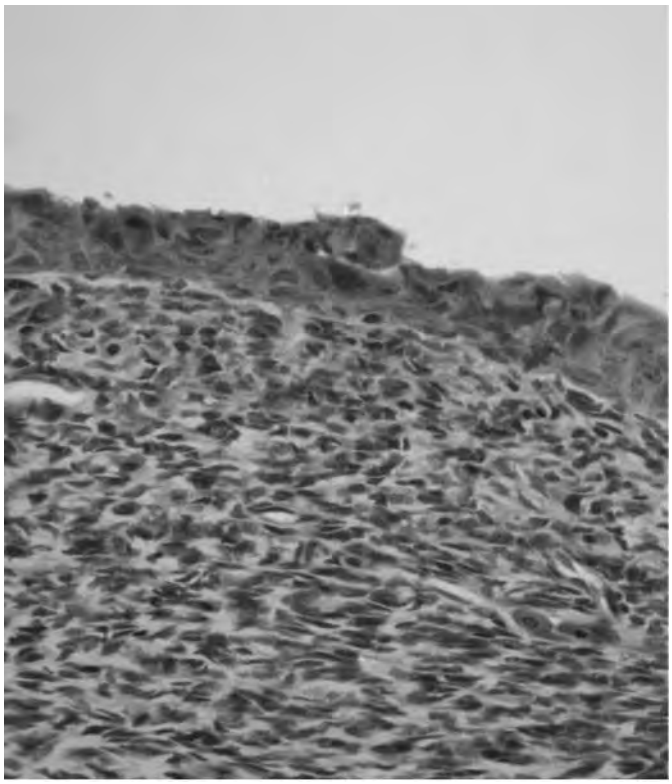


**Figure 8- Pharynx**

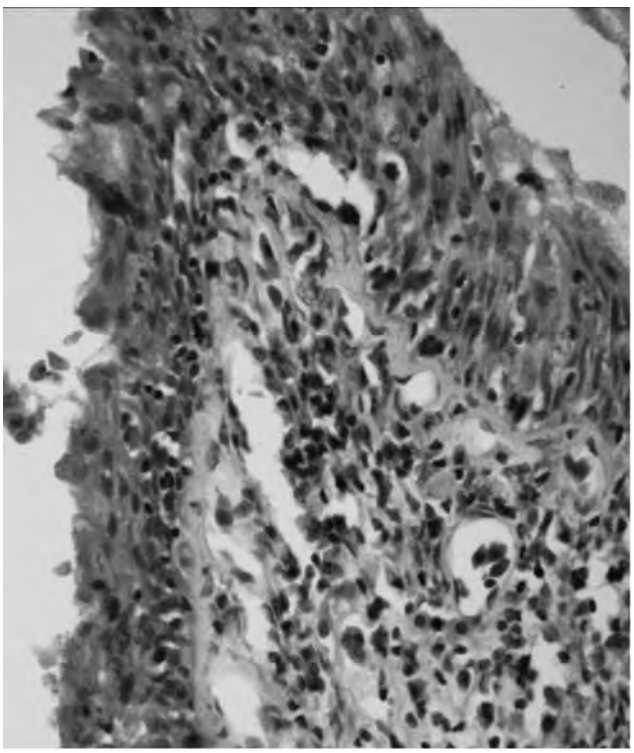


**Figure 9 -Cloaca**

## METHODS AND RESULTS

### Laboratory tests performed:

Tissue PCR +ve
(16S rRNA gene, Condon and Oakey, 2007 )

Conjunctiva – 14 from 14 samples tested positive for Chlamydia
Pharynx – six from six samples tested positive for Chlamydia

Antigen ELISA (Anti LPS) +ve

Conjunctiva – Three out of six tested positive for Chlamydia
Pharynx was not tested as it was discovered later after the ELISA test.

Mycoplasma PCR (tissue) +ve

Conjunctiva – one out of six tested positive for Chlamydia
Pharynx – nil samples tested positive

Viruses - Thin section EM +ve

No samples tested positive. However more work is needed. These results are from the early stages of testing. Crocodilian cell lines are being established and this will allow more virus work to be performed.

Survey work was also done in relation to the laboratory work to see if related factors could be detected.

Farms were surveyed late 2006 for any factors that could have influenced the Chlamydia outbreak.

Heron birds were considered as disease carriers and extended weeks of cold temperatures were also considered as an influence.

Herons were not carriers and the temperature influence needs more detailed research. No other related factors between farms could be discovered.

Gene sequencing of the crocodile Chlamydia isolated is as follows:

16S rRNA- 98% to 99% similarity to C Psittaci, C abortus and C rostinovo. Omp 2 – 91% similarity to C caviae and C psittaci..

Therefore the crocodile Chlamydia species is new and placed between C psittaci and C caviae.

**Outcomes:**

**Questions to answer:**

What is the prevalence (reservoir) of Chlamydia infection in various age groups in the normal farmed population?

What species/strains of Chlamydia are present and are these unique to crocodiles?

Is the infection common in the wild population and is the infection vertically transmitted?

To answer these questions, funding was sought and granted for a one year project. The project consists of two parts.

**1 year project:**

Part one-

- Introduce and validate molecular techniques for Chlamydial detection and identification

Detection:

- PCR for the omp 2 gene – primary detection method.

Identification of positives:

- RFLP of the omp 2 PCR product
- Sequencing of the omp2 PCR product
- PCR and sequencing of the 16S ribosomal RNA gene
- PCR and sequencing of the 23S ribosomal RNA gene

Part two-

Epidemiological investigation of Chlamydial infection in crocodiles.

Prevalence and strains of Chlamydial infection in farmed animals (ocular and pharyngeal swabs):

- Hatchlings – 2 farms: <1week, 1-4 weeks, 4-8 weeks, 8-12 weeks
- Grower animals – 2 year old animals - 4 farms in the NT, 2 farms in Qld, 1 farm in WA

Occurrence of chlamydial infection in the wild population:

- Ocular and cloacal swabs from trapped animals

Transmission of Chlamydia on or within eggs (shell and embryo swabs):

- Eggs collected from nests in the wild (2 per clutch, 30 clutches

## CONCLUSIONS

### Early Conclusion discovered in 2007:

- No positives detected in hatchlings pre-emergence from the egg – no vertical transmission could be detected.
- No positives in hatchlings under 40 days of age.
- Most positives detected in animals between 60-80 days of age.
- Number of positive animals varies in older age groups.
- Animals sampled from the wild, had positive detections from cloacal swabs.
- Animals sampled from farms, had positive detections from the ocular and pharyngeal swabs
- Infection of Chlamydia and clinical disease is rare under the age of 40 days.
- Clinical eye and throat disease is almost always associated with the presence of Chlamydia.
- Chlamydia is present in normal farmed animals but its prevalence varies widely between age groups and between farms.

### Related results to date:

- A bio security plan for the NT crocodile Industry has been developed. It outlines the bio-security measures to be taken in normal routine work procedures as well the measures to be taken in the event of a disease occurrence or outbreak.
- Finalization of a three year minor use permit through APVMA for the antibiotic Baytril; in the event of another Chlamydia outbreak.
- Crocodilian cell line cultures are being established as part of RIRDC/NTRIB funding, additional antibiotic testing can be performed.

(APVMA - Australian Pesticide Veterinary Medicine Authority)

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# Experimental induction of vitamin deficiency with diet in captive alligators.

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**ABSTRACT:** Previous studies show that mass mortality of American alligators (*Alligator mississipiensis*) in Lake Griffin in central Florida between 1998 and 2003 was due to neurological pathology and this pathology was associated with reduced levels of tissue thiamine (vitamin B1). Similar thiamine deficiency pathology is reported from captive marine mammals and free ranging salmonid fish that eat fish containing high levels of thiaminase. Gizzard shad (*Dorasoma cepedianum*), an abundant filter feeding fish, are common in alligator diets in Lake Griffin. We investigated whether gizzard shad have high levels of thiaminase, if feeding alligators gizzard shad would cause changes in alligator thiamine status and if thiamine deficiencies could be reversed by vitamin therapy and diet. We tested gizzard shad for thiaminase and demonstrated mean levels of 16,000 pmol/g/min which exceeds levels in alewife and smelt that cause thiamine deficiency in salmon that eat them. We held seven wild caught alligators in captivity and fed them only gizzard shad for 9-15 months. All seven showed significant declines in blood and muscle thiamine and three animals died showing symptoms similar to those seen in the field mortality event. Five of the seven alligators also showed diagnostic neural pathology at post mortem examination. Two alligators received thiamine therapy (injection and in the diet) starting in the 11$^{th}$ month of the experiment. These two alligators, which previously showed thiamine declines, restored their blood and tissue thiamine to normal levels and one of these did not show neuropathology, although the other did. We speculate on the relationship of lake eutrophication, gizzard shad abundance, blue green algal blooms and thiaminase levels in shad that might explain the observed field mortality of alligators as an end result of complex ecosystem interactions.

# American alligator growth: Determinate or indeterminate?

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**ABSTRACT:** American alligators (*Alligator mississippiensis*) have been suggested to exhibit indeterminate growth, i. e. they continue to grow throughout their entire lives. The objective of this study was to address the issue of indeterminate versus determinate growth in alligators from measurements taken over long term capture to recapture intervals. Capture-recapture data from 1980 to 2007 (27 years) were used to determine if alligators continued to grow after reaching adult size. Capture crews caught 49 adult alligators on the Yawkey Wildlife Center in coastal South Carolina (2005 – 2007), of which twenty-two were long-term recaptures. Six had not stopped growing since last captured (5 < 12, 1 < 25 years) but were not exceptions to growth parameters found in this study. Sixteen (6 males > 3.25m, 10 females > 2.36m TL) recaptures had no discernable growth in TL since previous capture (11 – 27 years). I conclude alligator growth is determinate. Alligators in this habitat showed strong site fidelity. Eleven females were recaptured at < 100m of their capture site over a period of 11 to 26 years. Three others were recaptured at locations 300m to 2km from previous capture sites. Individual males were recovered at or near their original capture sites over this same time period. Incidental to this study, three females were observed to have nested at intervals over a period of 24 to 26 years.

**Key words:** *Alligator mississippiensis*, American alligator, determinate growth, life history, site fidelity, movements, nesting longevity.

**RESUMEN:** Se ha sugerido que el caimán norteamericano (*Alligator mississippiensis*) muestra un crecimiento indefinido i.e. continúa creciendo durante toda su vida. El objetivo de este estudio fue tratar el tema del crecimiento indefinido de los cocodrilos vs. el definido a través de mediciones que se tomaron después de intervalos de larga duración entre captura y recaptura. Para determinar si los cocodrilos continuaban creciendo después de alcanzar su tamaño adulto, se utilizó información captura-recaptura de 1980 al 2007 (27 años). Los grupos de captura atraparon 49 cocodrilos adultos en el "Yawkey Wildlife Center" en las costas de Carolina del Sur (2005-2007), de los cuales 22 fueron recapturas de largo período. Seis no habían dejado de crecer desde la última captura (5<12, 1<25 años) pero no eran excepciones a los parámetros de crecimiento que se encuentran en este estudio. La recaptura de 16 (6 machos >3,25 m, 10 hembras >2,36m LT) no experimentaron un crecimiento perceptible en el LT desde la captura anterior (11-27 años). Mi conclusión es que el crecimiento de los cocodrilos es definido. Los cocodrilos en este hábitat mostraron una fuerte fidelidad con el lugar. Once hembras fueron recapturadas a < 100 m de su sitio de captura original durante un período de 11 a 26 años. Otras tres fueron recapturadas en lugares de 300m a 2 Km de los anteriores lugares de captura. Los machos individualmente fueron recuperados en su sitio original de captura o cerca durante este mismo período de tiempo. En forma incidental a este estudio, se observó que tres hembras habían anidado en intervalos durante un período de 24 a 26 años

**Palabras claves:** *Alligator mississippiensis*, caimán norteamericano, crecimiento definido, historia de vida, fidelidad con el lugar, desplazamientos, longevidad de anidamiento.

## INTRODUCTION

The American alligator inhabits various wetland habitats throughout the southeastern United States and west into Texas. Growth rate variability has been demonstrated in several states, including Florida (Jacobsen and Kushlan 1989), Georgia (Hunt 1990), Louisiana (Elsey *et. al.* 1992), North Carolina (Fuller 1981), and South Carolina (Wilkinson and Rhodes 1997). These studies demonstrated that growth varied in relation to alligator size, and thus age, between sexes, and among locations.

The relation between size and age was quantified for alligators in coastal South Carolina using mark-recapture data (Wilkinson and Rhodes 1997). Wilkinson and Rhodes (1997) developed growth curves by sex, of total length (TL) using the von Bertalanffy growth curve model (von Bertalanffy 1960, Fabens 1965, Kirkwood 1983). They found a mean of 34 years (3.46 m TL) for males and 24 years (2.51m TL) for females to reach asymptote size, which represents the mean size at which growth essentially stops.

Crocodilians have been suggested to exhibit indeterminate growth, i.e. they continue to grow throughout their entire life (Jacobsen and Kushlan 1989). However, Wilkinson and Rhodes (1997) had eight alligators among recaptures (2 M > 3.63m TL, 6F > 2.50m TL) that demonstrated no measurable growth for a period of > 10 years. This suggested alligator growth in length is determinate. According to the von Bertalanffy model, growth that approaches an asymptote and theoretically will continues to increase slowly throughout an individual's lifetime (Brisbin 1990). The curve will not reach zero growth. In reality this may not occur. The objective of this study was to address the issue of indeterminate versus determinate growth in alligators from measurements taken over long term intervals of captured and recaptured alligators.

## METHODS

The study was conducted on the South and Cat Island portion (6033 ha) of the Thomas A. Yawkey Wildlife Center, a South Carolina Department of Natural Resources managed wildlife refuge, located on the north central coast of the state. These lands are located between the Winyah Bay and North Santee River estuaries (33 degrees N). Mean tide range is 1.16m at the Yawkey Wildlife Center with a spring tide range of 1.34m (National Ocean Service 2006). The dominant vegetation in the surrounding tidal marsh (2524 ha) is smooth cordgrass (*Spartina alterniflora*). Managed impounded ponds (1012 ha) were typically maintained at water levels < 0.6 m depth and were vegetated with widgeon grass (*Ruppia maritima*), a submergent aquatic plant interspersed with emergent tall cord grass (*Spartina cynosuriodes*), salt marsh bulrush (*Scirpus robustus*) and smooth cord grass. Water salinity in impoundments ranged from 0 to > 35 ppt. Historically the Yawkey Wildlife Center has been maintained, both privately and by the state as a wildlife sanctuary. Alligators have been protected there from hunting for nearly 100 years.

As part of statewide alligator research projects, alligators were captured on this study area during 1977 – 1983 and 1993 with modified baited trip snares (Murphy *et. al.* 1983), snare

pole, snatch hooks, (Cherkiss *et. al*. 2004), and walk-through snares (Wilkinson 1994). Measurements to the nearest 0.1cm were taken of dorsal total length (TL) and ventral snout vent length to the posterior of vent (SVL), snout, tail, hindfoot length, (HF), neck, chest and tail girth. Where practical, alligators were weighed. Sex was determined and individuals were marked by toe clipping and notching dorsal tail scutes (Charbeck 1963). Alligators were also marked individually during early studies using colored, numbered tags affixed to nuchal and tail scutes and with a numbered monel fish tag placed in the webbing between the toes of the hindfoot. In all instances, capture method, location and date were recorded, and alligators were released at their capture sites.

During 2005 – 2007, alligator capture was undertaken using volunteer assistance on the Yawkey Wildlife Center with emphasis on recapturing previously marked alligators located while basking. The marking sequence was observable while the animals basked and indicated the approximate time when animals were first captured. Traps were situated to recapture animals with a long interval since last capture (> 12 years). When captured, unmarked alligators were sexed, weighed, measured, marked and released. Recaptures were individually identified, and their measurements and capture locations were compared with earlier records. Since long-time intervals were involved, time interval between capture and recapture were calculated in years, months and days. Distance between capture locations were calculated using 1” = 2000’ scale maps.

## RESULTS

Of 49 alligators captured during this study, 24 (10M, 14F) were recaptures of which twenty-two had intervals of > 11 to 27 years between captures. Alligator TL was compared between initial capture and subsequent captures to determine growth. Sixteen (6M, 10F) displayed no measurable growth for a period of > 11 to 26 years (6M > 3.25m, 10F > 2.36m TL) between initial capture and recapture during this study. There were six alligators (2M > 3.10m and 4F > 2.32m TL) that had grown since their previous capture (> 12 to 26 Years). The remaining two recaptures were first captured during this project and were not used in these calculations.

Distance between capture and recapture sites varied from 0 to 2 km. Eleven females were captured and recaptured at nearly the same site over a period of > 11 to 26 years while three others were recaptured at locations 300m to 2 km from their initial capture sites. Individual large males were captured-recaptured in relatively the same locations over this time period.

Incidental to this study are three females, two of which were observed to have nested at least three times over a period of 24 to 26 years. The other was observed to have nested twice over 25 years.

## DISCUSSION

Elements that contributed to the feasibility of this study were: (1) the study area has been maintained free from alligator hunting for a period of at least 95 years; (2) the alligator population on the area has been subject to scientific study during periods over 31 years: (3) individual alligators were permanently marked using a continuous standardized marking system which facilitated identification of individual alligators while basking; and (4) earlier

telemetry studies included data on alligator denning, basking and general feeding areas which identified parts of individual alligator home-ranges .

Of 22 recaptures, 16 (6 males 10 females) had no discernable growth in 11 to 26 years. These had reached maximum growth upon previous capture. Six alligators (2 males, 4 females) had not reached average asymptotic size at previous capture, but four were thought to have done so between previous and final capture (12 to 26 years). One male continued to grow throughout the study. It was first captured as a hatchling at the nest, and recaptured 26 years later (3.10m TL). Its length age relationship was eight years less than the average asymptotic age from previous models (34 years) (Wilkinson and Rhodes 1997). A second male was captured three times over a period of 25 years. At first capture it was 2.77m TL, an average length-age estimate of 18 years. At second capture, 13 years later, it had increased to 3.35m TL, a length-age estimate below male asymptote (34 years). It had increased to 3.70m TL 12 years later upon final capture, a length age relationship indicating it had reached asymptotic size between its second and final capture. However, affirmation of this would require future capture. One female in this group was first captured at 88.6cm TL and at second capture was 2.32m TL (12 years). This alligator was smaller than any other female in the recapture data set. An alligator this size would be expected to continue growth. Three females were small adult size females when first captured: 2.24m TL, 2.27m TL and 1.27m SVL (tail missing). These animals grew 0.1m TL, 0.09m TL and 0.04m SVL respectively (12 years). This growth would not be an exception to growth parameters in this study. I therefore conclude that alligator growth is determinate.

Terminal growth was variable for both sexes. Terminal growth for males in this study varied from 3.25 – 3.84m TL (range 0.61m), while terminal growth for females varied from 2.36 – 2.94m TL, a greater relative range than for males (0.58m).

Recaptured alligators displayed high site fidelity, 82 percent were recaptured <100m of their capture sites. This may be attributable to the habitat in the study area. There are 1012 ha of tidally controlled marsh impoundments (N=25) in the study area ranging from 5 – 170 ha and an estimated 20 additional freshwater rain-catches < .05 – 156 ha. These impoundments and freshwater rain-catches are the primary habitat used by alligators in the study area. Finfish and crustaceans are abundant in these impoundments including: striped mullet (*Mugil cephalus*), atlantic croaker (*Micropogonias undulates*), atlantic menhaden (*Brevoortia tyrannus*), spot (*Leiostomus xanthurus*), ladyfish (*Elops saurus*) and blue crab (*Callinectes sapidus*). These species tend to congregate at impoundment water control gates on the study area (N=68), especially during the high tides cycle when gates allow water seepage into impoundments. Alligators make periodic feeding forays to these fish and crab concentrations, where marked individuals may be located. Alligators also habitually use the freshwater rain-catches as haul-outs for basking and denning, especially during periods of drought when impoundment salinities exceed 12 ppt. Basking and denning locations of larger alligators are predictable, and the same location may be used by some individuals for years. Marked individuals can be predictably located at these areas. Additionally, travel routes between denning-basking sites and feeding locations are traditional which makes walk-through snares highly efficient. Walk-through snares were the most frequently used capture method (63 percent). This passive capture method is designed to snare an alligator as it moves normally from one location to another and was not thought to influence alligator habitat use. Our use of baited trip snares (27 percent) and snare pole (8 percent) was similarly not thought to

influence habitat use. Both methods were employed at sites where alligators were visually located during daylight reconnaissance.

Data were not collected relative to alligator home range during this study. Information gathered during earlier telemetry studies (Wilkinson 1983) was used to help locate specific alligators. Display of high site fidelity by recaptured alligators in this study is reflective of familiarity with their activities in specific portions of what may have been a larger home range. In undisturbed conditions, the data demonstrate that large, dominant alligators become behaviorally habituated and predictable, sometimes for years.

Continuous monitoring of alligator nesting was not conducted on the study area. Incidental to this study, three of the female recaptures were observed nesting over a span of 24 – 26 years. At initial capture one female (2.03m TL) was associated with nesting and later a crèche of hatchlings by means of radio telemetry and blood plasma analysis (Wilkinson 1983). Thirteen years later, it was recaptured at the same location and was 2.47m TL. It was observed later that season, associated with a crèche of hatchlings. Offspring genotypes are consistent with maternity by the female guarding the nest being the mother of the clutch (Davis, *et. al*. 2001). Upon final recapture totaling a 26-year span, it was again found associated with a crèche of hatchlings. The second female (2.64m TL), at first capture, was similarly associated with nesting and a crèche of hatchlings, and again upon final capture 25 years later. The third female (2.94m TL) was captured four times, and was the largest female alligator ever caught on the study area. This female was similarly associated with nesting when first captured, and has been associated twice over a span of 24 years with hatchling crèches. Growth studies of alligators in coastal South Carolina showed that it required, on average, 24 years (2.51m TL) for female alligators to reach asymptotic size (Wilkinson and Rhodes 1997). Nesting may occur as much as nine years earlier. The size of this female (2.94m TL), 0.43m TL above asymptotic size, indicates it had reached asymptote and was perhaps older when first captured. The additional years of known nesting suggest this female has continued to produce viable young at an age > 50 years.

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# Competing risk analysis of survival traits in *Crocodylus porosus*

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**ABSTRACT:** The heritability for overall survival in juvenile saltwater crocodiles has previously been estimated as 0.15 (SE 0.04). However, this heritability estimate was based only on whether an animal lived or died and not based on why the animal died. Thus, the objective of this study was to determine the heritability for various reasons for juvenile mortality in *Crocodylus porosus*. Data were collected from all juvenile crocodiles in the 2005, 2006 and 2007 cohorts at Darwin Crocodile Farm (n = 2,171) of known-parentage. The data were collected in a categorical manner as either congenital (hatchling abnormality), disease-related (confirmed by pathology), stress-related, runtism, no visible ailments (NVA; no obvious husbandry reason for death) or miscellaneous. Using a pair model, the heritability was 0.71 (SE 0.08) for all traits, whilst using an animal model, heritability estimates ranged from 0.39 (SE 0.03; Overall survival, congenital and stress) to 0.56 (SE 0.04; runtism). These estimates are higher compared to the previous published crocodile estimate and other livestock species. Further data is required to remove the confounding present in the current models.

## INTRODUCTION

Juvenile crocodile deaths still remains an issue of concern for Australian crocodile producers. Webb (1989) commented that producers should aim for 95% survival in the first year after hatch, but in reality, survival rates are more likely between 85-90% (Isberg et al. 2004). However, grouping all deaths into an overall category does not assist in our understanding of the predominant causes of death. The overall purpose of this study was to collect mortality data in a categorical fashion to overcome this deficiency. Herein are the results from the genetic analysis of this data.

## METHODS AND MATERIALS

Mortality records from 2,171 juvenile saltwater crocodiles of known-parentage from Darwin Crocodile Farm (Northern Territory, Australia) were collected from the 2005, 2006 and 2007 cohorts. Progeny records were from 67 “pair” families (53 sire families). After hatching, crocodiles were scute cut for individual animal identification.

Mortality records were collected on a daily basis by recording the animal’s unique scute cuts, and recording the reason for death using six categories as follows:
1. Congenital defects including unabsorbed yolk sacs, jaw deformities such as cleft palate and under-/over-shot jaws, spinal deformities, tail deformities such as no tail, partial tail, “curly” tails and any other physical defect.
2. Runtism was defined by an emaciated, non-thriving animal in comparison to others of similar age.

3. Disease-related was determined after pathology at Berrimah Veterinary Laboratory (BVL; Northern Territory Department of Primary Industries, Fisheries and Mines) and defined as independent to a management-induced stress. In most cases, subsequent administration of antibiotics was warranted.
4. Stress-related is defined whereby deaths occurred within a short time after a management-induced stress event. These include minimising size variation within pens (grading), moving animals between pens, hot water services failing or pens left without water.
5. No visible ailments (NVA) was used when no disease outbreaks were identified nor a stress incident noted. These deaths usually occur in random pens with no distinct trend in mortalities.
6. Miscellaneous is any other event that does not fit into the above categories. Animals that had not died (n = 1,764) and were still in the production system when the trial period ended (31st December, 2007) were right censored and coded zero (0). Table 1 shows the number of deaths in each category.

**Table 1.** Number of deaths in each category used in the survival analyses from 2,171 animals.

| Category | No. deaths | Overall deaths (%) | % of overall deaths |
|---|---|---|---|
| Overall | 407 | 14.96 | |
| Congenital defect | 7 | 0.26 | 1.72 |
| Runt | 201 | 7.39 | 49.39 |
| Disease-related | 37 | 1.36 | 9.09 |
| Stress-related | 62 | 2.28 | 15.23 |
| NVA | 93 | 3.42 | 22.85 |
| Miscellaneous | 10 | 0.37 | 2.46 |

Data were analysed using a Cox's Proportional Hazards Model in Survival Kit V3.12 (Ducrocq and Sölkner 1994; 1998) using two models.

1. Pair model

$$\ln[h_{ijk}(t)] = \ln[h_0(t)] + (ß_{HD}HDays_{jk} + ß_{No}NoFarm_{jk} + Year_k + Pair_j + Clutch_{jk})$$

2. Animal model

$$\ln[h_{ijk}(t)] = \ln[h_0(t)] + (ß_{HD}HDays_{jk} + ß_{No}NoFarm_{jk} + Year_k + Animal_j)$$

where $h_{ijk}(t)$ is the hazard function for the $i^{th}$ individual from the $j^{th}$ pair in the $k^{th}$ year at time t, $h_0(t)$ is the unspecified baseline hazard function, $HDays_{jk}$ is the number of days between hatching date and the 1st of January in that particular year for an individual from the $j^{th}$ pair in the $k^{th}$ year; $ß_{HD}$ is the regression coefficient for HDays; $NoFarm_{jk}$ is the number of live hatchlings in a particular clutch from the $j^{th}$ pair in the $k^{th}$ year; $ß_{No}$ is the regression coefficient for NoFarm; $Year_k$ is the fixed effect of the $k^{th}$ year (k = 2005, 2006, 2007); $Pair_j$ is the random effect of pair (assumed $N(0,\sigma^2Pair)$); $Clutch_{jk}$ is the common environment (random) effect of a clutch produced by the jth pair in the kth year (assumed $N(0,\sigma^2Clutch)$); and Animalj is the random effect of the jth individual (assumed $N(0,\sigma^2Animal)$). A 5% significance level was chosen to evaluate explanatory variables by backward elimination. Pair model log-survival heritability estimates were calculated as in Isberg et al. (2004), whilst the animal model estimates were calculated as

$$h^2_{log} = \frac{\sigma^2_{Animal}}{\sigma^2_{Animal} + \frac{\pi^2}{6}}$$

using the estimates of the variance component, $\sigma^2_{Animal}$.

## RESULTS AND DISCUSSION

The pair model baseline survival function for crocodiles between hatch and 1000 days is shown in Figure 1. This plot shows the probability of a crocodile surviving to any given day, and it demonstrates a high mortality rate over the first approximately 400 days. Isberg et al. (2004) reported the probability of an animal surviving to day 400 was 56% after removing genetic and non-genetic effects. This probability has been increased using the current data to 89% for overall survival. For the other traits, the probability of an animal surviving to day 400 are 100%, 93%, 100%, 99% and 97% for congenital defects, runt, disease-related, stress-related and NVA, respectively.

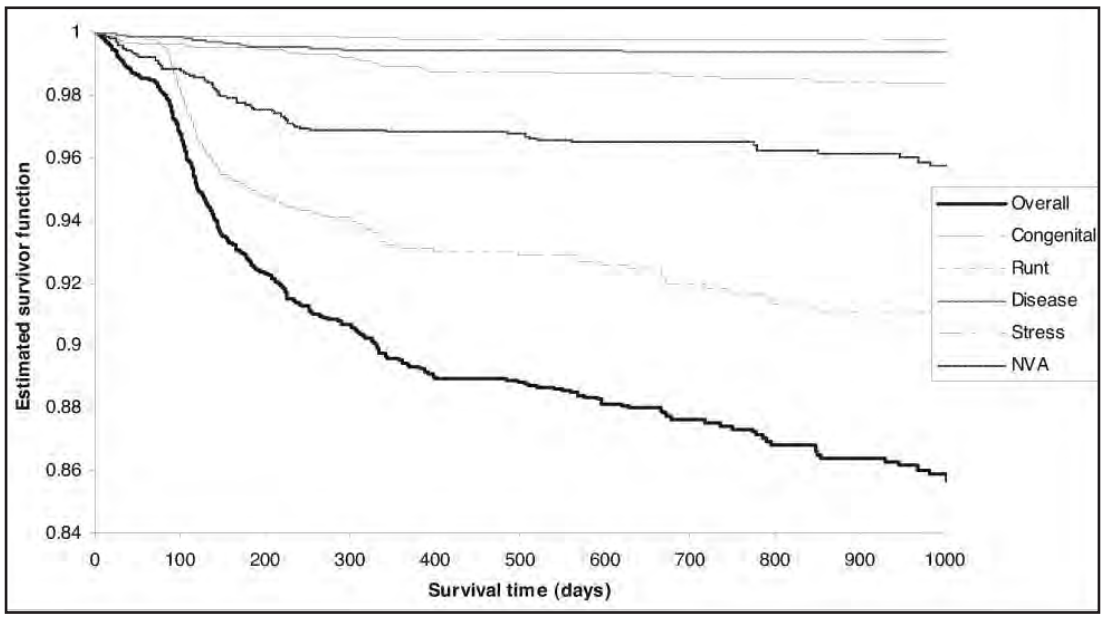



**Figure 1.** Baseline survivor function after removing all significant genetic and non-genetic effects for all survival categories between hatch and 1000 days.

Using the pair model, the number of live hatchlings produced in each clutch (NoFarm) was not significant for any of the survival categories, whilst using the animal model, NoFarm was significant for the runt, disease-related, stress-related and NVA categories. The date of hatch (Hdays) was found to be significant for the runt, disease-related and stress-related categories using the both the pair and animal model. Year was also significant for the runt pair model as well as overall, runt, stress-related and NVA animal models.

Using the pair model, the log-survival heritability estimates were all unexpectedly 0.71 (SE 0.08; Table 2), despite different models being significant and differences in the estimated variance components for pair and clutch. With exception, no significant random effects were found for the NVA survival category indicating that these are truly random events. However, using the animal model, the heritability estimates varied from 0.39 (SE 0.03) to 0.56 (0.04; Table 2).

**Table 2.** Heritability estimates (SE) for the survival categories using the pair and animal model. n.s. is where no random effects were fond to be significant, and thus heritability could not be estimated.

| Category | Pair model | Animal model |
|---|---|---|
| Overall | 0.71 (0.08) | 0.39 (0.03) |
| Congenital defects | 0.71 (0.08) | 0.39 (0.03) |
| Runt | 0.71 (0.08) | 0.56 (0.04) |
| Disease-related | 0.71 (0.08) | 0.49 (0.04) |
| Stress-related | 0.71 (0.08) | 0.39 (0.03) |
| NVA | n.s. | 0.42 (0.03) |

Overall, these heritability estimates are extremely large in comparison to the overall heritability estimate published by Isberg et al. (2004; $h^2$ 0.15 (SE 0.04)) and those for other livestock industries (Ducrocq et al. 2000; Knol et al. 2002; Southey et al. 2001). The similarity and magnitude of the pair model estimates may indicate confounding in the data set since there are only three years of data and clutch was modelled as an interaction between pair and clutch. Data will continue to be collected in this manner for future analysis. The animal model heritability estimates show differences between the different traits which was expected.

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# Early growth of Black caimans (*Melanosuchus niger*) in Bolivia

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**ABSTRACT:** One hundred and five Black caimans (*Melanosuchus niger*) were captured and marked between 1992 and 1996 at Laguna Cedral, within the Biosphere Reserve, Estación Biológica Beni, Bolivia. Thirty caimans were recaptured at least once, five were recaptured twice and two caimans were recaptured three times. Longest periods between the first and last captures were between 1445 and 1470 days. Smallest caimans were 19 cm long (body length) at first capture and largest recaptured caimans reached 53 cm in body length, at about 4.5 years of age. Growth rates ranged between 0.28 to 0.47 mm/day for about a year, for those caimans captured before their first year of age; while growth decreased to as low as 0.14 mm/day later in life. Distances between capture sites ranged between about 300 to 1500 m for caimans captured with a four-year interval. All caimans captured before their first year of age were recaptured at < 700 m from their initial capture sites within the next year and most of them were recaptured at < 500 m of their initial capture site. Only one caiman captured at another location, about 3 km from the primary study site, was captured four years later at Laguna Cedral.

# Hypoxia: Does it affect embryo differentiation rate or just growth?

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**ABSTRACT:** In crocodilians, the rate of embryonic development and consequently the duration of the incubation period are affected by temperature. Since incubation temperature has strong influences on embryo development by altering metabolism rate, we manipulated oxygen concentration in order to uncouple the effects of developmental rate from the direct effects of temperature. Here we consider whether oxygen concentration has influence on differentiation rate (or progression from one stage to the next) and growth (body mass and total length). Thus, we incubated *Caiman latirostris* eggs at two different temperatures (31ºC, 100% female-producing temperature, and 33ºC, 100% male-producing temperature) and at two O2 concentrations (15% and 21%). We monitored the developmental stages of these embryos within the thermosensitive period (stages 20-24). Incubation under hypoxia reduced embryonic growth, but it had no effect on differentiation rate. Our results suggest that both, temperature and oxygen concentration, affect yolk-to-tissue conversion rate (and thus embryo size), and differentiation rate is affected only by incubation temperature.

# Relationship between size of reproductive females and size and mass of eggs and hatchlings of Babilla (*Caiman crocodilus fuscus*) in Colombian Croco farm LTDA, Municipio Barranco de Loba, Bolivar, Colombia

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**ABSTRACT:** The Colombian Croco farm classifies its reproducers in homogenous groups according to size. For the nesting season in 2005 we collected data on 104 nests from females of different size groups.

The size rank of each group were: Group 1: small females with average 66 cm (SVL); Group 2: females with average 76 cm (SVL) and Group 3: large females with average 84.5 cm (SVL).

The first group (small females) 40 nests were selected; the second group 37 and the third (large females) 27 nests. For each group the following aspects were evaluated: eggs length, width and weight of each clutch, also hatchling length and weight.

The information was related to female size of the three reproductive groups. The data collected for each variable were statistically analyzed. Analysis of variance revealed that females size does not affect the egg length, but width and weight do show a statistically significant relationship. Larger females have eggs that are wider and heavier than smaller females. These results also show a similar relationship with hatchling length and mass.

# Physiological diagnostic and a comparison of growth and mortality induced by the effect of medicinal products on *Caiman yacare* hatchlings in Crocoland farm, Santa Cruz, Bolivia

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**ABSTRACT:** Mortality under captivity is often directly linked to conditions on the farm, where the effect of stress, high density levels, and bad sanitary cleaning and disinfection practices are further common sources of diseases that have to be treated with preventive handling actions to bring about an improvement on hatchlings and finally cure them. We analyze the effect of medicinal products and fluctuating temperatures over the development and mortality on hatchlings that, after 6 months of been born, still show a low growth rate level. We worked with 720 hatchlings, divided into 4 groups of 180, which were placed in 4 husbandry pools with different temperature degrees. Each one of the 4 groups were also subdivided and placed into 6 compartments with 30 hatchling, and supplying them with: G1: Anabolics, G2: Injectable B Complex, G3: Oral B Complex, G4: Suppressor, G5: Combination of the 4 medicines y G6: Standard group (without supplies). The preliminary results of the development were: 0.9 cm per month in G1 of Compartment 1, 3.7 cm per month in G2 of Compartment 2, 3.3 cm per month in G1 of Compartment 3 and 1.5 cm per month in G2 of Compartment 4. The optimum growth temperatures degrees fluctuated between 33ºC and 38 ºC, and the anabolics turned out to be the best encouraging medicinal product.

**RESUMEN:** La mortandad de cocodrilos en zoocriaderos se debe generalmente a condiciones específicas del criadero, donde el efecto del estrés, densidades elevadas y malas prácticas higiénico-sanitarias pueden desencadenar diversas patologías, ante las cuales se deben desarrollar alternativas y medidas de prevención, y en el mejor de los casos, mejoría y/o curación de los especímenes.

En este trabajo analizamos de los efectos que ejercen los medicamentos y temperatura sobre el crecimiento y mortandad en neonatos que, después de los primeros 6 meses, presentan un desarrollo deficiente. Se trabajó con 720 neonatos, separados en grupos de 180, y colocados en 4 bandejas con temperaturas diferentes. Cada uno de los 4 grupos, fue dividido en 6 subgrupos de 30 neonatos, suministrándoles: G1: Anabólico, G2: Complejo B inyectable, G3: Complejo B oral, G4: Antiparasitario, G5: Combinación de los 4 medicamentos anteriores, y G6: Patrón (sin medicamentos).

Los mejores crecimientos, en base a resultados preliminares, fueron de 0.9 cm/mes en el G1 de la Bandeja 1, de 3,7 cm/mes en el G2 de la Bandeja 2, de 3,3 cm/mes en el G1 de la Bandeja 3 y 1,5 cm/mes en el G2 de la Bandeja 4. Las temperaturas entre los 33 y 38 ºC son las más óptimas para el desarrollo y los anabólicos estimulan el crecimiento.

# Efficiency of Freeze - Dried Crocodile Blood in Iron Deficiency Anemia Male Rats

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**ABSTRACT:** Male weanling Sprague-Dawley rats were divided into AIN93G$^{+Fe}$, AIN93G$^{-Fe}$ and AIN93G$^{-Fe}$ + FzCB groups. After 4, 8 and 24 weeks, animals were fasted for 24 h prior to the experiments. The hematological values that indicated anemia status were evaluated. After 4 weeks, hemoglobin (5.59 + 0.25, and 5.80 + 0.29 g/dl) and hematocrit (26.46 + 6.45 and 17.10 + 0.83 %) values of AIN93$^{-Fe}$ and AIN93G$^{-Fe}$+FzCB groups respectively were significantly different ($P$<0.05) from AIN93G$^{+Fe}$ (15.14 + 0.43 g/dl and 41.52 + 1.09 %) group. Consequently, after 8 and 24 weeks, hemoglobin (15.67 + 0.49, 16.32 + 0.17; 13.7 + 1.65, 15.44 + 0.55 g/dl) and hematocrit values (43.45 + 1.31, 45.44 + 0.44; 38.38 + 4.29, 40.08 + 2.15%) of AIN93G$^{+Fe}$ and AIN93G$^{-Fe}$+FzCB groups respectively were differ ($P$<0.05) from AIN93G$^{-Fe}$ (8.88 + 2.52 g/dl and 26.46 + 6.45) group. The freeze-dried crocodile blood had no detrimental effect on histological change in intestine kidney and liver after 24-week. These data suggest the freeze-dried crocodile blood has efficiency for promoting hemoglobin and hematocrit values on iron deficiency rat that may be used as food supplement in anemia patient.

## INTRODUCTION

Iron deficiency anemia is the most prevalent nutritional deficiency and the most common cause of anemia in Thailand (Pattanee 2002). It is the most common micronutrient deficiency in the world today and impacts the lives of millions of women and children contributing to poor cognitive development, increased maternal mortality and decreased work capacity. It is characterized by a defect in hemoglobin synthesis, resulting in red blood cells that are abnormally small (microcytic) and contain a decreased amount of hemoglobin (hypochromic) (Provan 1999). The capacity of the blood to deliver oxygen to body cells and tissues is thus reduced. Iron is essential to all cells. Functions of iron include involvement in energy metabolism, gene regulation, cell growth and differentiation, oxygen binding and transport, muscle oxygen use and storage, enzyme reactions, neurotransmitter synthesis, and protein synthesis (Beard 2001). Measurement of hemoglobin or hematocrit is the most cost efficient and commonly used method to screen for anemia (Cook 1999). Iron therapy, in combination with dietary strategies to increase iron and vitamin C intakes, effectively treats iron deficiency anemia by raising the hemoglobin level and replacing iron stores.

Freeze dried crocodile blood is a natural product and can served as medicines for curing illness such as allergy and asthma, and may also prolong their life. It has been widely

consumed not only for its nutritious composition, but also for its claimed medicinal value (Siruntawineti *et al*. 2004). The practice of consuming crocodile blood for improving human health is found in the traditions of many Asian cultures. Recently, the Freeze dried crocodile blood production process has been development and the safety for crocodile blood consumption has been reported (Chaeychomsri *et al*. 2004, 2006). Our study aimed to determine whether the efficiency of freeze dried crocodile blood on iron deficiency male rat, Spraque-Dawley, to use as human food supplement by observing hemoglobin and hematocrit values in the rats.

## METHODOLOGY

*Preparation of Freeze-dried Crocodile Blood:* Crocodile blood was collected from Siamese crocodiles (*Crocodylus siamensis*) rose at Sriracha crocodile farm, Chonburi, Thailand using sterile technique. Fresh crocodile blood was weekly taken and kept at 4ºC in sterile containers. The freeze-dried blood was prepared in sterile conditions and stored at 4ºC until use.

*Laboratory animals:* The Animal Ethics Committee of Kasetsart University, Thailand approved the use of laboratory animals in this study. Fifteen male Spraque-Dawley were purchased from The National Laboratory Animal Center, Mahidol University, Salaya, Thailand. They aged 3-4 weeks with weight ranging from 45 to 60 g. The rats were randomly divided into 3 groups. Group 1 (AIN93G$^{+Fe}$) received high fat corn starch (HFCS) base on AIN93G$^{+Fe}$ (Reeves *et al*. 1993) diet (Table 1). Group 2 (AIN93G$^{-Fe}$) received HFCS base on AIN93G$^{-Fe}$ diet. Group 3 (AIN93G$^{-Fe}$+FzCB) received HFCS base on AIN93G$^{-Fe}$ diet for 30 days and followed by HFCS base on AIN93G$^{-Fe}$+Freeze dried crocodile blood 1000 mg/kg body weight. Rats were housed individually in hanging wire-mesh cages in a room with a controlled temperature of 25-29ºC and a 12:12-h light-dark cycle with 30-70% relative humidity. Animals were allowed unlimited access to food and distilled water. Daily food intake were recorded and body weights were recorded weekly

**Table 1.** Composition of diet

| **Dry Matter Ingredient (g/kg)** | **High fat corn oil (HFCS)1** | |
|---|---|---|
| | **AIN93G$^{+Fe}$** | **AIN93G$^{-Fe}$** |
| Casein[2] | 200.0 | 200.0 |
| L-Cystine[3] | 3.0 | 3.0 |
| Corn starch[2] | 529.5 | 529.5 |
| Sucrose[2] | 100.0 | 100.0 |
| Cellulose[2] | 50.0 | 50.0 |
| Soybean[2] | 70.0 | 70.0 |
| AIN-93G mineral mixture[2] | 35.0 | 35.04 |
| AIN-93G vitamin mixture[2] | 10.0 | 10.0 |
| Choline Chloride[3] | 2.5 | 2.5 |

1 Based on the AIN-93G diet. 2 Purchased from Diet (USA).
3 Purchased from Wako Pure Chemical Col., (Osaka, Japan). 4 Ferric citrate was omitted.

*Experimental procedure:* Beginning at 4 weeks of age, rats were fed the experimental diets for 24 weeks. After 4, 8 and 24 weeks, animals were fasted for 24-h prior to the experiments.

Rat blood was sampled at 4, 8 and 24 weeks. The blood sample was withdrawn from tail vein using ethylene diamine tetraacetic acid (EDTA) as anticoagulant. The hematological values of rat blood, complete blood count (CBC), white blood cell count (WBC), red blood cell count (RBC), Hematocrit (Hct), hemoglobin (Hb) concentration were performed by an automate hematology analyzer (Sysmex K-1000, Diamond Diagnostics, USA). All values were compared to control group at the same time of treatment and to the standard range for rats.

*Histopathological study:* The specimens were taken from intestine, kidney, and liver of rat after 24 weeks of treatment. These organs were then collected and fixed with Bouin's fixative. Tissues slides were prepared and stained with hematoxylin and eosin. The slides were examined by a pathologist.

*Statistical Analysis:* The data were analysed by one-way ANOVA. The significant differences between the experimental groups, at $P<0.05$, were compared by Duncan multiple range test. Each value represents Mean + SE.

## RESULTS AND DISCUSSION

Throughout the experiment AIN93G$^{+Fe}$ and AIN93G$^{-Fe}$+FzCB groups appeared healthy, inquisitive and active. No illness or death occurred. However, AIN93G$^{-Fe}$ appeared bad health and inactive. The body weight of AIN93G$^{-Fe}$+FzCB and AIN93G$^{-Fe}$ groups were significant difference ($P<0.05$) from AIN93G$^{+Fe}$ after 4 weeks of diet until the end of the study. These results indicated that AIN93G-Fe+FzCB and AIN93G$^{+Fe}$ groups had higher growth rates after fed freeze-dried crocodile blood than AIN93G-Fe group that had Iron deficiency anemia.

The hematological parameters specially in hematocrit and hemoglobin values of AIN93G$^{-Fe}$ and AIN93G$^{-Fe}$+FzCB treated groups revealed that after 4 weeks hemoglobin (5.59 + 0.25, and 5.80 + 0.29 g/dl) and hematocrit (26.46+ 6.45 and 17.10 + 0.83 %) values of AIN93$^{-Fe}$ and AIN93G$^{-Fe}$+FzCB respectively were significantly different ($P<0.05$) from AIN93G$^{+Fe}$ (15.14 + 0.43 g/dl and 41.52 + 1.09 %) group. After 8 and 24 weeks, hemoglobin (15.67 + 0.49, 16.32 + 0.17 and 13.7 + 1.65, 15.44 + 0.55 g/dl) and hematocrit (43.45 + 1.31, 45.44 + 0.44 and 38.38 + 4.29, 40.08 + 2.15%) values of AIN93G$^{+Fe}$ and AIN93G-$^{-Fe}$+FzCB respectively were significantly different ($P<0.05$) from AIN93G$^{-Fe}$ (8.88 + 2.52 g/dl and 26.46 + 6.45) group (Table 2). These results indicated that administration of the freeze-dried crocodile blood as food supplements had effects on hematological values. Moreover, our previous studies confirmed that the crocodile blood was free from parasites. Therefore crocodile blood should be safe for consumption as food supplement.

Treated rats of AIN93G$^{-Fe}$+FzCB group exhibited no alteration of intestine, liver and kidney after 24-week of daily feeding freeze-dried crocodile blood. These results indicated that freeze-dried crocodile blood had no detrimental effect on histological change in intestine kidney and liver in all treatments.

These data showed that freeze-dried crocodile blood had effect in hematological values especially in hemoglobin and hematocrit values on iron deficiency rat. Moreover, treated rats exhibited no alteration of intestine, liver and kidney after 24 week of daily feeding with freeze dry crocodile blood (Fig. 1).

Table 2 Mean hematological values of 5 Sprague-Dawley rats in each group after feeding 4, 8 and 24 weeks of experiment.

| Hematological | After 4 weeks | | | After 8 weeks | | | After 24 weeks | | |
|---|---|---|---|---|---|---|---|---|---|
| Value | AIN93 $^{+Fe}$ | AIN93 $^{-Fe}$ | AIN93$^{-Fe}$+FzCB | AIN93 $^{+Fe}$ | AIN93 $^{-Fe}$ | AIN93$^{-Fe}$+FzCB | AIN93 $^{+Fe}$ | AIN93 $^{-Fe}$ | AIN93$^{-Fe}$+FzCB |
| RBC ($10^6$/ml) | 7.55 ± 0.10 | 4.69± 0.08* | 4.80± 0.15* | 8.26 ± 0.20 | 7.05 ± 0.64 | 9.81 ± 0.31* | 8.65 ± 0.11 | 6.00 ± 0.92 | 9.14 ± 0.91 |
| HGB (g/dl) | 15.14 ± 0.19 | 5.58 ± 0.14* | 5.80 ± 0.14* | 15.67 ± 0.24 | 7.57 ±1.11* | 13.7±0.74 | 16.32 ± 0.17 | 9.69 ± 0.05* | 15.44 ± 0.55 |
| Hct (%) | 41.52 ± 0.48 | 16.90 ± 0.43* | 17.10 ± 0.41* | 43.45± 0.65 | 23.15 ± 2.95* | 38.38± 1.91 | 45.44 ± 0.44 | 21.65 ± 2.30* | 40.08 ± 2.15 |
| MCV (fl) | 55.00 ± 0.41 | 36.00 ± 0.35* | 35.70 ± 0.40* | 52.57 ± 0.55 | 32.75 ± 1.25* | 39.3 ±2.65* | 52.52 ± 0.34 | 35.80 ± 0.50* | 44.02 ± 2.34 |
| MCH (pg) | 20.02 ± 0.12 | 11.90 ± 0.10* | 12.10 ± 0.12* | 18.97± 0.23 | 10.70 ± 0.60* | 14.04 ± 0.97* | 18.86 ± 0.14 | 11.6 ± 0.46* | 17.02 ± 1.03 |
| MCHC (g/dl) | 36.42 ± 0.10 | 33.03± 0.27* | 33.90± 0.29* | 36.15 ± 0.18 | 32.65 ± 0.65* | 35.66 ± 0.25 | 35.88 ± 0.08 | 32.38 ± 1.20 | 38.70 ± 1.27 |
| Plt ($10^3$/ml) | 815.00 ± 74.25 | 1815.67 ± 72.45* | 1811.50 ± 68.38* | 738.00 ± 109.20 | 1394.50 ± 5.50* | 1126.00 ± 100.93* | 793.40 ± 76.62 | 1856.50 ± 152.50* | 1070.40 ± 110.34 |
| MPV (fl) | 8.4 ± 0.21 | 9.39 ± 0.12 | 9.73 ± 0.40* | 8.59 ± 0.28 | 11.90 ±0.38 | 8.90 ± 0.26 | 8.80 ± 0.36 | 14.18 ± 1.64 | 12.60 ± 0.37 |
| WBC ($10^3$/ml) | 12.50 ± 0.98 | 14.13± 3.96 | 17.05 ± 1.75 | 9.50 ± 1.14 | 13.90 ± 1.10* | 13.4 ± 0.80* | 8.78 ±0.87 | 5.42 ± 2.5* | 8.12 ± 1.09 |
| Neu (%) | 7.55 ± 0.89 | 20.03 ± 6.3*0 | 14.10 ± 1.4 0 | 8.53 ± 1.00 | 13.57± 3.80 | 8.31± 0.84 | 13.2 ± 3.50 | 9.93 ± 1.09 | 14.06 ± 3.08 |
| Lym (%) | 85.38 ± 1.40 | 66.10 ± 8.10* | 67.53 ± 5.10* | 86.37 ± 1.97 | 79.30 ± 5.20 | 80.74 ± 2.10 | 76.6 ± 9.56 | 76.95 ± 17.30 | 53.50 ± 14.06 |
| Eo (%) | 1.43 ± 0.24 | 1.56 ± 0.47 | 1.37 ± 0.29 | 2.02 ± 0.19 | 1.78 ± 0.40 | 2.03 ± 0.69 | 1.93 ± 0.23 | 3.49 ± 0.03 | 2.54 ± 1.63 |
| Ba (%) | 1.16 ± 0.18 | 1.12 ± 0.56 | 1.60 ± 0.58 | 0.63 ± 0.18 | 0.65 ± 0.25 | 2.06 ± 0.31 | 3.09 ± 1.43 | 3.35 ± 1.76 | 1.30 ± 0.37 |
| Mo (%) | 1.16 ± 0.50 | 11.16 ± 1.45 | 15.40 ± 3.79* | 2.44 ± 0.97 | 4.67 ± 1.52 | 6.84 ± 1.19 | 5.12 ± 1.29 | 5.15 ± 1.65 | 2.83 ± .0.67 |

*Significant different between group at the same time ($P$<0.05)
RBC= red blood cell, HGB= hemoglobin, Hct= hematocrit, MCV= mean corpuscle volume, MCH= mean corpuscle hemoglobin,
MCHC= mean corpuscle hemoglobin concentration, Plt= platelet, MPV= mean platelet volume, WBC= white blood cell,
Neu= neutrophil, Lym= lymphocyte, Eo= eosinophil, Ba= basophil, Mo= monocyte

In conclusion, the results in this study revealed that freeze-dried crocodile blood has efficiency for promoting hemoglobin and hematocrit values on iron deficiency rat in at least 4 weeks of and very useful for use as food supplement in anemia patient.

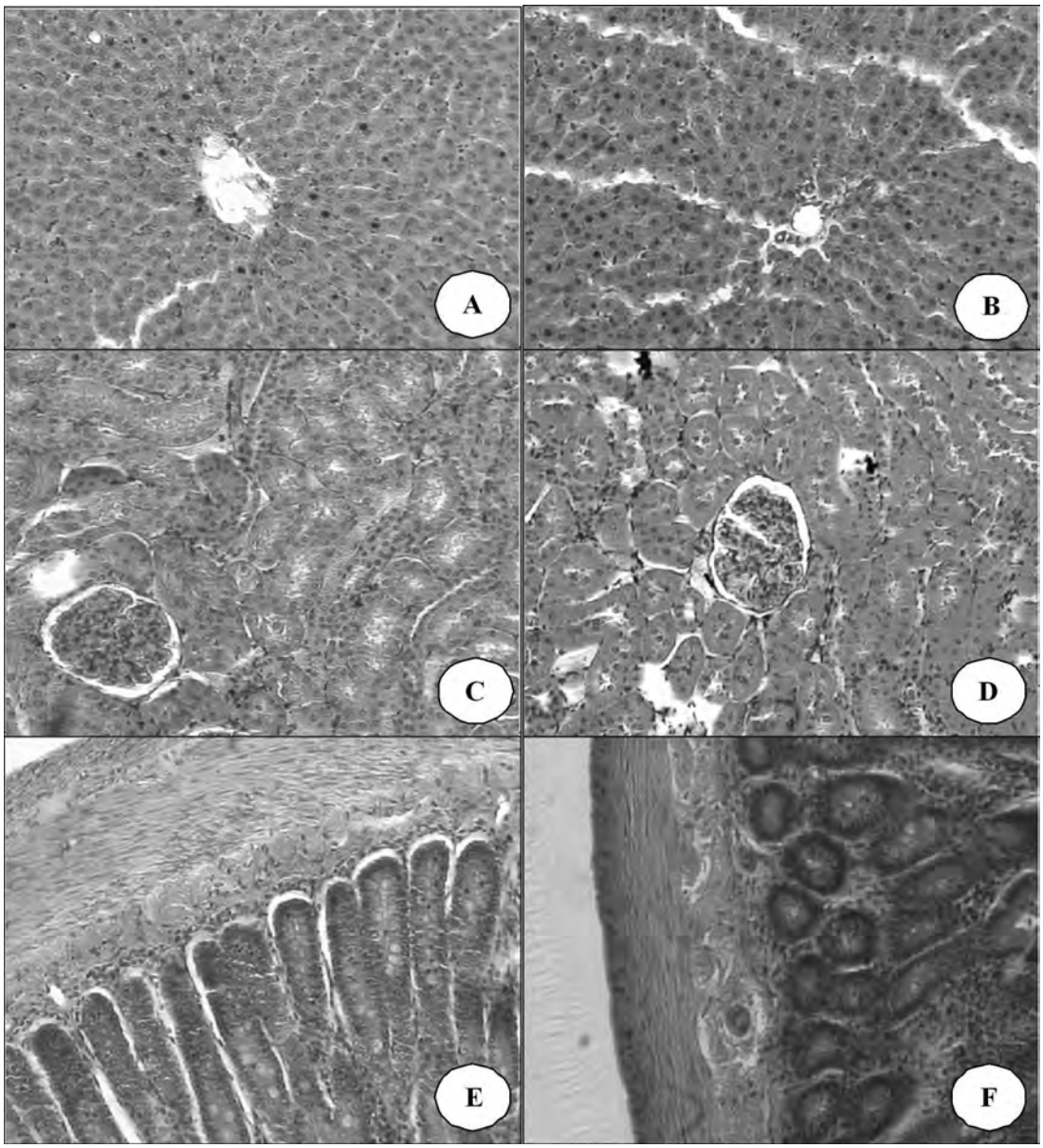



**Figure 1.** Histopathological examination of liver, kidney and intestinal of Sprague-Dawley rats after 24 weeks of freeze-dried crocodile blood consumption.

(A) Liver cell of control group (B) Normal sign of liver cell from freeze-dried crocodile blood consumption.
(C) Kidney cell of control group (D) Normal signs of kidney cells from freeze-dried crocodile blood consumption.
(E) Intestinal cell of control group (F) Normal signs of intestinal cells from freeze-dried crocodile blood consumption.

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**Key words:** Anemia; Crocodile blood; Rat; Iron deficiency anemia

# How populations of the Australian Saltwater Crocodile (*Crocodylus porosus*) have recovered: Baseline analysis of four major tidal rivers within Kakadu National Park, Australia.

**Britton, A.R.C., Lindner, G., Winderlich, S. (2008).**

**ABSTRACT:** The Australian Saltwater Crocodile (*Crocodylus porosus*) was protected in the Northern Territory of Australia in 1971.Since then its recovery has been dramatic, and was accompanied by extensive, replicable surveys across many significant tidal river habitats. Various aspects of this recovery have been presented over the years, and it has since become clear that recovering population trends not only follow similar patterns but also take many years to become fully apparent. This paper presents up-to-date results on the near-complete population recovery of *C. porosus* within major tidal rivers, and is particularly relevant because it concentrates primarily on rivers within Kakadu National Park that have not been exposed to significant post-protection harvest of either eggs or adults. At least one tidal river appears to have now reached carrying capacity, with others close behind it, and the resulting similarities in density changes and population structure may represent an idealised baseline trend for crocodile population recovery in such habitats.

# Ligawasan Marsh Wild Crocodile: Status of *Crocodylus mindorensis*

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**ABSTRACT:** Ligawasan marsh is strategically located at the central Mindanao river basin. Its physical characteristics and geographic location suggest a high potential to support wild populations of crocodiles particularly *Crocodylus mindorensis*.
Strategic survey, photo documentary and actual count captured their existence using equipment provided through a research fund by the Crocodylus Porosus Philippines, Inc. (CPPI). The presence of poachers, fishermen, farmers and local inhabitants inside and around the marsh are instrumental in describing habitats, volunteering live samples, locating nests and counting individual crocodiles.
The habitats were found in Sadsalan (the deepest part of the marsh), entering Cuyapon, Kabacan, North Cotabato, Talitay and Buliok at Rajah Muda side of Pikit, North Cotabato and Pagalungan, Maguidanao and tributaries.
The two largest crocodiles found before the study were *C. porosus* in Pagalungan side of the marsh. There were 265 *C. mindorensis* counted and 13 in captivity at the vicinity of the marsh from late 2007 and early 2008. A regular catch of foot long juveniles has been reported. Cultural myths, beliefs and environment have important role in the conservation and protection of their dwindling population in the marsh.

**Keywords:** Ligawasan marsh, crocodile, North Cotabato, Philippines, *Crocodylus mindorensis*

## INTRODUCTION

### Crocodile industry in the Philippines

The crocodile industry in the Philippines is a very lucrative industry due to the growing demand for skin, as raw material for quality handbags, boots, belts, briefcases and luggages in developed countries for their fashion industry.

Further, hotels and restaurants also include in their menu the exotic meat of these reptiles in their dining tables. Rapid commercialization of crocodiles can be seen on the growing numbers of crocodile farms in the provinces of Rizal, Palawan, Davao del Norte and the cities of Davao and Cagayan De Oro.

Though this actual scenario is good for the economic side of the Philippines but the conservation of these crocodiles in the wild is neglected and hence, there is greater likelihood that in the future these crocodiles particularly, the *Crocodylus mindorensis* will only be read

in books. If there is no action taken by both the government and the private sectors, these reptiles will no longer be seen in the wild. The present situation of these crocodiles in the wild is considered at very high risk of extinction due to the destruction of habitats caused by rapid urbanization, farming, negative local attitudes and ineffective management.

**Crocodiles in Mindanao**

The two species of crocodiles namely *Crocodylus mindorensis* and the *Crocodylus porosus* are both found in the island of Mindanao in the Southern Philippines. *C. porosus* for instance are mostly raised captive in the provinces of Davao del Norte and city of Davao for future commercial purposes while these are raised as pets in Bukidnon, Lanao del Norte and other provinces.

Information on *C. mindorensis* is quite silent or limited on its present situation although, it is believed to be in the wild particularly in the marshes of Agusan and Ligawasan and tributaries.

One proof that crocodiles exist in the wild particularly in Ligawasan marsh and its tributaries was documented in the national newspapers, the Philippines Daily Inquirer on May 15, 1994 (Maulana, 1994) and May 30, 1994 (Alconaba & Maulana, 1994) and also in the local newspaper Daily Express on May 23, 1994 (Duque, 1994). This wild wounded crocodile (believed to be *C. porosus*) measured 19 feet and 6 inches long and 3 feet wide with approximate weight of 800 kilograms. The fishermen who captured the crocodile witnessed fighting with another crocodile having an estimated length of 25 feet. This reptile is still roaming the Ligawasan marsh basin.

**The Ligawasan Marsh**

The Ligawasan Marsh has been identified as a distinct and unique region among the 15 bio-geographic regions of the Philippines (Wetlands in Asia). It covers about 288,000 hectares of which 43,900 hectares were declared a Game Refuge and Bird Sanctuary under the Forestry Administrative Order No.19 of December 26, 1940 (effective January 1, 1941). The Ligawasan Marsh Development Master Plan (1999-2025), prepared by the Region XII Office of the National Economic and Development Authority (NEDA) in November 1998, recommends designation of the marsh as a protected area under the National Integrated Protected Areas System (NIPAS) Act (Republic ACT No. 7586 of 1992).

Ligawasan Marsh is strategically located at the central Mindanao river basin covering 3 provinces such as: North Cotabato, Sultan Kudarat, and Maguindanao. It is within 19 municipalities and one city (or 190 barangays). It is the countrys' largest wetlands. It drains the rivers coming from the mountain ranges in the east – the Kidapawan and mount Apo ranges; north – the Carmen, Libungan, Bukidnon mountain ranges; and south – the Sultan Kudarat mountain ranges.

Potential food source

The migratory and endemic birds could be among the list of prey for the crocodiles. Tilapia, eels, mudfish, catfish and carp are also abundant in the marsh. Domesticated dogs and cats were listed to be attacked and eaten by the crocodiles. Reptiles like snakes and small lizards,

wild rodents, monkeys and other non-volant mammals could also be a part of their diet. Other dead animals may be driven to the marsh thru the tributary rivers.

**Habitat**

A game refuge and bird sanctuary was established in 1941. Meandering rivers provide sanctuary for the shy crocodiles. Mangroves, sedges water lilies, and grasses may increase the stealth ability of crocodiles to make them more effective predator. The margins of the marsh is being cultivated and planted with rice, corn, root crops, coconut, tobacco, oil palm, and banana. The margins of the marsh swell during rainy seasons. The highest rainfall (over 200 millimeters) comes during the month of July (based on the rainfall data of the past 4 years, USM). The rains during December also help in impounding water in the marsh. It supports a significant variety of wild plants and animals. But there is no solid scientific and statistical information on the marsh's biodiversity to strengthen *in-situ* conservation, and for appropriate water management to supply the water needed for the survival of its biodiversity.

This joint undertaking aims to enable the locals, farmers, barangay officials, and stakeholders to develop positive attitude towards self-reliance, appreciate their roles in conserving ecological resources through sustainable farming practices, upgrade their knowledge and skills in agro-livestock-fishery technology focused on crocodile farming system management and rural entrepreneurship.

**Goals**:

The purpose of this research is to verify if both *C. porosus* and *C. mindorensis* species co-exist in the marsh and its surrounding environs. Both species are still under pressure from poaching for commercial purposes, indiscriminate killing (in some areas), predated as a food source, and accidentally killed. It is believed that the Ligawasan Marsh still houses the largest wild population of *C. mindorensis* in the Philippines and it is the only remaining area where the interactive ecology of the two crocodiles in the Philippines can be studied.

**Methodology:**

I. Expected Results of this Project (First Year)

Through a proposed 50 days field survey work and 10 days extension work in the marshlands funded by CPPI:

1. Obtain baseline data on distribution and abundance of crocodiles in the marsh.
a. Field surveys
b. Questionnaire
2. Obtain baseline data on cultural beliefs about crocodiles and potential effect on conservation and/or sustainability. These are characterized by indigenous beliefs (IB) and indigenous knowledge (IK).
3. Collect biological information on crocodiles in the marsh.

II. Tentative Long Term Results – depending on first year activity and subsequent funding:

A. Development of a *C. mindorensis* rescue and rehabilitation center at USM.
   i. Potential of release back into the wild.
   ii. Release to the local hog industry for rearing.

B. Create a permanent study area/sanctuary in the marsh for *C. mindorensis*.
   i. Participation of indigenous inhabitants.

C. Investigate ranching strategies for *C. mindorensis* in protected areas.
   i. Livelihood and conservation activity through sustainability.

D. Commercial utilization of *C. mindorensis.*
   i. F2 progeny from farms
   ii. Sustained support for USM program.

## RESULTS AND DISCUSSION

### Ligawasan Marsh, potential haven of wild crocodiles

Ligawasan Marsh is a vast complex of river channels, fresh water, lakes, ponds and extensive marshes are the areas' main features. Its physical characteristics and geographic location suggest a high potential to support crocodiles. The marsh receives water from Libungan river, Malmar river, Pulangi river, Buluan river, Paglas river and Allah valley river. The water in the marsh is drained to the sea via the Rio Grande de Mindanao that empties into the Illana bay of Cotabato City.

It contains fresh water marshes with an abundant growth of *Eicharvia crassipes*, *Nymphaea tetregan* (water lilies), *Ipomoea aquatica (*kangkong), water hyacinths, swamp cabbages and redges. It also supports a great variety of wildlife including species of fish, reptiles, birds and mammals.

It produces a huge quantity of fresh water fishes like mud fish (locally known as Dalag), tilapia, cat fish (native and hybrid Hito), carps, freshwater eels, crabs and shrimps that are delivered daily in the cities of Cotabato, Kidapawan, Koronadal and Tacurong, Digos and Davao and the provinces of Sultan Kudarat, Maguindanao, South Cotabato, North Cotabato and Lanao del Sur. During the period of heavy rains, when the water level is high due to the swelling of its tributaries, most of inhabitants temporarily migrate to neighboring highland areas in search for alternative livelihood such as upland farming and daily labor in some commercial farms and businesses.

Majority of the inhabitants within the marsh are Maguindanaoan muslims although due to migrations some people particularly Ilocano, Cebuano and Ilongo Christian groups are also found in the villages surrounding the marsh.

The agricultural activities along these river tributaries decrease the volume of water that goes into the marsh. There are no precise data on the conditions and proportions of area that are permanently under water. But it is certain that the Ligawasan marsh swells during the

rainy season submerging some crops planted near the margins. The inhabitants were able to live with the periodic flooding by diverting their source of food and livelihood into fishing.

The Marsh is also home to some allegedly rebel factions who demand for an independent Islamic country in the Southern Philippines, the feared criminal groups, and the kidnapping gangs. While their presence, on one hand, is an opportunity for the conservation directions because poaching, collecting and trade of crocodiles from the marsh will not be physically pursued. Seemingly, on the other hand, bombs and explosives as war effects will be detrimental to habitat, food chain and ecological balance.

**Crocodiles**

The presence of crocodiles in the Ligawasan marsh has been noted during the Second World War. At the present time, there were captive crocodiles treated as pet near the marsh. Some poachers are also selling juvenile *C. mindorensis* around the municipalities near the marsh, these indicates that the *C. mindorensis* is actively reproducing in the wild.

It has been fairly documented that the biggest crocodile (*C. porosus*) was captured sold and died under the management of one resort in Pres. Roxas municipality and that this including another crocodile has been fighting when the other one was captured.

**Ecological issues and threats in the marshland for crocodiles**

- Plans and prospecting for natural gas exploitation
- Agriculture
- Siltation and eutrophication
- War (explosives, bombs, and bullets destroy the habitat)

There are other form of threats by which crocodiles in Ligawasan Marsh are exposed (Figure 1). Flooding from the municipalities that surround the marsh alters the ecological habitat of the crocodiles due to siltation and makes the marsh shallow.

Drought on the other hand provides opportunity for the locals for farming, while it is beneficial to the human populace it dries-off the marsh and when flood waters overcome the crops, the habitat for the crocodiles is altered. It also endanger the crocodiles because the farmed portions have been planted with oil palm and other permanent crops. The other threats are: alleged gas exploitation, wars around and inside the marsh where bombs are dropped the habitats and food chain are destroyed.

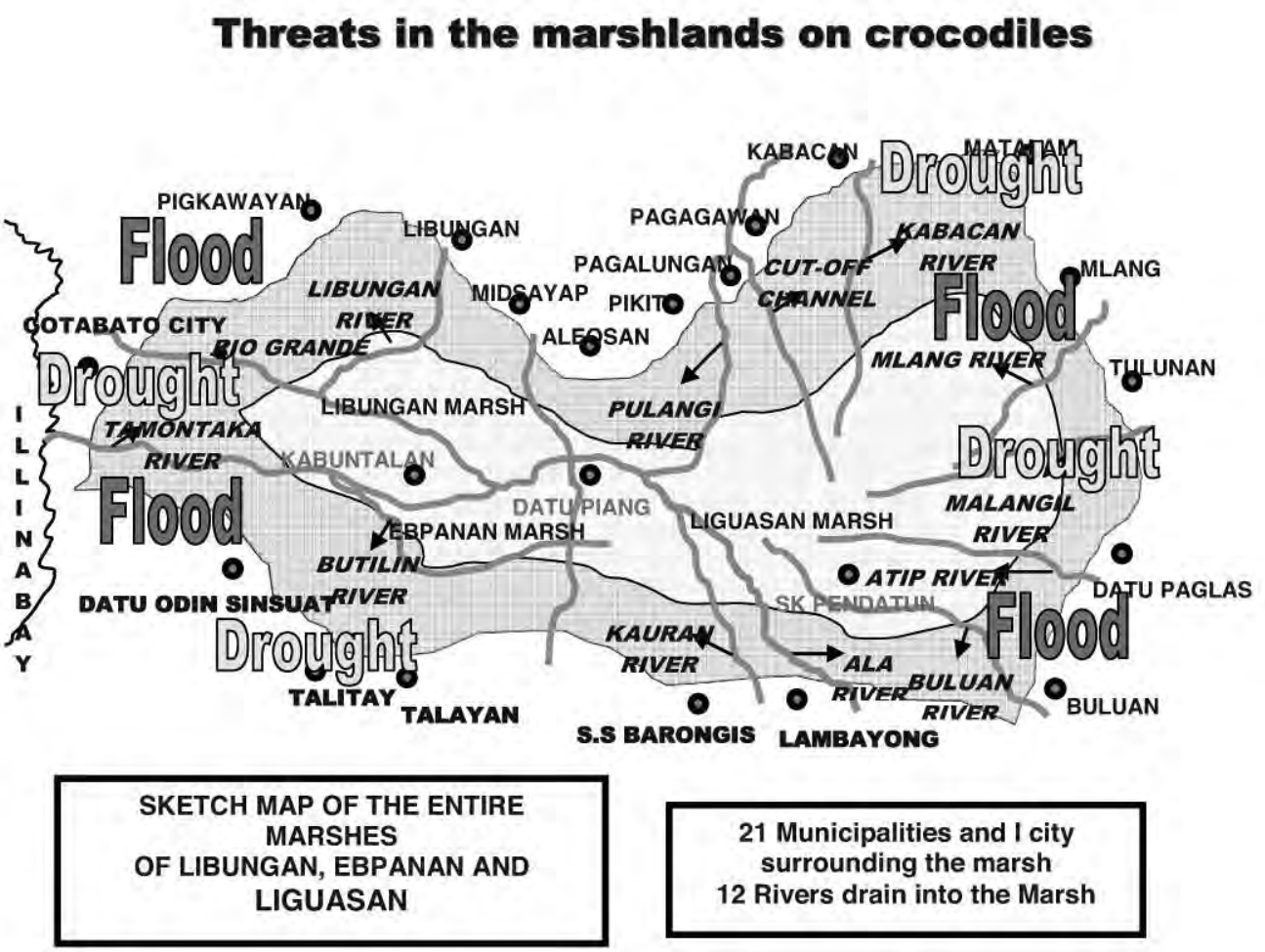



**Figure 1.** Sketch map of the marshes of Libungan, Ebpanan and Ligawasan by which wild crocodiles has been sighted and potential threats viewed.

**Survey results on sightings and attitudes of locals.**

The study focuses on the two species that are present in the area, *Crocodylus mindorensis* also known as the Philippine crocodile and the *Crocodylus porosus. C. porosus* is common in the crocodile industry for its meat and skin. The species is spread not only in the Philippines but also in other areas of the Asia-Pacific region. On the other hand, *C. mindorensis* is endemic in the Philippines and it is believed that its number is decreasing due to the destruction of its natural habitat and being captured for sale as pets. Further, the actual number of these crocodiles in the wild is unknown. There are claims of their presence in some areas in the wild but are unverified. In Mindanao, *C. mindorensis* are mostly found in Ligawasan marsh and its tributary rivers, although baseline information about their presence is limited due to the location and the political situation of the area.

Table 1 presents the situation of the crocodiles based on the actual sightings of people who are resident in the area and their attitude towards the crocodiles.

Respondents that actually sighted crocodiles in the area of Ligawasan marsh and its tributaries as well as respondents who owned crocodiles in captivity were randomly selected on location.

**Table 1.** Status of wild crocodiles in Ligawasan marsh and selected tributaries indicating the number, sizes and type as perceived by randomly selected respondents. (Note: Size in length – unless measured, approximated as small = 1 foot; medium = 1-2 feet; large = 2-3 feet; extra large = 3-4 feet; jumbo= 4-5 feet; very very large = 5 feet and above, unless specified the crocodiles counted are *C. mindorensis*)

| LOCATION | SIZE OF CROCODILE | NUMBER SIGHTED |
|---|---|---|
| **Ligawasan Marsh** | | **n=191** |
| - Cuyapon | Small to large | 29 |
| - Kabacan | Small to medium | 5 |
| - Sadsalan | Small to very large | 117 |
| - Talitay | Small to very large | 16 |
| - Buliok | Small to very large | 22 |
| - Pagalungan | Very very large | *C. porosus*= 2 |
| **Tributary** | | **n=74** |
| - Pres. Roxas | Medium to very large | 5 |
| - Omonay | Very large | 25 |
| - Tambad | Medium to very large | 21 |
| | Medium to very large | *C. porosus*=5 |
| - Matalam | Small to large | 8 |
| - Pulangi | Medium to large | 15 |
| **Total** | | **N= 265** |

This survey was done in the following places: Barangay Cuyapon, Kabacan which is an entrance barangay of the Ligawasan Marsh, President Roxas town and barangay Tambad, Carmen and Pagalungan. Kabacan, President Roxas and Carmen are all towns of Cotabato province and Pagalungan in Maguindanao Province.

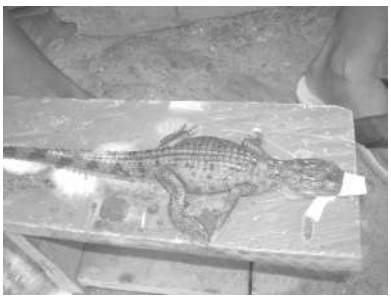
Pres. Roxas and Carmen were surveyed as part of the tributary (Pulangi river) of the Ligawasan Marsh. The survey was also done in barangay Omonay of Damulog, Bukidnon which is just a neighboring barangay of Tambad, Carmen which share Muleta river, a tributary of Pulangi river and where crocodiles have been sighted with nests.

The result of the survey showed that there were 265 sightings of the crocodiles, 252 are found in the wild and 13 under captivity. Most appeared in the afternoon as well as noon time and mostly were alone. The size of the crocodiles ranges from 1 foot to 19.5 feet (small to very very large). Most of the crocodiles diet were found in the fresh water particularly fish, which are in abundance in the Ligawasan Marsh.

The sighted wild crocodiles were confirmed as *C. mindorensis* (n=258) and *C. porosus* (n=7). Some eggs were collected by the locals and one clutch in a nest was allowed to hatch under the supervision of the researchers. These results were collected and recorded during the night and day surveys for at least 10 expeditions in the site of study.

These crocodiles were given approximate sizes from the actual measurements under captivity. The respondents gave their estimate of the size of the crocodiles. These were transformed into the proximate values prepared by the team. The sizes of *C. mindorensis* classified ranged from small to large while *C. porosus* were medium to very very large.





It was observed by 'Mr. B' (oldest poacher, collector for more than 30 years) that two different crocodiles were found in the marsh the black/yellow (friendly, with mythological connotation as reincarnate of humans), white (ferocious, shy, huge). This observation was confirmed by him after the visit at Crocodile Park in Davao as: *C. mindorensis* for black yellow while white as *C. porosus*. Another "Mr. T' was born twin to a male crocodile who is still in contact with him. During the full moon, the twin crocodile will be offered with live chicken and cooked rice and 'Mr. T' will observe the twin crocodile to appear and they have connections again. The twin crocodile always watches over him and his family around the Pulangi river, even when they go fishing, the crocodile leads them to where the big catch is.



Most of the small sizes (juveniles – one foot long) has been regularly caught and put in a screen cage lowered in the canal waterway leading to the entrance of Ligawasan via Cuyapon side until a buyer pays the catch.

These juveniles are usually sold to pet shops and collectors around Kabacan. The large to very large were caught only when orders are posed by a middleman. A large number of these sizes were delivered to operators of parks.

**Table 2.** Summary on the occupation and tribal affiliation crocodiles in the wild.

| OCCUPATION OF RESPONDENTS | Number of respondents | TRIBAL AFFILIATIONS |
|---|---|---|
| Students | 7 | Maguindanao (n= 31) |
| Farmers | 9 | Igorot (n = 2) |
| Mayor | 2 | Ilonggo (n = 9) |
| Fishermen | 8 | Ilocano (n = 15) |
| Businessmen | 2 | Cebuano (n = 3) |
| Barangay official | 2 | |
| Soldier | 1 | |
| Agriculturist | 1 | |
| MILF | 28 | |
| | N = 60 | |

The respondents were classified according to their occupation and tribal affiliation (Table 2). It showed nine varied occupational groupings of the respondents who are mostly Ilocanoes followed by Maguindanaons who are related to their cultural identifications. Ilocanoes are industrious farmers and Maguindanaons establish houses in banks of water bodies and basically use fishing as source of livelihood. The rest of the respondents are residents in the area, traders, and family members of settlers or government officials.

**In Barangay Cuyapon and Poblacion, Kabacan**

Some captured crocodiles are caught by the local poachers ('Mr. B' and son) in Barangay (village) Cuyapon, Kabacan, Cotabato (which is one of the entrance to the Ligawasan Marsh) and were sold in the town Poblacion area for a price from Php 750 (US$15) to Php1500 (US$30) per piece for a 12-inche size crocodile. All of these captured crocodiles were *C. mindorensis*. Middlemen of poachers are selling these crocodiles (size=12 inches) in the cities and provinces to crocodile farms and pet lovers and shops for a price of Php 3500 (US$71). A *C. mindorensis* was found as pet in Poblacion, Kabacan about 3 years old, 7 feet long which was captured in the Malmar area (Malitubog-Maridagao rivers), a tributary of the Ligawasan marsh.



Entrance to the marsh

'Mr. B' and sons of Cuyapon, Kabacan



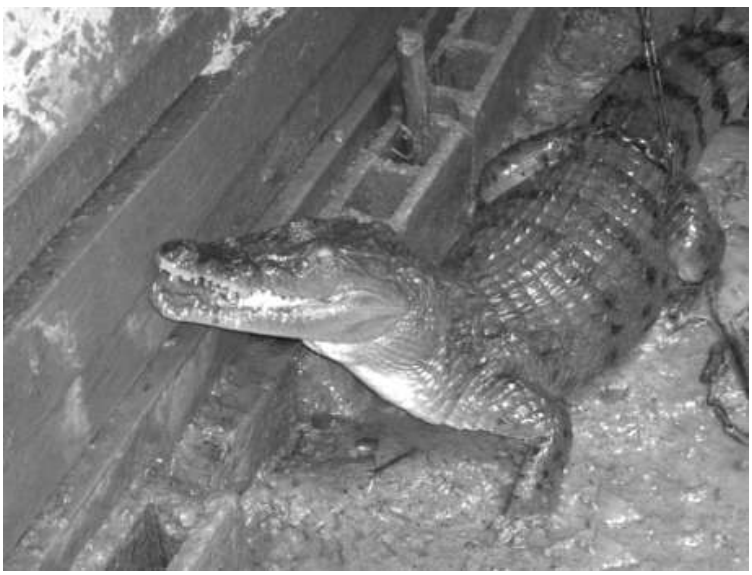
'Mr. B' illustrates how crocodile lay eggs and how many in a clutch . According to him, eggs are arranged by layers, the top most layer indicates the number in the clutch. If 2 eggs are on top, 20 eggs are laid; if 3 eggs on top, 30 eggs are laid. He collects eggs as well if ordered, it costs 20 pesos per piece.



discussions and lectures about crocodile conservation, the respondents were given a tour to the Davao Crocodile Park for exposure and confirmation about the looks and sizes of the crocodiles sighted, counted and collected,




(especially on species identification). This proved to be a good measure to confirm the volunteered answers to the questionnaires and focus group discussions. Information, Education, Communication (IEC) materials were provided to the participants for advocacy and complementation. The children in the foreground are young students who witness frequently the presence of the crocodile in Tambad every time they bring their carabaos to pasture along the bank of Muleta river.



In Barangay Pisan, Kabacan Cotabato, a chained male *C. mindorensis*, caught using electrocution. This guy together with another (escaped) was caught close to a duckery project during a big flood. He was believed to have swam upstream via the Pulangi river. He was placed in a mini park zoo but due to the method of restraining and catching, he did not survived long

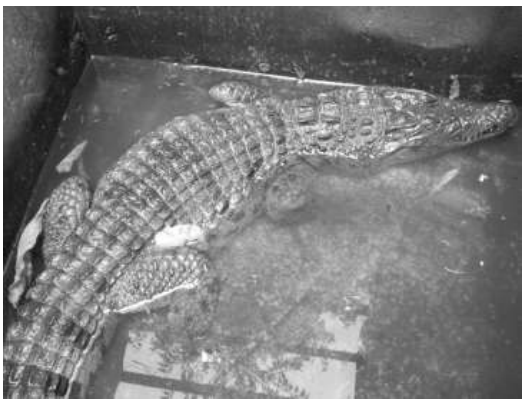
**In Sitio Sadsalan, Cuyapon, Kabacan**



Sitio Sadsalan is located in the mid-point of Ligawasan marsh and believed to be the deepest part of the marsh. This served as the breeding habitat of crocodiles and a 'holy' place for the locals. Occasional visit by locals from the MILF camp has proved that crocodiles of the black/yellow (*C. mindorensis*) and white (*C. porosus*) used the place for their nest. It has thick floating vegetation that formed into islets. The crocodiles used the rotting plant parts as nests. The locals stressed that the place should be vacated before 4:00 o'clock in the afternoon because the water become turbulent and only few can manage to get out safely from the place. They believed that the very very large crocodile will appear and make the water turbulent with huge waves when they fight. But logically, this is so because the islets move due to strong wind velocity and waterways close up where you can not retrace your passage. Much more, security is not guaranteed at any time. There were 15 bancas of full security force that accompanied the research team to the area in case an encounter happens. The largest number of sightings of wild crocodiles was recorded in this place.



**In Kabacan, North Cotabato**



Four *C. mindorensis* crocodiles were bought from hunters in Maridagao river area. They measured 3-4 feet long. Two males died due to fighting while one female was kept in an enclosure in the farm. The other male crocodile was placed in a cement water tank that served as cage and fed with refused from the food store. The owner is willing to donate to the University if there is a need. He got a license from DENR to keep the crocodiles. This is where trading of juveniles and mature crocodiles take place.



**In President Roxas, North Cotabato**

In President Roxas, two captured *C. mindorinses* are found under the custody of the local government unit. The lengths of these two crocodiles are around 7 feet. We also interviewed the former mayor who bought the biggest crocodiles (*C. porosus*) in the Philippines

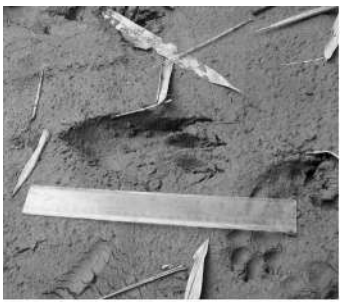


so far being reported whose length is 19 feet and 6 inches and 3 feet wide and weighed 800 kilograms. This crocodile died in 1994 due to gunshot wound (Alconaba & Maulana, 1994). The only remaining part of this huge reptile is the skull (measures almost 2 feet) and 2 teeth.

**In Tambad, Carmen and Omonay, Damulog**

In the boundary between barangay Omonay and Tambad along Muleta River, it was reported that a man (one of our respondents) was attacked while in the river and was bitten in the right leg in the year 2005. One respondent also reported that they shot a 200 kilogram, 9 feet black crocodile (allegedly, *C. porosus*) in September 2006. This croc was slaughtered and was eaten. In Tambad area, along the Muleta River which was surrounded by bamboo trees and corn fields, a couple of nests was seen by the researchers. Crocodile marks were seen near the banks of the river. It was reported that the 22 eggs of this crocodile was taken by one of our respondents (a crocodile poacher). He sold those eggs to the local people for 20 pesos (0.45 cents in US dollars) each. We recovered two eggs from one of the locals (our respondent) who said he wanted to hatch it but he is not successful. The eggs measured almost 3 inches (7 centimeters). The other nest was allowed to hatch and 10 juveniles were monitored from the nest while the mother crocs watch nearby. Footprints of the mother crocodile was observed in the shoreline of the river.





The army detachment in barangay Omonay also reported that crocodiles were seen in the vicinity of the camp. The commanding officer said that in daytime they saw crocodiles in the Muleta River. During the night at around 10 p.m. and at dawn around 2 a.m., crocodiles he said were seen crawling in the slope of the hill where the detachment is located. The army's response is to drive them away by shooting them with their guns.

**In Talitay, Rajah Mudah, Maguindanao**

Two separate day-surveys were conducted at Talitay, Rajah Mudah with the support of the local barangay officials (Barangay Capt., Odin Abubakar) and residents on board a 8-sitter banca (small wooden boat). The respondents in barangay Talitay showed the habitat and locations by which crocodiles were caught, captured, and sometimes killed when they attacked ducks farms. However, these crocodiles have been victims of air bombings in the area during the clean-up procedures



by the military where alleged kidnap gangs and rebels were suspected to be hiding. The floating vegetations and islets were totally destroyed. The crocs were forcibly driven towards SK Pendatun, Maguindanao side of the marsh. The team was not allowed to visit due to security reasons, big waves and turbulent waters impassable to small boats.

**In Buliok, Rajah Mudah, Maguindanao**

A day survey was conducted in Buliok, Rajah Mudah. Negotiations took place with MILF leaders. The visit was organized with the troop leaders as guide. The hunting grounds for crocodiles were shown. Fishing is the source of livelihood of the locals. The guide showed how crocodiles and juveniles were trapped in the fishing gears. There is a sustained fish catch that proves abundance of fish food and food chain. An occasional catch of juveniles entrapped in the fishing gears was observed and these juveniles were sold together with the fishes caught.



**In Matalam, North Cotabato**

An owner of a resort housed a large male and medium size female in a mini zoo captured from the Pulangi river. There were four crocs (*C. mindorensis)* at the start but sold the other two to pet collectors. The collection is one of the attractions in the resort. This also attracts poachers and hunters to bring more catch but due to the small space the owner refused the other crocs, it cost ranging from five to ten thousand pesos a crocodile.

**Myths, legends and other telltales surrounds the wild crocodile**

There were several myths and legends noted during the interviews with the people in Ligawasan marsh and the Pulangi (Camp Mantawil), these are:

- The crocodiles could be the “real crocodile” or “pagali”. The pagalis are men that shifted to a crocodile form. They are supernatural that guard the natural environment.
- Crocodiles are kept for “good luck”.
- The reproductive organ of the male crocodile is used as aphrodisiac, it makes the penis erect for days. Men put this in the side pocket before sex to enhance sexual performance.
- The crocodile tooth is gathered and used as necklace, it serves as amulet.
- Every turn of the Pulangi river is a territory of a crocodile family.
- There was a large crocodile with a length of 28 -30 feet, and its upper arm is as big as a sack of rice.
- Never kill or harm the crocodile because crocodiles will come and attack you for vengeance.
- There is a “holy” place in the water as territory of the largest crocodile in the marsh where they gather at 4 pm, so leave the place before that time because if not, big waves and the turbulence of the water will consume you.
- A man was born twin to a male crocodile. The crocodile become buddy of the

man, they lived together, the man can command other crocodiles to do errand for him, every harvest time and full moon an offering of rice and a chicken will be killed for the crocodile, the crocodile knows it is for him and he will come out of the water during that ritual. No one person was attacked by crocodile in the area ever.

- No artifacts of crocodile should be carried in a boat or "banca", crocodiles will attack the vessel.
- Crocodiles are ferocious and will eat humans, humans feared the crocodiles, they are not touched hence.
- The mother crocodile eat the eggs and hatchlings when the eggs hatch and only those who escaped survived. This controls the crocodile population.
- The number of the topmost layer of eggs in the nest is the corresponding count of eggs in the clutch. If 2 eggs are found on top, the total count is 20, if three, 30 eggs, and if 4 , 40 eggs.

Myths and beliefs record from local people in the sanctuaries or habitats of crocodiles are contributory to their survival. Some myths and beliefs are good but some are considered threat to crocodiles. The Maguindanaoan people for instance do not want to harm the crocodiles since they argued that the spirit of the crocodiles sometimes becomes human. Others said that they want to capture crocodiles and raise them as pets since they will give good luck in terms of financial prosperity to the family. Others said crocodiles protect human from any sickness and diseases since crocodiles absorbed all those sickness and diseases in their body while other just leave them alone since crocodiles they said will not harm if they are not harmed. Further, some respondents said the sexual organ of the male crocodile if eaten can give then more vigor in their sex life while others said that if they kept the sexual organ as an amulet will make them more attractive to women. A respondent also said that crocodiles have some sort of center teeth whose function he said will tear humans. Finally, one of our respondents said that crocodiles are friends since they help by eating pests.

**Extension Works**

Advocacy on conservation and creation of volunteer teams to provide locals information about the crocodiles and why they should be conserved and protected were conducted in four areas of the survey sites.

A series of community immersion and seminars were conducted by the team to introduce the research project and to involve the locals that surround the marsh and immediate tributary for massive campaign on conservation of wild crocodiles. The research team used radio broadcast for awareness and focal group discussions after a schedule is established in the barangays. The barangays involved were Cuyapon, Omonay, Tambad and Ugalingan. These have been mostly identified as habitat and places where crocodiles has been sighted, collected, trapped and sold. It is also the residence of identified poachers, collectors and sellers of live crocodiles.

Added as an attraction for the locals is giving trainings in handling their livestock for deworming, medications, feeds, and feeding of livestock and poultry. The team gave also consultations for the farming activities of the settlers.

Posters on conservation and protection of the wild crocodiles were developed in consultation with the locals. These were reproduced in tarpaulins and hung in strategic places in the barangay.




**Figure1.** Samples posters in tarpaulin on crocodile conservation and protection for advocacy in Ligawasan Marsh, tributaries and barangay surrounding the marsh.

## CONCLUSION AND RECOMMENDATIONS

The result of this survey gave us baseline information that indeed *C. porosus* and *C. mindorensis* are in the wild in the Ligawasan Marsh and its tributaries in Mindanao. The official recorded count of 191 wild crocodiles in Ligawasan marsh and 74 wild crocodiles in its tributaries are limited only to 6 sites in the marsh and 5 in the tributaries. It is also limited to 60 individuals who were approached by the researchers at the time of day and night surveys.

It is recommend that a sanctuary or conservation area and a rescue center will be established in the vicinity with the University of Southern Mindanao in Kabacan, Cotabato that will serve as frontline area for research, conservation, protection and education for this endangered but unique crocodile species in the Philippines.

Further, the long term research activity for the wild crocodile research should be funded and conducted to be able to sustain the conservation of the wild crocodiles in the largest marshland in the country.

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# Status of *Caiman latirostris* and *Caiman yacare* populations in North Argentina

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**ABSTRACT:** In past years, caiman populations were utilized without a solid basis of biological knowledge, nor the status of the populations. Fortunately, this situation has changed, and today, studies are necessary in order to access to wildlife to undertake a sustainable use program. In Argentina, it is a requirement for Ranching programs that monitoring of wild populations in each Province be undertaken; so available information about the status of both caiman species distributed in Argentina (*Caiman latirostris* and *C. yacare*) have increased. In this work we present surveys results, done during 2007 in Formosa and Salta Provinces, and in 2008 in Corrientes Province, Argentina. In the three Provinces, wild populations look healthy, presenting higher densities in Formosa (up to 144 ind. $km^{-1}$). In Salta Province, we found principally *C. latirostris*. In Formosa, results were more variable, existing places with abundant populations of *C. latirostris* and some others presenting higher densities of *C. yacare*. In Corrientes Province, *C. latirostris* was found in the southwest portion, Yacare caiman was found in the northeast of Corrientes Province, and both species share distribution in the northwest part.

# Distribution and population status of spectacled caiman (*Caiman yacare*) and black caiman (*Melanosuchus niger*) in the Mamoré and Iténez river basins, Bolivia.

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**ABSTRACT:** There are five species of caimans registered for the Bolivian territory. The spectacled caiman (*Caiman yacare*) and the black caiman (*Melanosuchus niger*) are sympatric species which have generated special interest in Bolivia, due to their potential for their sustainable use. At the same time, both species are the most affected because of the illegal hunt. Populations of *C. yacare* and *M. niger* have been evaluated during the dry season in aquatic habitats of the up basin of the Mamoré river an in the mid basin of the Itenez river. The different types of habitats were classified for both areas. In the Mamoré basin, 89% of the caiman population belonged to the specie *C. yacare* and 11% to *M. niger*, meanwhile in the Itenez river 95% of the population belonged to the specie *C. yacare* and 5% to *M. niger*. In both basins a remarkable inter-specific segregation was observed. We discuss the environmental and geomorphological factors that might influence in the differences observed between both basins and in the habitat selection of both species.

**Key words:** Distribution, *Caiman yacare*, *Melanosuchus niger* , Mamoré river, Iténez river.

**RESUMEN:** En el territorio boliviano se han registrado cinco especies de caimanes. El lagarto (*Caiman yacare*) y el caimán negro (*Melanosuchus niger*) son especies simpátricas que en los últimos años han generado interés en Bolivia, debido a su alto valor de conservación y el potencial de realizar un aprovechamiento sostenible. Al mismo tiempo, son las dos especies más afectadas por la caza legal e ilegal. Se evaluó las poblaciones de *C. yacare* y *M. niger* en hábitats acuáticos durante la época seca en la cuenca alta del río Mamoré y en la cuenca media del río Iténez. Se realizó una tipificación de hábitats en las dos zonas. En la cuenca del río Mamoré, el 89 % de la población de caimanes pertenece a la especie *C. yacare* y 11% a *M. niger*; mientras que en el río Iténez, el 95% de la población pertenece a *C. yacare* y 5% a *M. niger*. En ambas cuencas se observó una segregación inter-específica notoria. Se discuten los factores ambientales y geomorfológicos que influyen en las diferencias observadas entre ambas cuencas y en la selección de hábitats por ambas especies.

**Palabras clave:** Distribución, *Caiman yacare*, *Melanosuchus niger*, río Mamoré, río Iténez.

# Preliminary information about distribution and abundance of the Black Caiman *Melanosuchus niger* in Beni, Bolivia

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**ABSTRACT:** Among the crocodile species present in the low lands Bolivia, its possible to find *Melanosuchus niger*, evenly distributed, with populations that are severely diminished due to excessive pressure between 1950 and 1970, entering the Red List of the UICN under the category of Endangered in 1982. In Bolivia, since 1980 the specie was already considered rare, and was even included in the Red Book of Vertebrates. As a result of new rules of protection, and their inclusion in the CITES appendix I in 1975, the population of Black Caiman is slowly coming back to his natural distribution and natural habitats. They were then classified at the low risk level by the UICN, even though for Bolivia detailed information doesn't exist yet. The results of abundance and distribution studies obtained during the crocodile surveys, during 2006 and 2007, in 324 sample points in Beni department, show a total of 3052 Black Caimans in 2369.454 km of shoreline and at 43% of the surveyed sites, had an estimated density between 0.02 and 63.97 ind/km. This suggests that the populations are recovering, but they are not abundant in all their original geographic range.

## INTRODUCTION

Widely distributed, the black caiman populations (*Melanosuchus niger*) were under great hunting pressure during 1950's to 1970's due to the leather commerce. In consequence, the populations were greatly reduced in most of its original distribution range (Asanza 1992), becoming one of the species included in1982 in the IUCN Red List with the category of "Endangered".

In Bolivia, where the species was historically abundant in the north and east of the country (Ross 1998), the commercial hunting was intensified since 1942 (Aparicio y Ríos 2006). According with these authors, an estimate of 781 leathers of lagarto (*C. yacare*) and black caiman were extracted daily until 1950, from the Beni provinces. For *M. niger*, these numbers were decreased between 1956 to 1973 to about 12 caimans per day; meanwhile, between 1974 to 1977 the collected data point a number of 152 caimans per day.

The harvest of wildlife animals of M. niger was allowed in Bolivia until 1979 (D.S. 08063, 1967), establishing a minimal size of 2,5 m of total length and forbidding the hunting between December and July. Nevertheless, these regulations had minimal or none effect controlling the intense exploitation that the species was suffering (Ross 1998). In the present days, the black caiman is completely protected by the Supreme Decree 16606 (1979), and posteriors, becoming the species part of the Red Book of Bolivian Vertebrates in 1980.

Besides some small scale poaching continued (King y Videz-Roca 1989) and still continues today (CPIB *com. per.*), in a general way, and as a result of the protectionist measures

applied and the incorporation of the species in the 1975 CITES I appendix, the black caiman populations started recovering and returned to some of their original distribution range (Da Silveira 2002), becoming in 2000 into the "low risk" category of the UICN.

In Bolivia, the information related with the *M. niger's* population status is scare and is in part supported by qualitative reports. The census performed between 1986 –1987 showed that the species was still in its historical area of distribution, but in very low numbers, dominating the population young sub-adult individuals (King y Videz Roca 1989). Posterior counting showed the presence of local abundant populations in specific areas of Beni and Santa Cruz Department lowlands. But, these surveillances were performed in protected areas: Estación Biologica del Beni (Pacheco 1993), Reserva de Vida Silvestre Ríos Blanco y Negro (FAN y WCS 1994), y Reserva Inmovilizada Iténez (Liceaga *et al*. 2001). Besides other reports that suggest the presence of localized populations in some flood lagoons of the Iténez river, inside the Noel Kempff Mercado National Park (Ross, 1998) and in the Isiboro - Sécure Indigenous Territory and National Park (TIPNIS) (FAUNAGUA *et al.* 2005). We can also include the data collected in the North of La Paz, inside the Tacana I TCO (Llobet 2005).

In order to update and increase the knowledge about the conservation status of this species in Bolivia, the results for abundance and distribution of the *M. niger* are presented here, from the surveillances performed during 2006-2007 in the Beni Department. Information that belongs to crocodilian studies performed to establish the 11 different Lagarto's (*Caiman yacare*) Management Plans in this department, the studies correspond to: Municipio de Loreto and the Original Communitarian Lands (TCO for *Tierras Comunitarias de Origen* in Spanish) Sirionó, Itonama, Baure, Cayubaba, Canichana, Movima I, Movima II, Tacana III, Joaquiniano and Moré.

**Study Area**

Located in the northeast of Bolivia and being part of the Amazon Basin, the Beni Department is part of a large system of flood plains with periodical floods from the overflow of rivers that run through it. The study area is located inside this department, including the Municipio of Loreto, and the Original Communitarian Lands (TCO) Sirionó, Itonama, Baure, Cayubaba, Canichana, Movima I, Movima II, Tacana III, Joaquiniano y Moré. For the TCO's besides its territorial extension the bodies of water traditionally used to harvest *C. yacare* were also considered (Fig. 1).

In a general way, the Bolivian Amazonian plains have a tropical climate, with an intense rainy season in the summer. About 60 – 80% of the rains occur between December and March, and at the same time the temperatures are the highest (Navarro y Maldonado 2002). The highest water levels are generally registered between January and March, as a result of the runoff of the rains produced in los Andes.

**METHODOLOGY**

The field work was performed in the low water season, during the months of July and September of the years 2006 (Municipio de Loreto) and 2007 (TCOs). The samplings

consisted in nocturnal surveys in row guided canoes using dazzling methods, following all the recommendations for this kind of studies (Woodward y Marion 1978, Woodward 1987, Coutinho y Campos 1996, Pacheco 1996), avoiding high wind-wave days and one week wait after cold weather fronts. This standardization of the surveys minimized the methodological bias (Pacheco 1994).

At the same time, the following classification for the bodies of water was used: a) rivers or continuous water courses; b) streams; c) fluvial lagoons, very close related with important water courses, belong to old river streambeds or cut meanders; d) tectonic lagoons, with large surface but with an homogenous deep, these lagoons are in contact with the superficial freatic layer that is between 0,5 – 2 m deep, that assures a stable water level throughout the year, besides the seasonal precipitation (Poully et al. 2004); and e) small lagoons, lentic bodies of water with a perimeter less than 1 km independently its origin. This classification corresponds to the one used by the Natural History Museum Noel Kempff Mercado (lowlands scientific authority) in the process of designing monitoring tools for the *Caiman yacare* (Museo de Historia Natural Noel Kempff Mercado 2005).

Inside this classification some modifications were added: a) white-water rivers, rivers that contain great amounts of load from the runoff of rains in the high Andes, and with lots of sediments (Mamoré and Beni river basins); b) clear-water rivers with low levels of load (Iténez river basin); and c) black-water rivers, originated in streams in the plains and richer in humus' acids.

The method used to enumerate the caimans was the incomplete counting or relative abundance index (ind/km). For the totality of the places with black caiman presence, the factors included in the statistics were the type of water (basin), type of water body and the history of usage of crocodilian species in the area (administrative unit), because the relative abundance data didn't follow a normal distribution, it was calculated through non-parametric statistics (Kruskal-Wallis, Spearman correlation coefficient).

To determine the population structure categories of size of 60 cm. were used. The population size structures obtained were compared without considering young individuals, because this group is under a high mortality that produces great fluctuations among months (Velasco y Ayarzagüena 1995). Those caimans that weren't close enough to allow correct size estimation, were included in the category "eyes only" (King *et al*. 1990, Pacheco 1996), and were considered in the total density estimations of each place.

At the same time, during the counting the presence of *C. yacare* was considered, this species has an overlapping distribution range with *M. niger* in the study area. Both species can be easily differentiated in the field, and considering other crocodilian species in the study assures not to overestimate the black caiman for this species (CITES 2007), and allow us evaluate the relationship between both, calculating the proportion of species for each survey. The comparing between relative observed densities were performed without considering class I individuals (individuals with TL < 59 cm. for *M. niger* and TL< 50 cm for *C. yacare*) because the aim was to find potential competence relationships between the two species (Dueñas 2007).

## RESULTS

For the population studies of *M. niger* and *C. yacare* a total of 2.369,45 km of shore were surveyed in the different bodies of water, spread around 324 sampling points (fig. 1). In the Table 1, the obtained results are summarized.

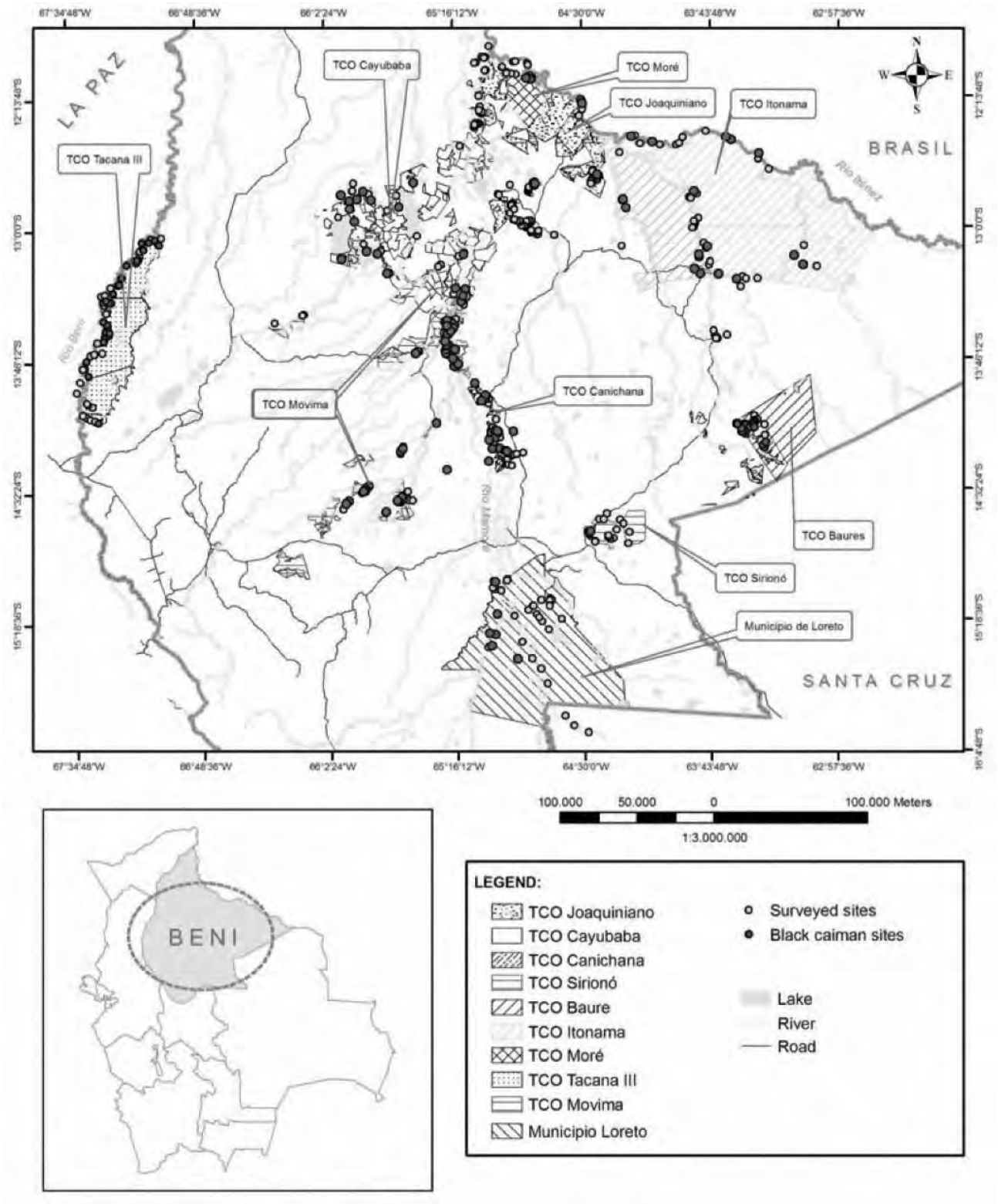



**Figure 1.** Observed distribution of *Melanosuchus niger* in the Beni Department.

**Table 1.**Relative abundance indexes for *Melanosuchus niger* related according to basin and study area.

| Study area | Basin | Sample (km) | Sampling points | Places with caimans | Nº Caimans | Density range (ind*km$^{-1}$) | Ind*km$^{-1}$ | Clases II-V*km$^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| Tacana III | Beni | 447.72 | 59 | 28 (47.46%) | 165 | 0.08-4.58 | 0.63 | 0.62 |
| Sirionó | Mamoré | 68.954 | 13 | 1 (7.69%) | 1 | 0 -1.21 | 1.21 | 1.21 |
| Joaquiniano | Iténez | 83.22 | 21 | 4 (19.05%) | 27 | 0.6-4.90 | 0.80 | 0.71 |
| Moré | Iténez | 67.29 | 20 | 2 (10%) | 7 | 0.29-0.50 | 0.41 | 0.41 |
| Baures | Iténez | 122 | 31 | 10 (32.26%) | 1212 | 9.77-63.97 | 31.64 | 12.01 |
| Itonama | Iténez | 419 | 38 | 24 (63.16%) | 230 | 0.13-15.75 | 0.81 | 0.63 |
| Cayubaba | Mamoré | 343 | 24 | 19 (79.17%) | 754 | 0.02-25.00 | 0.71 | 0.68 |
| Movima | Mamoré | 479.93 | 57 | 34 (59.65%) | 557 | 0.1-34.20 | 1.47 | 1.38 |
| Canichana | Mamoré | 183.24 | 33 | 12 (36.36%) | 92 | 0.1-14.60 | 0.97 | -- |
| Loreto | Mamoré | 155.1 | 28 | 5 (17.86%) | 7 | 0.18-10.00 | 0.38 | 0.38 |
| TOTAL | | 2369.454 | 324 | 139 (42.90%) | 3052 | 0.02-63.97 | 2.14 | 1.46 |

## Abundance and distribution

A total of 3052 black caimans were counted, with a presence in 43% of the places studied. The relative abundance indexes are between 0,02 and 63,97 ind/km.

The observed distribution was not uniform among variables nor inside them. The media of relative abundance for the 139 sampling points with presence of black caiman was 4,21±9,50 ind/km. High population densities were found in the Iténez basin (63.97 ind/km in the tectonic lagoon La Porfía, TCO Baures; 15.75 ind/km in the fluvial lagoon Bahía Puerto Chávez, TCO Itonama) and in Mamoré basin (34.20 ind/km in the fluvial lagoon Laguna Bella, TCO Movima; 25.00 ind/km in the tectonic lagoon El Triunfo, TCO Cayubaba), but the registers of low population densities were larger: in 75 surveys less than 1 ind/km were counted, meanwhile in other 21 surveys moderate densities were observed (1.0 – 2.0 caimans/km) (figures 2 & 3).

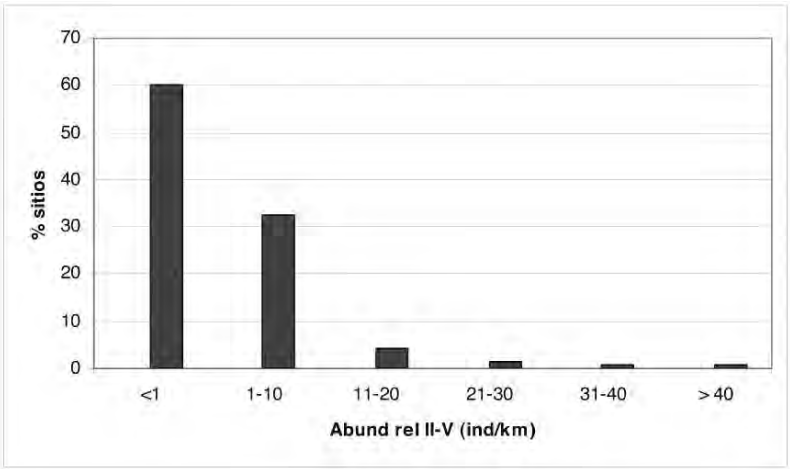



**Figure 2.** Observed percentage of relative abundance of *M. niger.*

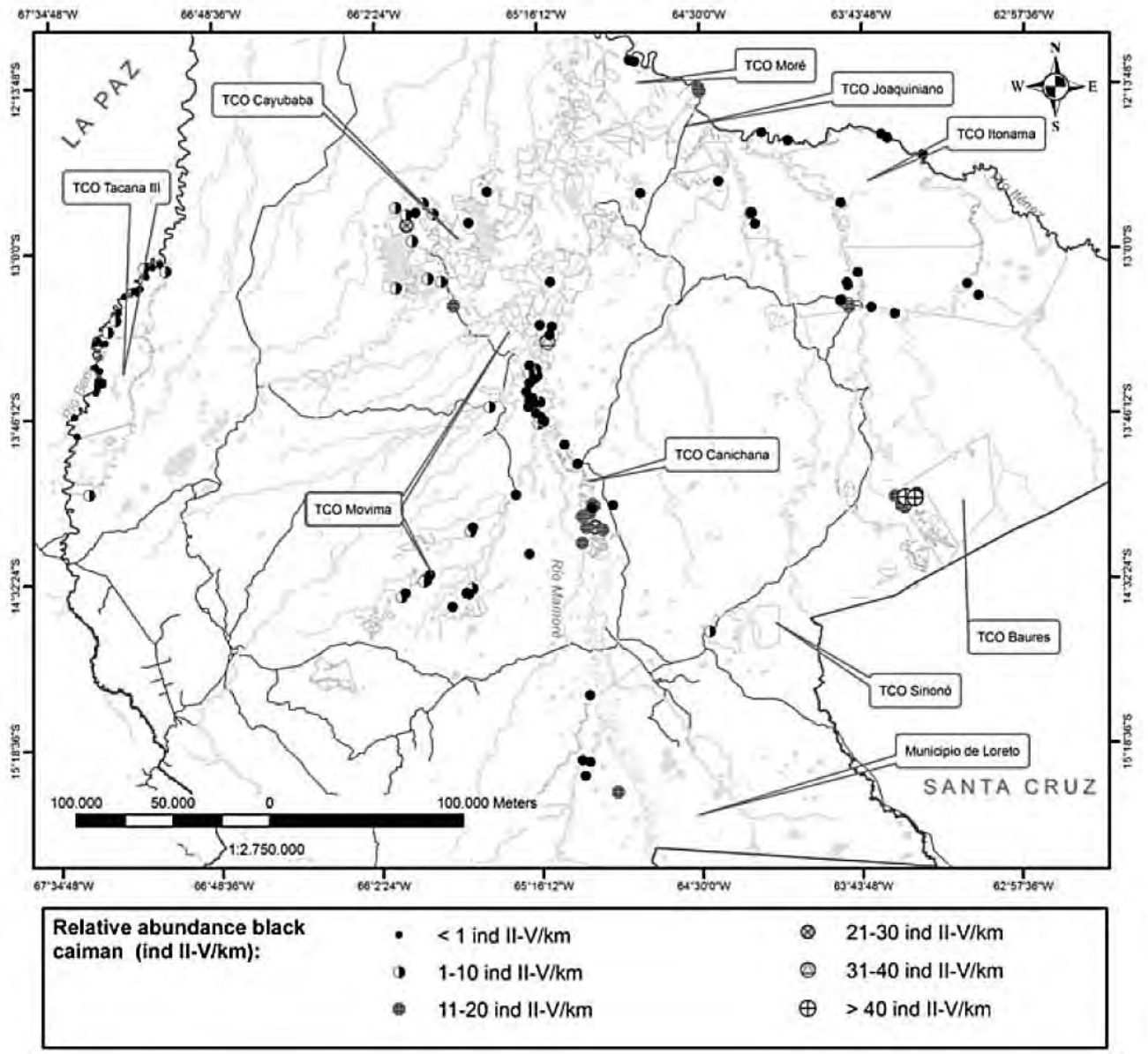



**Figure 3.** Observed distribution of relative abundance of *Melanosuchus niger* in the Beni Department.

The spatial variation of estimated relative abundance of *M. niger* was analyzed for each studied TCO or Municipio, among bodies of water and among basins (Fig. 4).

Highly significant differences were observed for the administrative units and for the types of bodies of water, the first is related with the second, because the 578 classes II to V caimans registered in the Baures TCO were registered in tectonic lagoons. In this kind of body of water the highest relative abundances were registered (8.14±11.52 caimans/km), exceeding in 71% of the observations 3 caimans/km. The relative abundances were similar among fluvial lagoons (2.15±4.80 caimans/km) and streams (2.80±0.95 caimans/km) and lower in rivers (1.06±4.58 caimans/km). The small lagoons weren't considered because there was only one register for black caiman for them.

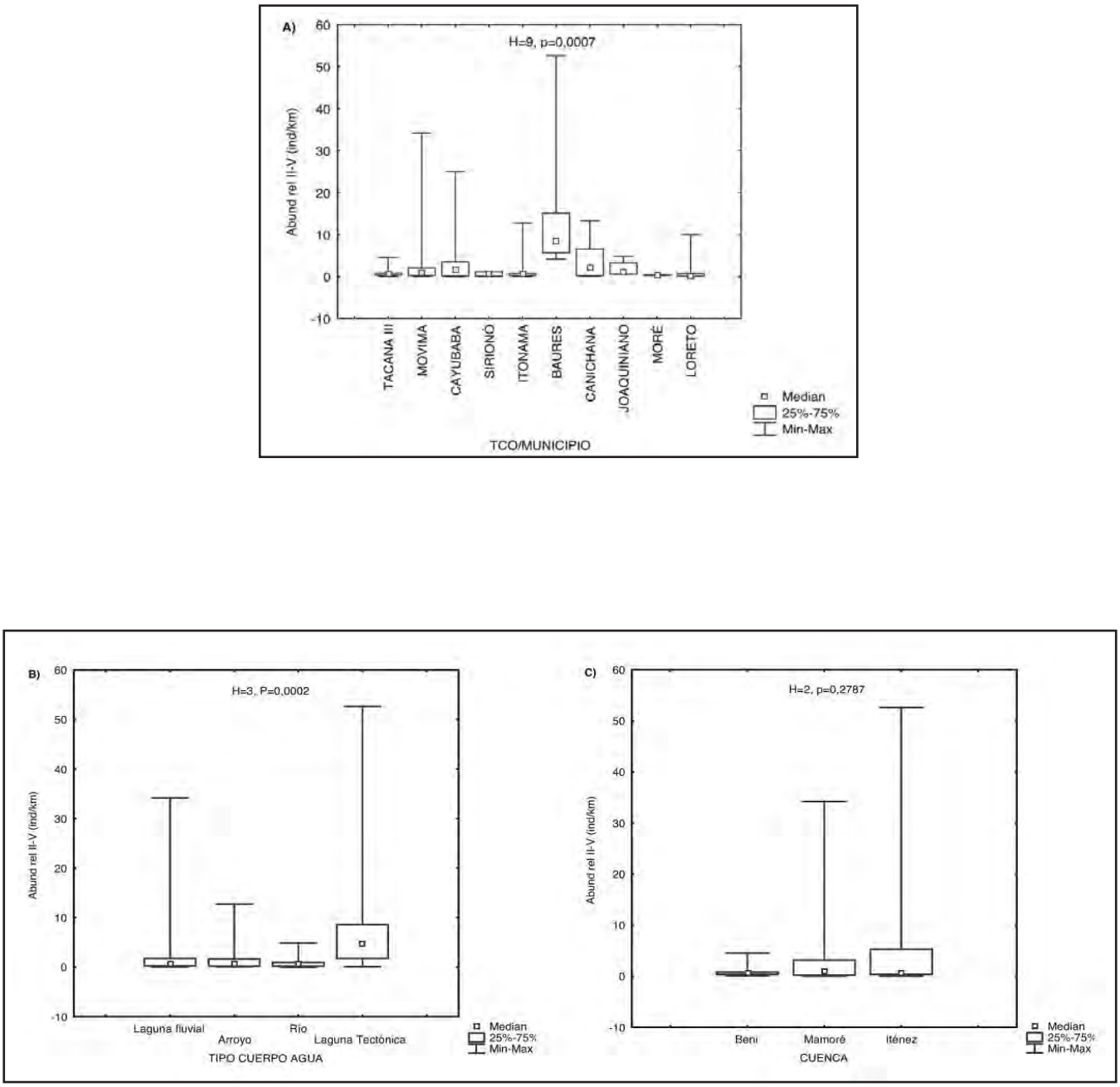



**Figure 4.** Variation of the general relative abundance estimated for *M. niger* for: A) administrative unit; B) type of bodies of water; and C) basin.

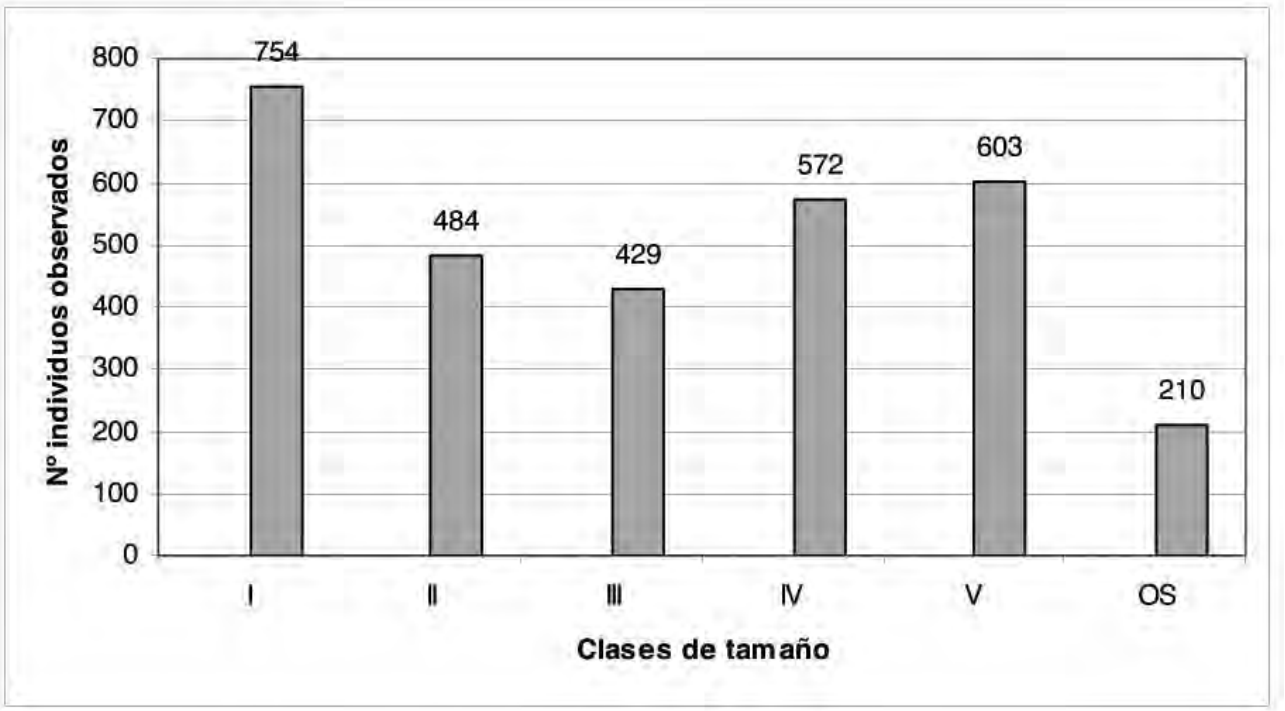
The correlation analysis establishes statistically significant correlation only among relative abundance classes II-V and type of body of water ($p = 0.048$). However, the presence or absence of black caiman, according to the same analysis carried out considering the entirety of the developed samplings, it establishes highly significant correlation between the presence of black caiman and basin type ($p = 0.0016$), like between presence of black caiman and type of body of water ($p = 0.005$). When excluding the tectonic lagoons of the analysis, present in the basins Mamoré and Iténez, this correlation disappears. While the developed

same analysis excluding the class individuals I would show highly significant correlation between occupation and half basin of the river Mamoré (p =0.000023).

**Population structure**

The estimate of the population structure was based on obtained data of 2842 black caimans on the 3052 sighted (93%). Remaining 210 *M. niger* was counted as "eyes only".

The 5 considered size classes were observed in the three basins and the different types of bodies of water, but not in the 10 administrative studied units. The most variable and abundant class (26.53%) were the neonates (class I), continued by the individuals of more size, classes V and IV (Fig. 5).




**Figures 5.** General population structure of *M. niger* for Beni department.

The population structure without keeping in mind neonates varied among spaces, bodies of water and basins (Fig. 6).

In the different types of bodies of water the population is composed mainly for individuals of great size. However, in the whole basin of Iténez, as well as in TCOs Itonama, Joaquiniano and Baures located in this basin, prevalence of sub-adults or smaller individuals is observed. Except for Baures's TCO, in those that relative low densities of *M. niger* were also observed.

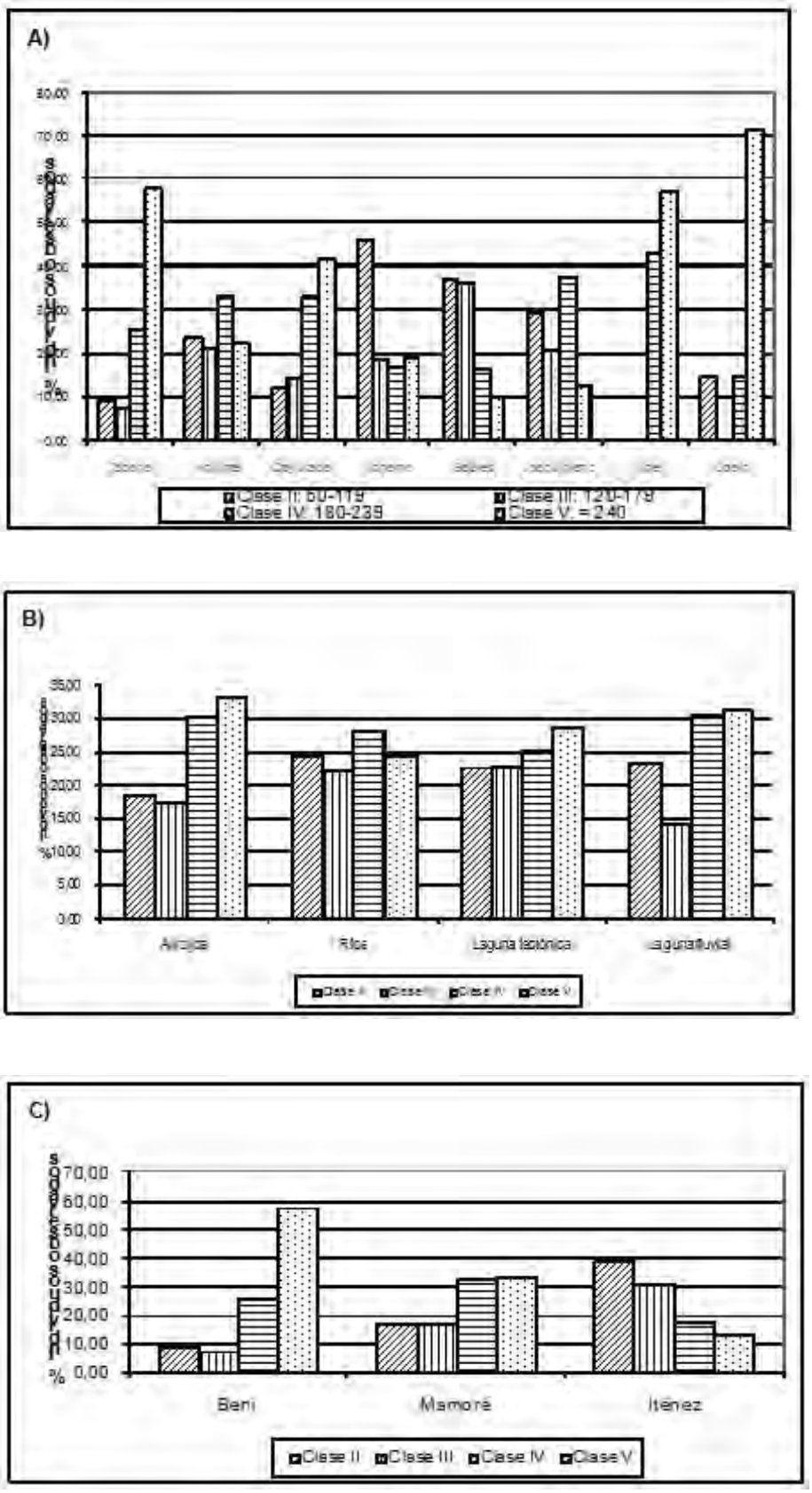



**Figure 6.** Presence of the different population strata of *M. niger* according to: A) administrative unit; B) type of body of water; and C) basin.

**Relationship with *Caiman yacare***

In the area of study two gregarious species share lakes, rivers and streams: *M. niger* and *C. yacare*. No other crocodilian species has been detected during the works. A total of 81672 individuals of both species were identified during the counts. Their distribution and abundance was not continuous throughout the whole study area. *C. yacare* was observed (97%) species and the most abundant (abundance average 45±104.24 individuals II-IV/km). *M. niger* was observed in 43% of the counts (2.94±4.47 individuals II-V/km) (Fig. 7, Table 2).

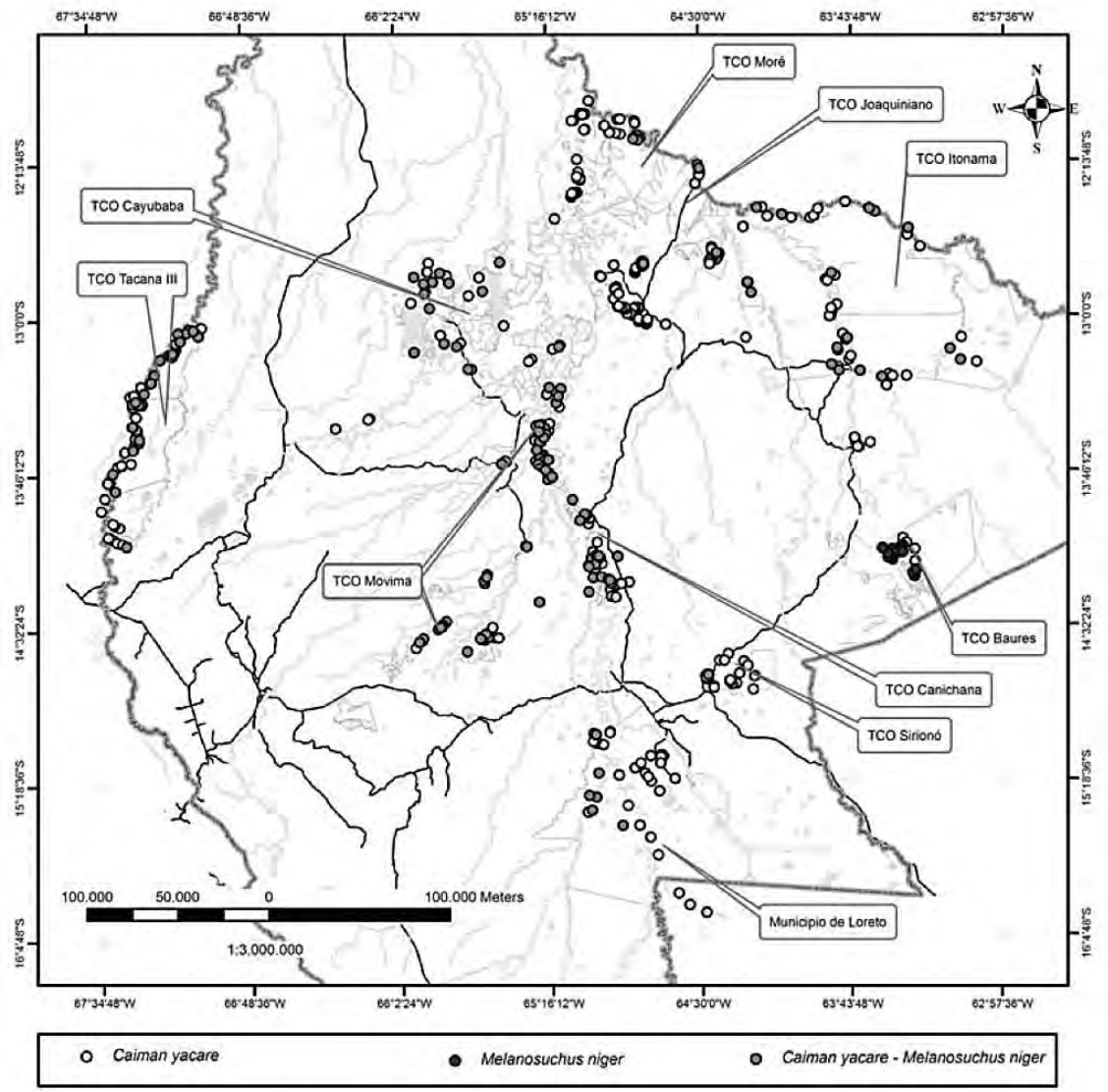



**Figure 7.** Space relationship between *C. yacare* and *M. niger.*

When comparing the composition of species, it was found that *C. yacare* and *M. niger* was only sympatric in 39.51% of the studied places, meanwhile that in the 57.41% of the studied places *C. yacare* was the only found species and only in 3.09% of the places *M. niger* appeared as only species, settling down an association coefficient of 0.44. No other crocodilians species were observed during the fieldworks (Fig. 7).

**Table 2.** Relationship of proportions observed among *M. niger* and *C. yacare*.

| | | Abund rel (ind/km) | | % | | |
|---|---|---|---|---|---|---|
| **TCO** | **Bodies water** | **Lagartos** | **Caimanes** | **Lagartos** | **Caimanes** | **Proportion among species** |
| **Tacana III** | Fluvial lagoons | 61.46 | 0.29 | 99.18 | 0.82 | 0.008:1 |
| | Streams | 50.39 | 0 | 100 | 0.00 | 0:1 |
| | Rivers | 41.38 | 0.34 | 99.21 | 0.80 | 0.008:1 |
| **Movima** | Fluvial lagoons | 14.68 | 0.22 | 91.89 | 8.11 | 0.09:1 |
| | Small lagoons | 13.33 | 0 | 100 | 0.00 | 0:1 |
| | Streams | 1.26 | 0.47 | 96.19 | 3.91 | 0.04:1 |
| | Rivers | 2.25 | 0.82 | 94.46 | 5.54 | 0.59:1 |
| **Cayubaba** | Fluvial lagoons | 56.01 | 0.2 | 99.62 | 0.38 | 0.04:1 |
| | Tectonic lagoons | 7.43 | 2.58 | 69.94 | 30.06 | 0.43:1 |
| | Small lagoons | 119.44 | 0 | 100 | 0.00 | 0:1 |
| | Streams | 37.25 | 12.75 | 74.5 | 25.50 | 0.34:1 |
| | Rivers | 27.16 | 0.11 | 98.42 | 1.58 | 0.02:1 |
| **Sirionó** | Fluvial lagoons | 3.82 | 1.21 | 99.93 | 0.07 | 0:1 |
| | Streams | 30.18 | 0 | 100 | 0.00 | 0:1 |
| | Rivers | 34.95 | 0 | 100 | 0.00 | 0:1 |
| **Cayubaba** | Fluvial lagoons | 56.01 | 0.2 | 99.62 | 0.38 | 0.04:1 |
| | Tectonic lagoons | 7.43 | 2.58 | 69.94 | 30.06 | 0.43:1 |
| | Small lagoons | 119.44 | 0 | 100 | 0.00 | 0:1 |
| | Streams | 37.25 | 12.75 | 74.5 | 25.50 | 0.34:1 |
| | Rivers | 27.16 | 0.11 | 98.42 | 1.58 | 0.02:1 |
| **Itonama** | Fluvial lagoons | 16.92 | 0.8 | 98.51 | 1.49 | 0.02:1 |
| | Streams | 17.02 | 0.31 | 98.2 | 1.80 | 0.02:1 |
| | Rivers | 15.47 | 0.25 | 98.51 | 1.49 | 0.02:1 |
| **Baures** | Fluvial lagoons | 0.33 | 8.59 | 2.75 | 97.25 | 0.03:1 |
| | Streams | 6.67 | 0 | 100 | 0.00 | 0:1 |
| | Rivers | 17.63 | 0 | 100 | 0.00 | 0:1 |
| **Canichana** | Fluvial lagoons | 47.73 | 1.02 | 98.12 | 1.91 | 0.02:1 |
| | Small lagoons | 82.21 | 0 | 100 | 0.00 | 0:1 |
| | Streams | 9.86 | 0 | 100 | 0.00 | 0:1 |
| | Rivers | 13.22 | 0 | 100 | 0.00 | 0:1 |
| **Joaquiniano** | Fluvial lagoons | 73.75 | 0 | 100 | 0.00 | 0:1 |
| | Tectonic lagoons | 7.35 | 0 | 100 | 0.00 | 0:1 |
| | Small lagoons | 1643.41 | 0 | 100 | 0.00 | 0:1 |
| | Streams | 2.17 | 0.2 | 97.24 | 2.75 | 0.03:1 |
| | Rivers | 10.08 | 0.3 | 95.51 | 4.49 | 0.05:1 |
| **Moré** | Fluvial lagoons | 39.14 | 0.04 | 99.82 | 0.08 | 0.002:1 |
| | Tectonic lagoons | 78.67 | 0 | 100 | 0.00 | 0:1 |
| | Small lagoons | 50.4 | 0 | 100 | 0.00 | 0:1 |
| | Streams | 36.6 | 0 | 100 | 0.00 | 0:1 |
| | Rivers | 10.1 | 0.5 | 95.28 | 7.72 | 0.05:1 |
| **Loreto** | Fluvial lagoons | 16.59 | 0.08 | 99.62 | 0.38 | 0.004:1 |
| | Tectonic lagoons | 10.58 | 0.2 | 97.92 | 2.08 | 0.02:1 |
| | Small lagoons | 190 | 10 | 96.61 | 0.00 | 0:1 |
| | Streams | 36.6 | 0 | 100 | 0.00 | 0:1 |
| | Rivers | 10.1 | 0.5 | 96.61 | 3.39 | 0.04:1 |

A relationship among both species cannot be determined, neither to establish an association pattern for type of body of water and basin, due to the variation in the composition of species among bodies of water, basins and studied areas (Fig. 8), and due to the reduced populations of *M. niger* detected.

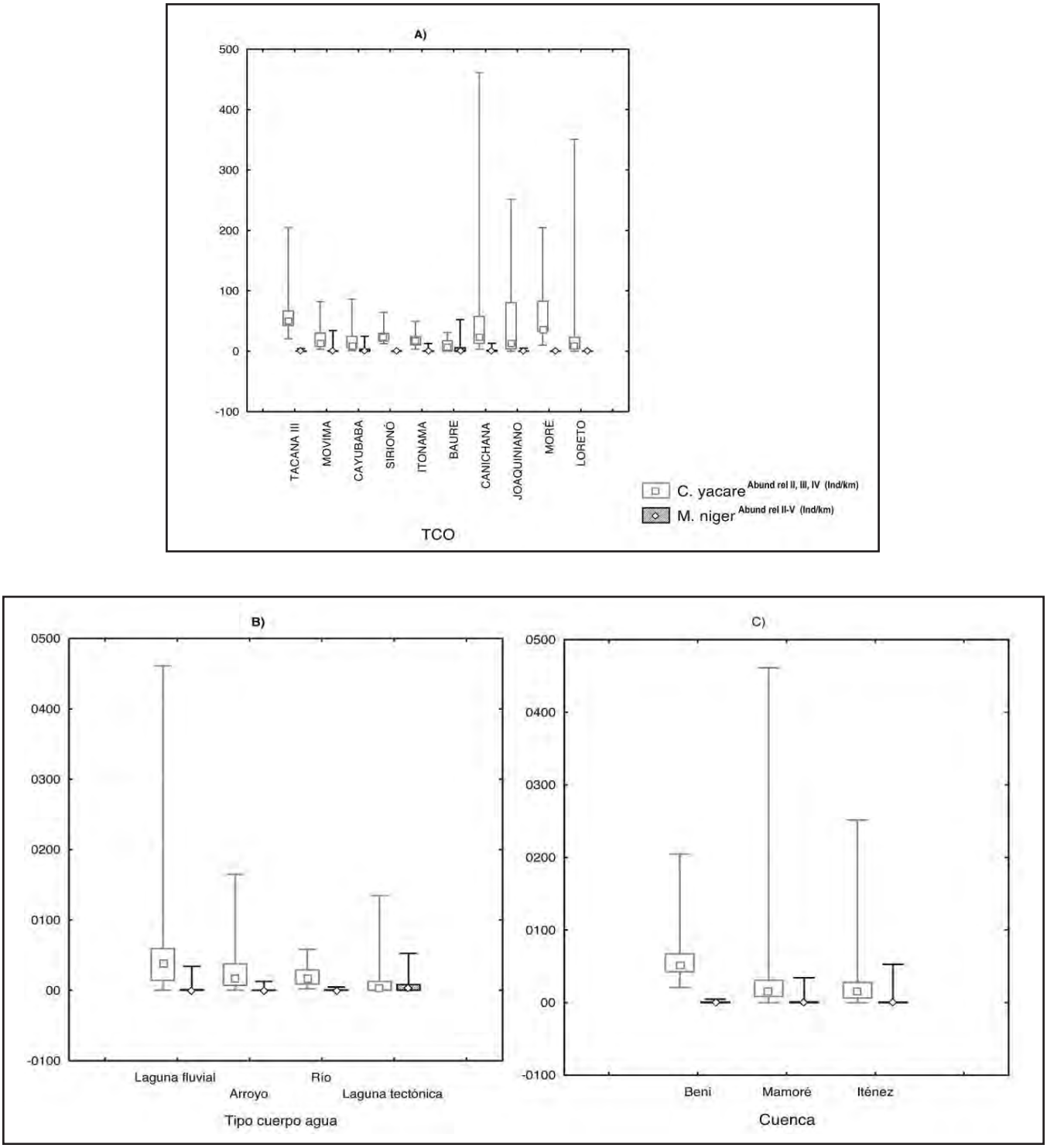



**Figure 8.** Variation of the estimated relative abundance by species in: A) different studied administrative units; B) different bodies of water; and C) the three sampled basins.

## DISCUSSION

Distribution and relative abundance

*M. niger* turned out to be moderately a common species in the study area (43% of the sampling places), spreading to occupy relatively not very accessible areas, associated mainly with lagoon-like bodies of water, especially tectonic lagoons. It was also observed that in certain areas with intense harvest of *C. yacare*, the species is common (half-tributary system of the Mamoré river). Another factor to consider in the distribution of this species is the type of water. The largest tendency of occupancy observed in the basins of rivers of white waters (46.3% of the studied areas in the Beni and Mamoré basins instead of the 36.4% of the places of the basin of Iténez river) it has already been presented in other works (ex. Rebêlo and Lugli 2001, CITES 2007). The aquatic ecosystems formed by rivers and streams of black waters, poor in nutrients, are considered unable to maintain big populations as those reported for the ecosystems of white waters. According to these authors, the caimans would be limited by the productivity of the habitats that they occupy and, therefore, the rivers of white waters, rich in nutrients, would not be limiting the development of their populations.

The estimated relative abundance without considering juvenile (0 -52.64 caimans / km) with an average of 1.27 caimans / km (2.94 ind / km considering only the places with caiman presence), compared with other populations where the species is distributed, goes from abundant to low.

For the Beni department, the results presented by Pacheco (1993) in the Biological Station of Beni indicate densities in lagoons of 0.47-19.5 caimans / km, while in rivers they turned out to be smaller to 1.4 caimans / km. For the Wildlife Reservation Ríos Blanco y Rio Negro in Santa Cruz, the densities obtained in the Blanco and Negro river there were 1.4 caimans / km and 0.9 caimans / km respectively (FAN and WCS 1994). Meanwhile other works in the Itonama TCO (Liceaga et al.2001) obtained a range of 0.06-6.67 caimans / km (average 1.51±2.68), with presence of *M. niger* in 45.5% of the studied places.

These results contrast with those recently obtained in other areas of their distribution range. The night counts performed in five Brazilian states during 2004 and 2005 (CITES 2007) showed presence of *M. niger* in 94% of the studied places, estimated density between 2.1 and 466.5 ind / km and an average of 48.2 caimans / km. For Ecuador, the described range of densities was of 8.27 - 4.09 ind / km (Villamarín-Jurado 2006).

We should remember that the population's estimation of *M. niger* presented doesn't constitute a complete population census, but an indicator of the minimum number of caimans that are in the area. The methodological deficiencies for the nocturnal surveys (Magnusson 1982b, Larriera *et al.*1993, Abercrombie and Verdade 1995, Pacheco 1994, Pacheco 1996), added to problems of accessibility for the investigators to reach the habitats, it implies that part of the caimans will remain without being detected. Tendency to underestimate the population that is also seen in cases of populations with very low densities, since it greatly decrease the probability of observing an individual. Therefore, we can suppose that the presented densities are conservative, being difficult to establish the relationship between the index of abundance and the real density present in the area (Hutton and Woolhouse 1989).

The indexes of relative abundance of caimans offer a limited data, if the distribution of frequencies of classes of sizes of the population under study is unknown (Magnusson 1983). For a group of animals in which the reproductive event is strongly related with the size / age, the estimate for the population structure allows us to obtain an approach to its conservation state (Hines 1992, Pacheco and Llobet 1998, Dueñas 2007), to detect the reproduction occurrence (Magnusson 1982b), to study the effects of the poaching (Cintra 1989, Mourão *et al.*1996), to determine the differences of use of habitat for populations of different strata (Campos *et al*.1994) and to make precise decisions about the handling of these species (Borteiro 2005). On the other hand, the estimates of curves of population are an indirect indicative of the fluctuations in the abundance of a certain crocodilians populations and are of difficult interpretation (Bayliss 1987).

The curves of *M. niger* in the different types of studied bodies of water are seemingly healthy, the same for the Tacana III, Cayubaba and Movima TCOs. For Moré TCO I and the Loreto Municipality, although the population structure appears healthy, the size of the sample is small (n =7). While the distribution for sizes observed in the Joaquiniano, Itonama and Baures TCOs could be interpreted as populations under some level of exploitation or in recovery.

If we analyze the distribution of sizes for basins, the abundance of caimans of big sizes present in the Beni and Mamoré basins suggest populations with good conservation state. However, if we consider the population structure in the Iténez basin, where the Joaquiniano, Itonama and Baures TCOs would be located, next to Moré I TCO, this would be dominated by individuals of smaller size. Now then, in spite of the potential existence of a small use of black caiman at local level, this species, in Bolivia, is protected, being illegal its commercial use.

The same as observed in other towns of Amazonia (Rebêlo and Lugli 2001), the factors that limit the populations of *M. niger* can be independent of the human exploitation. Mentioned TCOs, without big habitat alterations neither hunt pressure on the species, neither present evidences of a wide recovery of the populations of black caiman, what could suggest that in atmospheres of black waters their recovery can be slow.

Another aspect to consider, according with Dueñas (2007), a healthy crocodilians population's curve is characterized by the presence of a bigger frequency of small sizes, continued by a gradual decrease of the largest sizes. According with this, the population structure observed in the Iténez basin would be showing a population with exponential growth, 85% represented by the small and medium size classes (classes I, II and III).

However, it is understood that habitually the drop of observed small-sizes caimans could reflect a bias due to the visibility or as a result of the behavior of this size class. The individuals of Class I are in isolated areas of low depth and with dense vegetation; and not in open areas where the sampling is carried out, reason for which this size class is not habitually considered in the evaluations of population structures, in fact, for the case of Baures TCO, where 81% of the caimans of the basin were located, it was possible to carry out complete and detailed counts of the three tectonic lagoons where these showed up, included the vegetated banks. The caimans observed in the Beni and Mamoré basins,

correspond to more than 56% of the observations carried out in rivers, streams and fluvial lagoons, with numerous lagoons and annexed small lagoon to the main streambed and areas covered by vegetation, favorable habitats for individuals of smaller size.

On the other hand, the most accessible habitats, are marginal habitats for caimans of intermediate sizes (Dueñas 2007). In accordance with Thorbjarnarson and Da Silveira (2000), the dominant males and the reproductive females for this species would be in flooded areas of difficult access and with certain independence of the hydrologic fluctuations of the rivers, with more presence of males in habitats of open waters, while the females would be located in areas embraced by aquatic vegetation (Da Silveira 2001). This can be the case of the tectonic lagoons, located in poorly communicated areas and with smaller tradition of harvesting crocodilians (Baure TCO and Cayubaba-higher area) a population of *M. niger* has been maintained protected for several years in this area. In these lagoons the 51.23% of the individuals class IV and V were detected.

Finally, considering the high percentage of bigger classes registered, together to the observation of nests of *M. niger* in the reproductive epoch of 2006-07 in all the studied TCOs, except for the Moré and Itonama TCOs, would be indicators that the species is reproducing with success. (Asociación Boliviana de Conservación and Central de Pueblos Indígenas del Beni 2008),

**Relationship *M. niger* and *Caiman yacare***

The interactions of crocodilians species in Amazonia are barely known and the studies that examine the patterns of the communities have provided controversial conclusions (Medem 1971, Magnusson 1985).

The importance of the inter-specific competition in the crocodilians communities organization is of great interest, but difficult to establish by simple presence of differences in the use of resources among species, this is not a competition evidence, and if factors like predation, food, climate or others maintain the densities in low levels, the competition factor would be insignificant (Rebêlo and Lugli 2001).

Nevertheless, the same as it has been observed in other studies (Magnusson 1982a, Brazaitis *et al.*1988), where the ecological competition with a more common and smaller size *C. crocodiles*, that could be playing an important role in the natural slow recovery of *M. niger*; in the studied area *C. yacare* could be filling this paper. In accordance with available information (Rebêlo and Lugli 2001), numerous *C. crocodilus* next to few "surviving" *M. niger* from the commercial hunting they were subjected, could be contributing (as predator or competitor) to block the recovery of the populations of *M. niger*. In the present work, the similar species *C. yacare*, that was abundantly observed in all the administrative units studied, could be exercising this competition, impeding the recovery of bodies of water in those that *M. niger* should be abundant, according to these same authors, it is considered that reduced populations of *M. niger* are unable to affect the distribution of *C. crocodilus* (in our case *C. yacare*), although we cannot confirm this.

This could explain the observed tendencies of bigger presence of *M. niger* in areas of current intense hunt of *C. yacare* inside the mark of the National Program of Sustainable Use of

*Lagarto*, like it is the case of the central area of the river Mamoré, an area of historical importance for the hunting of both species and, at the moment, for the harvest of *C. yacare*.

However, since there are not enough evidences on the relationship among both species, the differences of distribution between these sympatric populations should be attributed to habitat preferences (Magnusson 1985). Similar studies (Rebêlo and Lugli 2001) establish that a micro-habitat analysis could suggest certain tendency to a partition of the habitat among both species. The preference of *C. yacare* for low depth and slow waters habitats and the preference of *M. niger* for deep waters, would imply that, in spite of sharing the same bodies of water in several counts, *M. niger* and *C. yacare* would not be sharing the same micro-habitats. This differences in use of micro-habitats and the difference of the adults' sizes could reduce the effects of sharing the habitats and of feeding of similar preys.

## CONCLUSIONS

It’s ignored the level of risk that the black caimans experienced in the past in the department of Beni. The information about the population situation of *M. niger* is scarce and most of it is sustained by qualitative type reports.

The presented data, although it’s preliminary and requires of more research, provide a first detailed vision of the community of *M. niger* in Beni. A dispersed population in the flood plain but apparently not abundant, except some local areas whose abundance varies in association with different habitats and it can be affected by other species, as *C. yacare*.

The results suggest that, although the populations are recovering, the species is not still abundant in all its distribution range, but it is locally. However, facing the results of studies developed among 1986-1987, to the present time, even though the population stays reduced, larger prevalence of mature individuals is observed. On the other hand, the presence of nests and/or neonates of *M. niger* in 6 of 8 studied TCOs, suggests that the species is reproducing with success in these areas.

We can also consider that, without imminent threats of habitat destruction, large areas of pristine habitats of difficult access and with a minimum threat of poaching, could have a favorable situation for the recovery of this species that is staying in the far away habitats of difficult access. However, it is also important to remember that the presence of small dispersed populations throughout the department, probably result of the overexploitation that *M. niger* suffered in the past, doesn't imply that the species has recovered in all its range of historical distribution.

The objective of this type of surveys carried out is to estimate the abundance and to make surveillance through time. It is necessary to incorporate this species to the general considerations of the management plan of crocodilians of the Beni department and of the entire country, and to increase the knowledge about the species. The population's surveillance should be increased in area and time to have a wider understanding about the status of the species.

The above-mentioned recovers special importance in a department in which, the evidences of growing populations in some located areas, increase the interest of incorporate this species

into the commercial use. For this reason, since *C. yacare* and *M. niger* are sympatric species and both have commercial value, the National Program of Sustainable Use of *Lagarto*, should be redirected to at least consider both species. This consideration would facilitate controlling the harvested *M. niger* that is illegally sold as big leathers of *C. yacare* by some specific leather-rescuers, situation that might also be affecting the recovery of the species.

The absence of information about the population state throughout the whole range of distribution of the species is the biggest obstacle for the development of a management and conservation program. For this reason, the departmental interest in developing a management program based on the commercial controlled use, should be sustained with appropriate information about the population state of *M. niger* in the region. The information provided in this article should be complemented and incorporated into the political framework to be developed for the use of crocodilian species in Bolivia.

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# Wicked Problems with Wicked Crocodiles

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**ABSTRACT:** The CSG and its members are involved with solving crocodilian conservation problems around the world. Humans generally attribute negative values to crocodiles (*wicked crocodiles*), which favour eradication, and so incentives to value crocodiles positively, favouring conservation, are often needed. The successful management of crocodilians typically involves dealing with an array of interacting variables (social, economic, biological), all subject to risk and uncertainty. These complex, dynamic and ever-changing problems can be defined as *wicked problems* (Rittel and Webber 1973). Yet the CSG and it members have met with considerable problem-solving success Relative to the 1960s, 13 of 23 species have increased in abundance and 19 species are not considered threatened with extinction within the next 50 years. With 4 other species, such optimism may not be justified. The main lesson learned from conserving and managing crocodilians is that the human variables associated with conservation are much more problematic than the biological variables.

## INTRODUCTION

The theme of the 19th Working Meeting of the IUCN-SSC Crocodile Specialist Group (Santa Cruz, Bolivia, 2-7 June 2008) is "*Lessons Learned on Conservation and Management of Crocodiles*". The CSG and its members have played a pioneering role in generating new knowledge and learning lessons about the conservation and management of crocodilians, and are the major international "institution" involved with crocodilians. So it is a pleasure to address this issue here, and highlight some of the key issues I think are important. The most important lessons we have learned is that the biological variables, although challenging, can often be quantified in a way that allows accurate prediction. The human variables in contrast, are much more unstable and vulnerable to spontaneous change.

### The CSG

The IUCN-SSC Crocodile Specialist Group (CSG) has evolved into a problem-solving organisation, focused on conserving the world's crocodilians. The membership of the CSG involves an array of people (337 members from 52 countries in May 2008) from the private and public sectors, from one or more of a series of primary disciplines (biologists, wildlife managers, NGOs, consultants, crocodile keepers, zoological park staff, crocodile farmers, tanners, manufacturers, educators, lawyers). All are problem-solvers - sometimes problem creators - in their own right.

The CSG was formed under the auspices of the Species Survival Commission (SSC) of the International Union for the Conservation of Nature (IUCN), as a loose and informal group, mainly of biologists, with expertise on crocodilians. The idea was that the CSG could provide the SSC and IUCN, on a voluntary basis, with credible scientific information about the status

of crocodilians, particularly for the IUCN Red Data Book – now the IUCN Red List of Threatened Species. However, creating the CSG had unforseen consequences. There were no NGOs with specialist conservation credentials nor interest in crocodilians in the late 1960's, and so the CSG, as a consortium of like-minded individuals, deeply concerned about crocodilian conservation, informally assumed that role. With many of the more charismatic wildlife species, NGO interest was already established, and so the role played by SSC Specialist Groups was perhaps not so broad.

The SSC and IUCN benefited from the scientific information the CSG provided and welcomed onservation action undertaken by CSG members. The CSG benefited from the credentials implicit in operating what was essentially a specialist NGO under the auspices of the SSC and IUCN. It is an unusual but effective alliance, the strength of which, from a CSG perspective, lies in: the governance freedom extended to the CSG by the SSC; the diversity of CSG membership; the camaraderie which now exists between CSG members; the dedication of CSG members to crocodilian conservation; their pride in association with the SSC and IUCN; their willingness to assist *gratis*; the free and open exchange of information that takes place between most CSG members; and, the transparency with which the CSG conducts its business.

Both the formal function of the CSG (providing scientific information to the SSC and IUCN) and the informal function (pursuing conservation action), clearly assists the SSC and IUCN to fulfil their Missions with regard to world crocodilians, in an efficient and cost-effective manner.

**Conservation and Changing Values**

For the CSG to pursue conservation, and solve conservation problems, it is clearly important they have a clear view of what conservation is and what it is not. This is of course subject to strong and variable personal views, within and outside the CSG. My own opinions, which I believe are widely shared by CSG members, is that *conservation action* is directly to human *values*. Throughout history people have never conserved anything that they did not value positively, and they have destroyed and eradicated many items that had negative values or no recognised value (neutral values). So on this basis, conservation can be defined as: *the sum total of actions taken to preserve and maintain items which are valued positively.* It does not really matter if the values are *utility* values (use values), or intrinsic values - what matters is that the net values are positive and can drive conservation action. If the values are negative they drive eradication.

The values people attribute to crocodilians vary from culture to culture and have evolved and changed over time. The earliest hunter-gathers apparently coexisted with crocodiles, but this does not mean they were motivated by conservation ideals to do so - they had no other option. The crocodiles were incapable of catching and eating all the people, and the people, with the technology of the day, were incapable of catching and eating all the crocodiles. An uneasy alliance, constrained by technology, in which both sides received benefits (occasionally eating each other), which came at a cost (occasionally being eaten). The problem that people from bygone years faced, which still exists today, was essentially: *how do we coexist with crocodiles?* The solution was: *with caution, skill and knowledge built on experience.*

The era of colonisation brought with it improved technologies for killing crocodiles over time, increasing popularity of sport-hunting, increased effectiveness of pest eradication programs and even the use of crocodile skins for leather in the 19th Century. It reduced crocodilian populations in many areas, but from a population viewpoint, the numbers killed were largely sustainable by the wild populations. Not so with large-scale commercial exploitation fuelled by international trade in the 20th Century, particularly after World War II. Wild populations plummeted around the world and by the early 1960s, when the IUCN Red Data Book was launched (1963), crocodiles were all considered vulnerable to extinction.

In terms of values, prior to the 1960s the *positive* values attributed to crocodilians by people were based on utility (= use) values. Crocodiles were a source of protein (meat and eggs) and products (mainly skins). These values could only be obtained by killing crocodilians. The consequences of unrestricted killing of crocodilians (extinction) was not generally appreciated before the 1960's. The *negative* values were that crocodilians preyed on people and domestic livestock, competed for food resources, restricted access to wetlands, and were generally a dangerous nuisance: values which could also only be avoided by killing crocodilians. The relationship between crocodilians and the values people attributed to them thus revolved around people killing crocodiles.

The public awakening about *extinction* in the 1960s resulted in *intrinsic* values being added to the list of positive values. People may not have particularly liked crocodiles, but neither did they like the idea of them going extinct and being lost to the world forever. In addition, with depleted crocodile populations everywhere, the negative values were decidedly reduced: less crocodiles, less problems. The rebalancing of net values meant that after the 1960s, increasing efforts to “save” crocodiles from extinction were possible - the era of conservation had begun.

The high *intrinsic* values associated with avoiding the extinction of wildlife spawned movements such as animal rights and animal liberation, and greatly increased interest in animal welfare. It led to many more organisations pursuing conservation, but with members often united through sharing a particular *intrinsic* value (little “c” conservation organisations) rather than through sharing a primary commitment to pragmatic conservation goals (big “C” conservation organisations).

This difference in conservation focus was to become very important to the CSG, because when conservation efforts with crocodilians succeeded, and depleted wild populations did recover, the positive values were eroded (extinction was no longer a possibility), but the negative values increased (more crocodiles more problems). That is, net values declined, favouring eradication rather than conservation. This led to the promotion of *sustainable use* programs, where crocodiles could once again be killed for trade, adding new positive values. It required a commitment to sustaining the uses, which was a technical challenge overcome in different ways in different programs.

The reintroduction of killing crocodiles to provide incentives to conserve crocodiles, was supported by big “C” conservation organisations, but strongly opposed by little “c” organisations. Their moral and ethical positions were the priority and they did not sanction the killing of animals for any reason, including improved conservation. It led to significant conflict within what is generally considered the conservation community, which was

essentially conflict about competing values rather than conservation itself. That conflict continues today. Every proposal to CITES involving the consumptive use of crocodiles is formally opposed by consortiums of little “c” conservation NGOs.

**Wicked Crocodiles**

In terms of implementing conservation programs for crocodilians in the field, CSG experience and common sense both dictate that it is critically important to understand the values local people attribute to crocodilians. These will mostly be negative ones. People do not have to view many crocodile attacks on people, nor discuss the frustrations and dangers crocodiles cause farmers and fishermen, to realise why. That crocodiles are normally viewed as *wicked crocodiles* by the majority of people is a matter of accepting reality.

Accordingly, despite most CSG members holding the *intrinsic* values of crocodilians highly, they often need to exploit *utility* values to make crocodile conservation programs work, particularly in areas where the people coexisting with crocodilians live in poverty. The two sets of values are not mutually exclusive and never have been. Hunters tend to hold their prey in high esteem.

**Wicked Problems**

Protecting severely depleted crocodile populations has sometimes led to rapid population recoveries, in which case more sophisticated management programs incorporating sustainable use have usually been implemented. But legal protection of highly depleted wild populations does not always result in a recovery. Where the human populations are high, where wetland habitats are restricted and heavily used by people, where crocodile populations were depleted long ago, the challenges of making protection work can be extreme. Regardless of whether trying to promote population recovery, or manage recovered populations, it soon becomes apparent that conserving crocodiles involves suites of different variables (social, cultural, legal, economic, political, biological), all interacting in often unknown but dynamic ways, all subject to risk (known low probability events occurring) and uncertainty (the “wild cards” - new variables not previously recognised as being important). These types of problems with crocodile conservation are identical to those encountered in the field of urban planning, where plans made for the future are invariably confounded by key variables (assumptions) not lasting the test of time. They have been aptly termed “wicked problems” (Rittel and Webber 1973). Most of the problems the CSG has had to deal with are thus *wicked problems with wicked crocodiles.*

The challenge with wicked problems is to accept that they cannot be solved by a classical reductionist (bottom up) approach to problem-solving, in which it is assumed that if you know all the component parts you will be able to predict how the program as a whole will operate. This type of highly dynamic problem, with different dimensions of the problem changing continually, is best approached through scenario planning. Deriving different management scenarios, and then subjecting them to detailed scrutiny from the top down. When a scenario is selected and implemented, it should be with full knowledge that circumstances will change and flexibility and adaptability will ultimately determine whether the goals of management are sustained over time. What this means in practice is that a precautionary approach is more about having protocols in place to detected and respond to

change, rather than a highly prescriptive way forward, implying (completely erroneously), that all key variables are known and understood.

Based on CSG experience, the single key ingredient for successful conservation programs for crocodiles is usually the presence of an individual champion with vision and leadership skills, who can both keep the original conservation objectives in sharp focus and retain institutional memory - a person rather than a position. Institutional memory of how a program responded to perturbations caused by risk and uncertainty in the past is a major safeguard for achieving long-term sustainability in the future. There are also some general rules born of CSG experience:

1. You can only become skilled at managing wildlife by managing wildlife, rather than planning to manage it.

2. You cannot avoid making mistakes, and nor will you be able to avoid being criticised for making them. If you cannot tolerate criticism, get out of the crocodile conservation game.

3. The game rules for any conservation or management program will change continually.

The political variables impacting on a crocodilian management program are but one set of variables vulnerable to change. Within forums such as CITES, decisions affecting local management programs can be made to achieve other political goals, with those voting on whether to support or reject a proposal often having only a rudimentary knowledge of the real substance of the issue. Little “c” conservation organisations opposed to killing animals for any reason are often more effective in such forums than big “C” organisations trying to promote management programs that include some controlled killing. Even at national and local levels, political support for a program can wax and wane depending on the passing parade of political players and the degree to which decisions are based on political opportunism, even jealousies, rather than conservation *per se*. The resources provided to establish a program, which often includes a large commitment to monitoring initially, will often be withdrawn or reduced when the program is operating. The vagaries of politics are arguably a much more important “threat” to conservation programs than the risk of overharvest. The ability to sustain a management program will ultimately be linked to the ability to fight against some political changes and adapt to others. However, the political variables are only one of many sets of variable subject to continual change.

**Indicators of Conservation Success**

In the 1960s the global wild populations of almost all crocodilians of commercial value for their skins were depleted, many dramatically so, relative to their status in the 1940s. This is clearly reflected in the proceedings of the 1st Working Meeting of the CSG in 1971. In the 40 years since the 1960s, despite significant variability between countries, there have been significant changes in status with most species (Table 1).

**Table 1.** Probable changes in global population size since the 1960s for 23 species of crocodilians. “Secure?” refers to whether the global population is considered secure against extinction within the next 50 years.

| Species | Probable Trend | Secure? |
|---|---|---|
| *A. mississippiensis* | Improved | Yes |
| *A. sinensis* | declined (now improving) | No |
| *C. crocodilus* | improved | Yes |
| *C. latirostris* | improved | Yes |
| *C. yacare* | improved | Yes |
| *P. palpebrosus* | declined (?) | Yes |
| *P. trigonatus* | declined (?) | Yes |
| *M. niger* | improved (SU) | Yes |
| *G. gangeticus* | improved (now declining) | No |
| *T. schlegelii* | declined | Yes |
| *C. acutus* | improved | Yes |
| *C. cataphractus* | declined (?) | Yes |
| *C. intermedius* | improved | Yes |
| *C. johnstoni* | improved | Yes |
| *C. mindorensis* | declined (now improving) | No |
| *C. moreletii* | improved | Yes |
| *C. niloticus* | improved | Yes |
| *C. novaeguineae* | declined (stable) | Yes |
| *C. palustris* | improved | Yes |
| *C. porosus* | improved | Yes |
| *C. rhombifer* | declined (?) | Yes |
| *C. siamensis* | seriously declined | No |
| *O. tetrapsis* | declined (?) | Yes |

By 2008 13 of the 23 species of crocodilians (57%) have probably increased in abundance relative to population levels in the 1960s and 10 (43%) have probably decreased. However, 19 species (83%), regardless of whether increased or decreased relative to 40 years ago, for a variety of reasons, are still considered secure - not threatened with extinction globally in the next 50 years. Four (4) species (17%) remain problematic (Table 2).

They *may* be secure given the conservation programs now in place, but they remain CSG priority species for action (Table 2).

**Table 2.** Crocodilian species that may not be buffered against extinction within 50 years, that are priorities for CSG conservation action. CSG assessment in the IUCN Red List is "critically endangered" (CR) for all four.

| Species | Problem | Action |
|---|---|---|
| *A. sinensis* | Seriously depleted in wild, remnant habitat remaining, public opposition to reintroductions (CR) | Encourage existing programs; More habitat dedicated, more releases to wild, more incentives to public, increased public education |
| *C. mindorensis* | Seriously depleted in wild, remnant habitat remaining, public opposition to reintroductions (CR) | Encourage existing programs; More habitat dedicated, more releases to wild, more incentives to public, increased public education |
| *G. gangeticus* | Seriously declining wild population, restricted range (CR) | Encourage existing efforts to determine reasons for decline, more basic ecological research, encourage population increase, repopulation in other parts of range. |
| *C. siamensis* | Serious population decline in Cambodia; wild crocs to village farms in Kalimantan (CR) | Boost wild population in Cambodia with captive-raised juveniles marked to distinguish in trade; Dialogue between SE Asian countries re trade; initiate research in Kalimantan to quantify status. |

## DISCUSSION

The CSG currently has 337 members, from 52 countries, who on average have more than 10-years experience with the conservation, management and sustainable use of world crocodilians. This means at least 3370 years of experience, which even if valued at $10,000 per person per year, provides a knowledge base that has conservatively cost more than $30 billion dollars to assemble, and may cost $3 billion each year to sustain and grow. It is the CSG members and their staff, students and operational budgets, spread throughout the world, that are at the coal-face of conserving and managing the world's crocodilians - the front-line of dealing with *wicked problems with wicked crocodiles*. They are the people providing the new knowledge and new lessons about how to conserve crocodilians.

The CSG assists its members, by facilitating the exchange of information between them, providing a forum through which members can learn from each other, and addressing problems that individual members cannot deal with effectively: for example “CSG National Reviews with Recommendations”. The CSG provides the SSC and the IUCN with a unique opportunity to source knowledge on crocodilians for use within the international forums in which they operate, well-distanced from the ground roots “problems” which many CSG members have to deal with.

Those CSG members dealing directly with management programs, especially programs incorporating sustainable use, have the skills needed to evaluate management scenarios realistically. They have a practical understanding of the strengths and weaknesses of different approaches, the types of mistakes that can and will be made, the most likely roadmaps to success, and the critical role that social, cultural, economic and political variables can and will play. The biological variables, although important, usually prove to be the least problematic in sustaining a management program.

It is now clear that long-lived, late-maturing crocodiles are as amenable to sustainable use as any other species, *if* the rate of harvest can be controlled. This is clearly a challenge in some national contexts because of a range of capacity problems, many of which cannot be rectified simply in order to make crocodilian management more successful. The same capacity problems effect education, health, infrastructure development, etc. Hence the types of management applied in different national contexts need to be tailored to local circumstances. There is no single "ideal" program.

From a CSG perspective, it is frustrating that we still have species of crocodilians that were *critically* endangered in the 1960s and remain so today. These species are all now being assisted by dedicated conservation programs, which was not the case in the 1960s, so advances have been made. But bold, new and innovative approaches may be needed if significant changes in status are to be achieved and sustained in the future. The main practical issues that need to be investigated if new conservation scenarios are to be identified and implemented have little to do with biology:

1. Who benefits financially, in direct and indirect ways, from maintaining the *status quo* and resisting change?
2. Are the beneficiaries the ones that need to benefit in terms of the incentives required to enhance a population recovery?
3. Is there a “legal assumption” involved, in the sense of laws restricting conservation program options that can be applied?
4. To what extent do intrinsic versus utility values exist in the community?
5. Is the flawed approach based on a strong philosophical or religious commitment to particular types of solutions?
6. Is the maintenance of a flawed solution implicit in fund-raising?
7. Is the issue a politically sensitive one for local politicians?
8. Is the issue an important or trivial one for local people?
9. Are there commercial industry ramifications?
10. Is the management capacity present to undertake different approaches?
11. Is gratuity involved in decision-making?
12. To what extent does poverty influence the actions of people?

13. Are there any cultural links with the species?
14. Are land tenure issues involved?
15. Is illegal trade involved?
16. To what extent is trade (illegal or legal) the primary or secondary cause of the population's *failure to thrive?*
17. Are the reasons for failure being allocated objectively or are convenient *public enemies* being used to divert responsibility?
18. Does the legal system have the capacity to achieve compliance with the laws?
19. Are cultural sensitivities to problems so great that they cannot be addressed objectively?
20. Are local people educated?
21. To what extent can concerns about wildlife be prioritised relative to other basic humanitarian needs such as health, education and food security?
22. Is there sufficient genuine interest by those required to conserve the species to negotiate increased involvement from communities?
23. Do the authorities understand the difference between population dynamics and dynamic populations?

The ability to implement new approaches to conservation, even if urgently needed to avoid the extinction of a critically endangered species, may be far more constrained by social, cultural, economic and philosophical barriers than by biological ones. This is the main lesson I have learnt about the conservation and management of crocodilians. It is people who usually create conservation problems, but it is also people who usually solve them, and people who prevent potential solutions even being tested. The problems are truly *wicked.*

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# A New Orinoco Crocodile (*Crocodylus intermedius*) Population in the Apure Llanos. Venezuela.

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**ABSTRACT:** The Orinoco crocodiles introduced at El Frío Biological Station, Caño Guaritico Wildlife Refuge and surrounding areas have formed a "new population". These crocodiles come from wild eggs, wild hatchlings and hatchlings from captive adults. At the beginning of the Venezuelan Conservation Program the number of crocodiles in the area was almost zero. 2,282 juvenile crocodiles were introduced at the study area between 1990 and 2006. We define a population of this species as a group of 300-500 individuals older than one year, which includes at least 25-30 breeding females. The introduction Program was evaluated through night and day surveys along 476 km of water courses between October 2006 and January 2007. The minimum population size is estimated as 400 crocodiles with at least 31 breeding females. The estimated introduction rate is 17,8 %. In Venezuela occur three Orinoco crocodile populations: Cojedes, Capanaparo and Guaritico (new), which shows a fast increasing in individuals and geographic expansion. El Frío Biological Station develops a "ranching" of eggs from the new population, with exclusive conservation purposes. This is the first case in the world where a wild crocodile population is made up from captive individuals.

## INTRODUCTION

*Crocodylus intermedius* is the great crocodile of the Orinoco basin. Its historic distribution is restricted to the huge plains below 200 m over the sea level typical of this river, although exist some records of its presence up to 400 m. According to naturalists from 18th and 19th centuries the species was very abundant both in Venezuela and Colombia. From 1929 to the early 60's an uncontrolled commercial hunting depleted the species in most of its habitat; just two important populations survived in Venezuela (Cojedes-Sarare and Capanaparo) and some small groups or isolated individuals in different places like Tucupido, Camatagua or Manapire (Ayarzagüena 1987; Thorbjarnarson and Hernández 1992; Seijas et al. 2002).

The conservation activities started in Venezuela in the late 70's, along the 80's four captive-breeding centres were established, and in 1989 Caño Guaritico Wildlife Refuge (CGWR) was designed as a specific area for the Orinoco crocodile conservation. Most of the restocking efforts have been taken in CGWR and in adjacent areas like El Frío Biological Station (EFBS). In this area the species was almost extinguished 20 years before (there are three unconfirmed reports of crocodiles from local people). 2.282 juveniles have been introduced between 1990 and 2006 (Velasco pers. comm.).

The study objective is to show up the results obtained by means of the restocking program in CGWR and surrounding areas and, on the basis of that, demonstrate the existence of a new population for this species.

Previous census (Lugo 1998; Chávez 2002) evaluated the success of the introduction program in CGWR and EFBS. The present study is more extensive in time and space, and it is actualized, which is really important in a population that is continuously growing since the restocking program started.

## STUDY AREA

The study was carried out in the southwest of Venezuela, into the Flooded Llanos region (Velasco and Ayarzagüena 1995), in an area around 5.000 km$^2$, which includes CGWR and EFBS (Fig. 1).

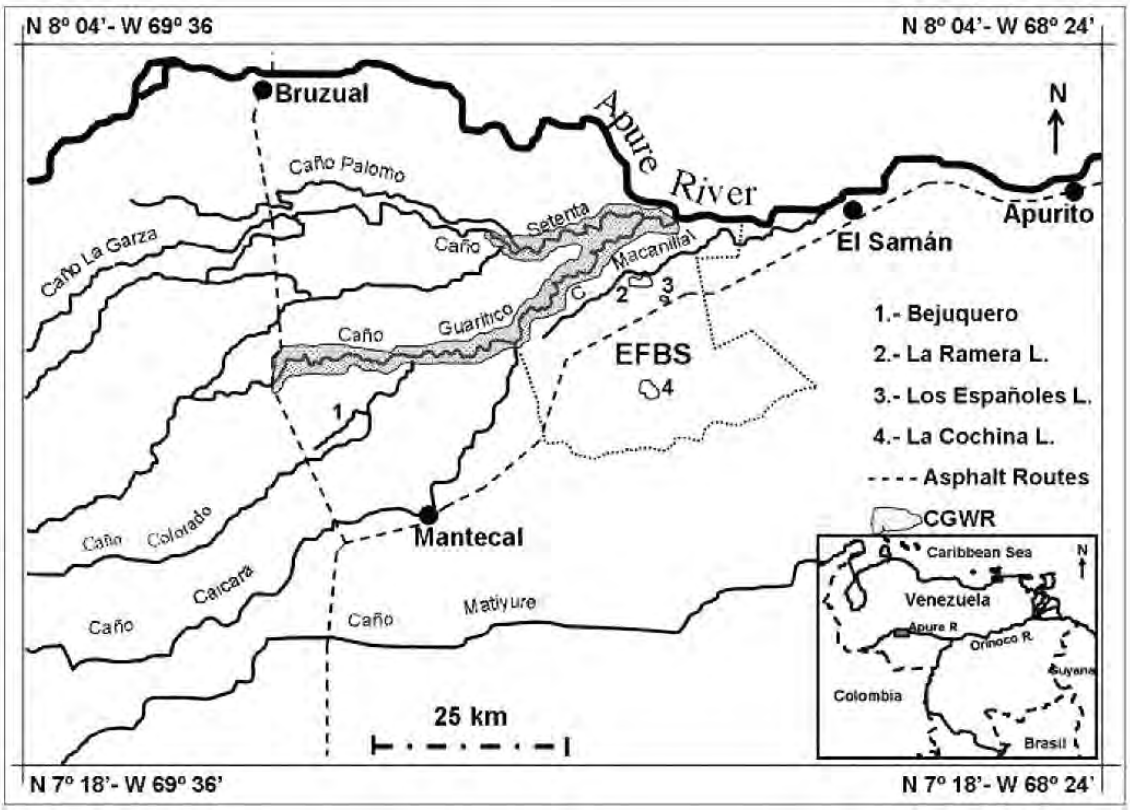



**Figure 1**. Map of the study area, includes CGWR, EFBS and other surveyed sectors. The water flows from west to east.

There are two clearly-defined seasons in the region, a dry season between December and April, and a rainy season between May and November. The almost flat relief and the rich clay substrates promote that, during the rainfall peak (July-August), 80 % of the territory is flooded up to one meter by the rainfalls water and the overflow of main rivers and *caños*.

*Caño* is a water course characteristic of the huge plains, with seasonal flow that canalizes the waters from rivers and lagoons overflows.

The savannah vegetation is typically herbaceous, but rivers and caños are flanked by gallery forest, mangle (*Coccoloba obtusifolia*) grows over the banks and some *caños* and lagoons present floating vegetation (*Eichhornia sp.*).

The surveyed *caños* in this study flow across or between private cattle ranches (*Hatos*).

## MATERIAL AND METHODS

From October 2006 to January 2007 nocturnal spotlights surveys were carried out from aluminium or wooden boats powered by 30 or 40 hp outboard engine. Crocodiles were detected and differenced from caimans (*Caiman crocodilus*) with the help of a 1.000.000 candles torch connected to a 12V battery. The observed crocodiles were recorded on a GPS Garmin E-Trex, which was also used to measure distances, speed, and time employed. The in-navigable sectors of Caño Macanillal and Los Españoles lagoon were surveyed by foot or by horse. La Cochina lagoon was surveyed from the shore by 4WD vehicle.

The census team was composed of the investigator and local people (except in EFBS) like fishermen, tourist guides and/or security employers, who know the area and the exact points where the crocodiles used to be. In the results, "observed crocodiles" are those that were already sighted. "Reported crocodiles" are those that were not seen for us, but are usually observed by local people. The preference of this species for concrete points during the dry season supports the reliability of this information.

The crocodile density is expressed in Individuals/km (Ind./km), except in the lagoons where is not possible to express density, because of its highly variable perimeter. The census was carried out looking for the minimum level water that allows the navigation. As is well-known, water level has great influence in crocodile detection (Woodward and Marion 1978; Hutton and Woolhouse 1989; Da Silveira *et al* 1997; Ron *et al* 1999; Da Silveira *et al* 2008).

The population size estimation is calculated by two methods: a) Assigning the average density obtained in the census to sectors which we were informed that contain crocodiles but could not be surveyed, b) Estimating the survival rate of introduced crocodiles in small well-known areas, and considering that rate for the total number of introduced crocodiles at the whole study area.

## RESULTS

### Population Size

115 crocodiles over 80 cm total length were observed along 476 km surveyed; if we add 96 "Reported crocodiles" that were not observed, we obtain 211 "registered crocodiles" (Table 1).

**Table 1.** Observed, Reported and registered (observed + reported) crocodiles during the census at different body waters.

| Date | Surveyed Area | km | Observed | Reported | Registered | Ind./km |
|---|---|---|---|---|---|---|
| 10/10/2006 | C. Terecay | 33 | 0 | 0 | 0 | 0,00 |
| 10/10/2006 | C. La Aguada | 6,9 | 1 | 1 | 2 | 0,29 |
| 11/10/2006 | C. Palomo (Section A) | 21,1 | 0 | 0 | 0 | 0,00 |
| 02/11/2006 | C. Garza (Section A) | 11,4 | 1 | 3 | 4 | 0,35 |
| 03/11/2006 | C. Palomo (Section B) | 8 | 0 | 0 | 0 | 0,00 |
| 07/11/2006 | C. Setenta | 6 | 2 | 6 | 8 | 1,33 |
| 15/11/2006 | C. Garza (Section B) | 42,2 | 3 | 15 | 18 | 0,43 |
| 18/11/2006 | C. Matiyure | 17,3 | 6 | 4 | 10 | 0,58 |
| 19/11/2006 | C. Molino Mocho | 9,6 | 1 | 2 | 3 | 0,31 |
| 24/11/2006 | C Bejuquero/C. Colorado | 26,1 | 3 | 2 | 5 | 0,19 |
| 27/11/2006 | CGWR C. Setenta | 39,5 | 5 | 7 | 12 | 0,30 |
| 01/12/2006 | CGWR C. Guaritico | 128 | 27 | 36 | 63 | 0,49 |
| 31/01/2007 | R. Apure (Section A) | 61,2 | 3 | 7 | 10 | 0,16 |
| 28/01/2007 | R. Apure (Section B) | 50,3 | 7 | 13 | 20 | 0,40 |
| 18/01/2007 | C. Macanillal | 15,3 | 30 | - | 30 | 1,96 |
| 03/01/2007 | La Cochina L. | - | 6 | - | 6 | - |
| 15/01/2007 | La Ramera L. | - | 16 | - | 16 | - |
| 20/01/2007 | Los Españoles L. | - | 4 | - | 4 | - |
| **Total** | | **475,9** | **115** | **96** | **211** | |

We observed crocodiles in all the surveyed areas, except in Caño Terecay and Caño Palomo, which both are narrow, shallow and do not represent an appropriate habitat for the species. If these *caños* and the crocodiles observed in the lagoons are removed from the analysis, the average density obtained in the study area is 0,45 Ind./km. The highest density was observed in Caño Macanillal (1,96 Ind./km).

**-Size population estimation based on potential habitat and census.**

We consider that in the study area there are around 648 km of river courses that are potential habitat for the species. 234 km (34%) of several sections of the *caños* Caicara, Setenta, Bejuquero, Guaritico and La Aguada could not be surveyed because they were inaccessible or because they become dry before than expected. These *caños* were surveyed for us in other accessible sections and in all of them we observed crocodiles; in addition, reports from local people confirm the presence of crocodiles at these not surveyed sections. If we apply the average density obtained in the census (0,45 Ind./km) to the not-surveyed sections, we obtained 105 estimated crocodiles, that added to the 211 “registered crocodiles”, resulted in a population size of 316 crocodiles.

**- Size population estimation based on survival rate of introduced crocodiles.**

As we mentioned at the Introduction, the whole crocodile population comes from individuals raised in captive- breeding centres; if we estimate the survival rate of the introduced

crocodiles, we can have a good estimation of the real population size. Some areas allow a quite good estimation because few crocodiles have been released in them and, in addition, are monitored constantly (Table 2).

**Table 2.** Estimated survival rate in small and well-defined areas.

| Area | Introduced crocodiles | Observed crocodiles | Survival Rate (%) | Estimated Crocodiles | Estimated Survival Rate (%) |
|---|---|---|---|---|---|
| C. Garza | 67 | 6 | 9 | 21 | 31,3 |
| La Cochina L. | 16 | 4 | 25 | 6 | 37,5 |
| C. Matiyure and C. Caicara | 69 | 5 | 7,2 | 15 | 21,7 |
| La Ramera L. | 72 | 14 | 19,4 | 16 | 22,2 |

In the four areas the survival rates oscillate between 21,7 and 37,5 %. Using the lowest value (21,7 %) as indicative of the survival rate for the 2.282introduced crocodiles, the population size estimation will be 495 crocodiles; if we use the highest one (37,5 %), the theoretical population size will be 856 crocodiles.

**Breeding females**

Between 2005 and 2006 at least 22 different females breed in EFBS and CGWR, added to nine nests referred to us for fishermen and local people. 21 nests were observed at EFBS and the rest in other parts of the study area (Table 3).

**Table 3.** Observed, referred and estimated (observed + reported) nests in the study area between 2005 and 2006.

| Area | Observed Nests | Reported Nests | Estimated Nests |
|---|---|---|---|
| C. Terecay | 0 | 0 | 0 |
| C. La Aguada | 0 | 0 | 0 |
| C. Palomo | 0 | 0 | 0 |
| C. Garza | 0 | 1 | 1 |
| C. Matiyure | 0 | 4 | 4 |
| C. Bejuquero and C. Colorado | 0 | 0 | 0 |
| C. Setenta | 0 | 0 | 0 |
| C. Guaritico | 1 | 2 | 3 |
| Apure R. | 0 | 2 | 2 |
| C. Macanillal (EFBS) | 10 | 0 | 10 |
| La Cochina L. (EFBS) | 0 | 0 | 0 |
| La Ramera L. (EFBS) | 10 | 0 | 10 |
| La Entrada L. (EFBS) | 1 | 0 | 1 |
| **Total** | **22** | **9** | **31** |

Some of the eggs founds in EFBS nests are collected to continue with the captive-breeding program.

## CONCLUSIONS

The values showed by the different estimation methods oscillate between 316 and 856 crocodiles. With this in mind, we estimate the population size present in CGWR and EFBS in, at least, 400 non-hatchling crocodiles, which includes around 30 breeding females.

This new population is comparable to the other two described ones for Venezuela, Cojedes – 547 crocodiles- (Seijas and Chávez, 2000) and Capanaparo -536 crocodiles-(Llobet and Seijas, 2002).

400 crocodiles is equivalent to 17,8 % survival rate of the introduced crocodiles, three times more than the estimated rate of survival of wild ones (Antelo in prep), which clearly shows the importance of the captive breeding conservation program.

The new population represents the successful introduction of a man-eater species in the wild, being, possibly, the first case around the world in which a new crocodile population is made up exclusively by individuals raised in captivity.

According to previous census (Lugo 1998; Chávez 2002), the information from fishermen and our observations, this population shows a fast increasing in individuals and geographic expansion.

We finally conclude that this success, which highlight at international level, has only been possible because of the substantial collaboration of several public and private institutions: Venezuelan Ministry of Popular Power for the Environment, FUDENA, FUDECI, FLASA, WWF-USA, AECI, GECV, and of course the four captive-breeding centres: Fundo Masaguaral, UNELLEZ, Agropecuaria Puerto Miranda and EFBS.

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# Population Status and Evaluation of the releasing program of Orinoco's caiman, (*Crocodylus intermedius*) in the Cojedes River, Venezuela.

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**SUMMARY:** In order to continue with the management strategies aimed to conserve and recover the populations of Orinoco crocodile in the Cojedes River, we estimated the abundance and population structure including spatial and temporal variations. Twenty five nocturnal surveys were performed in five different segments of the river (43 Km) between December 2005 and May 2006. The abundance indexes show intervals of 0.35 to 5 individuals/km, with a media of 2,9 ind/km for the most representative segments. There were no significant differences in the population structure between segments, and in all the cases the Class V adults, and Class II juveniles dominate the populations. From 61 different captured animals 5% belong to the reintroduction program, representing a 1,5% of the 207 animals reintroduced in may 2005. The dispersion of juvenile crocodiles (as wild as freed) shows that these animals use a relative small area of action. The growth rate of the freed crocodiles in the Cojedes river is between 2,8 and 11,4 cm/year. The development of the juvenile crocodiles in the Cojedes river seems to be very slow, and far below the potential growth shown by the species, therefore, the reintroduction of more animals in this area should be reviewed and/or refocus.

**RESUMEN:** Para continuar con las estrategias de manejo orientadas a la conservación y recuperación de las poblaciones del caimán del Orinoco en el río Cojedes se estimó la abundancia y estructura poblacional incluyendo variaciones espaciales y temporales. 25 muestreos nocturnos fueron realizados en 5 sectores diferentes (43 km) del río entre diciembre de 2005 y mayo de 2006. Se encontraron índices de abundancia entre 0,35 y 5 individuos/km, con un valor medio de 2,9 ind/km para los sectores más representativos. No se observaron diferencias en la estructura poblacional en los diferentes sectores y en todos los casos la población estuvo dominada por individuos adultos Clase V, y juveniles Clase II. De los 61 caimanes distintos capturados el 5% correspondieron a individuos provenientes del programa de liberación, se calculó una fracción de permanencia de 1,5% de 207 individuos liberados en mayo de 2005. La dispersión de caimanes juveniles (silvestres y liberados) demuestra que tienden a permanecer en un área de acción relativamente reducida. La tasa de crecimiento en cm/año de los caimanes liberados en el río Cojedes están entre 2,8 y 11,14, el crecimiento de los caimanes juveniles del río Cojedes parece ser muy lento, y muy por debajo del potencial mostrado por la especie, por lo que la liberación de animales en esta localidad debe ser revisada y/o reorientada.

## INTRODUCTION

The Orinoco's caiman is one of the most seriously endangered crocodilian species of the world, the commercial overexploitation since 1930's until the end of 1950's decimate the population all over the distribution area (Thorbjarnarson 1992, Ross 1998, Seijas 1998). Besides the legal efforts taken up to date, the population recovery has been too slow (Seijas 1998) and the species is still endangered for a factors combination as the habitat destruction, egg harvest, poaching, intentional deaths and other incidents (Thorbjarnarson y Hernández 1992).

In the Cojedes river system of Venezuela, the largest known reproductive population is found, making it the key place for the recovery of the species (Seijas 1998).

Several evaluations had been performed in the Cojedes river, providing valuable data about the most important wild population of the country (Godshalk 1978, Ayarzagüena 1987, 1990, Thorbjarnarson y Hernández 1992, 1993a, 1993b, Seijas 1993, 1998, Seijas y Chávez 2000).

In the Cojedes river system, historically, some special characteristics were present that lead to the residence of the most important caiman population, as the poor communication systems from important commercial roads of the country to the river, the constant riverbed changes with a complex meander system, navigation channels and old meanders, after a flood period. Another important factor that helps with the residence of the caiman in the Cojedes river, is that the Cojedes State remains as one of the least populated places of Venezuela (Seijas 1998).

Actually, the Cojedes river is under constant human pressure, caused specially by important intensive-agriculture areas close to the river, and large urban and industrial locations (Godshalk 1978, 1982, Ayarzagüena 1987, 1990, Seijas 1998). In the last 50 years, the human activities had changed the habitat characteristics of the Orinoco's caiman in the Cojedes river. Some changes modified the water quality of the river, meanwhile others altered the physical characteristics of the river by channels, deviations, dams, and dredges (Seijas 1998, Seijas y Chávez 2002, Mendoza y Seijas 2007).

The actual population status of the Orinoco's caiman in the Cojedes river is probably a consequence of the historical factors mentioned before, and the interactions of the species with the new factors directly or indirectly related to the human presence and activities. (Seijas 1998). Today, the Cojedes river isn't under any protective or special administrative regimen, even after several years of studies and the development of proposals, the protected area declaration for this location hadn't been achieved yet, determining the necessity of the project to achieve the creation of this area.

The present work, developed with the economical support of the Wildlife Conservation Society (WCS) and FUDECI, was made aiming to establish the population status of the Orinoco's caiman (*Crocodylus intermedius*) in the Cojedes river, with the objective to obtain necessary actualized information, in order to build a better vision about the conservation status, as to evaluate the population restoration efforts in the area helping to support technically the continuity of the work or to refocus it in order to increase its success.

## STUDY AREA

The Cojedes river belongs to the Turbion-Cojedes river system, that extends through several regions and flows into different relieves, ecosystems, canopy structures and types of human activity. The study area is placed south Lagunitas(Libertad)-El Amparo- Santa Cruz road, south to San Rafael de Otono National Road, part of the Cojedes river known as Caño de Agua, from the referencing point of La Batea, to the south passing a divide called Caño Amarillo, takes part of the Sarare river, that runs parallel to Caño de Agua and to a section of Caño Amarillo.

Most of the Cojedes river belongs to an area known as Tropical Dry Forest (Ewel y Madriz 1968). In the study area we find station homogeneity, with two well defined weather stations, as is common in the Venezuela plains. The rain station, which extends from May to October, and the dry station that takes place from December to March, being April and November transitional months between stations. According with the information obtained from the Environment Ministry, the average rain fall of the area is 1328.1 to 1335 mm (1967 to 2004).

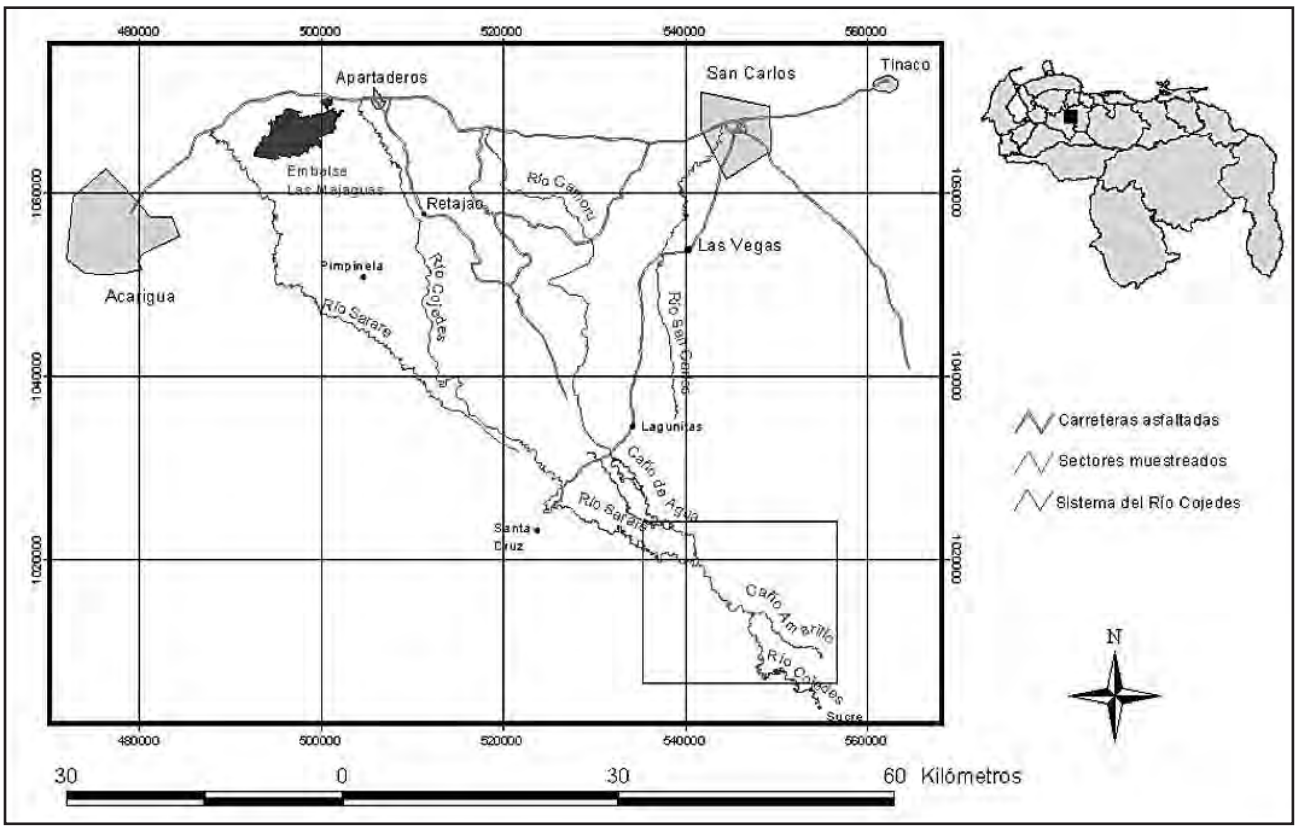



**Figure 1.** Study area relative location in Cojedes river System (Portuguesa and Cojedes limits estados, Venezuela).

## METHODOLOGY

### Study Area Delimitation

For this study five sections were selected: 1) La Batea – Confluencia, in which 5,5 km of river were sampled, from La Batea point to the confluence of the Sarare river and Caño Amarillo, downward the river; 2) Sarare. In this section 3,1 and 3,8 km were sampled, from the confluence of the Caño de Agua and Sarare river, upward the river; 3) Merecure-Caño Amarillo, in this section 12,3 km were sampled, from the confluence of Caño de Agua and Caño Amarillo, downward the river; 4) Caño Amarillo, in this section 7,5 km were sampled from the divide of Caño Amarillo and Caño de Agua, downward the river; 5) South Cojedes, in this section 14 km were sampled, part of the Cojedes river from the divide of Caño Amarillo and Caño de Agua downwards the river. (Figure 2)

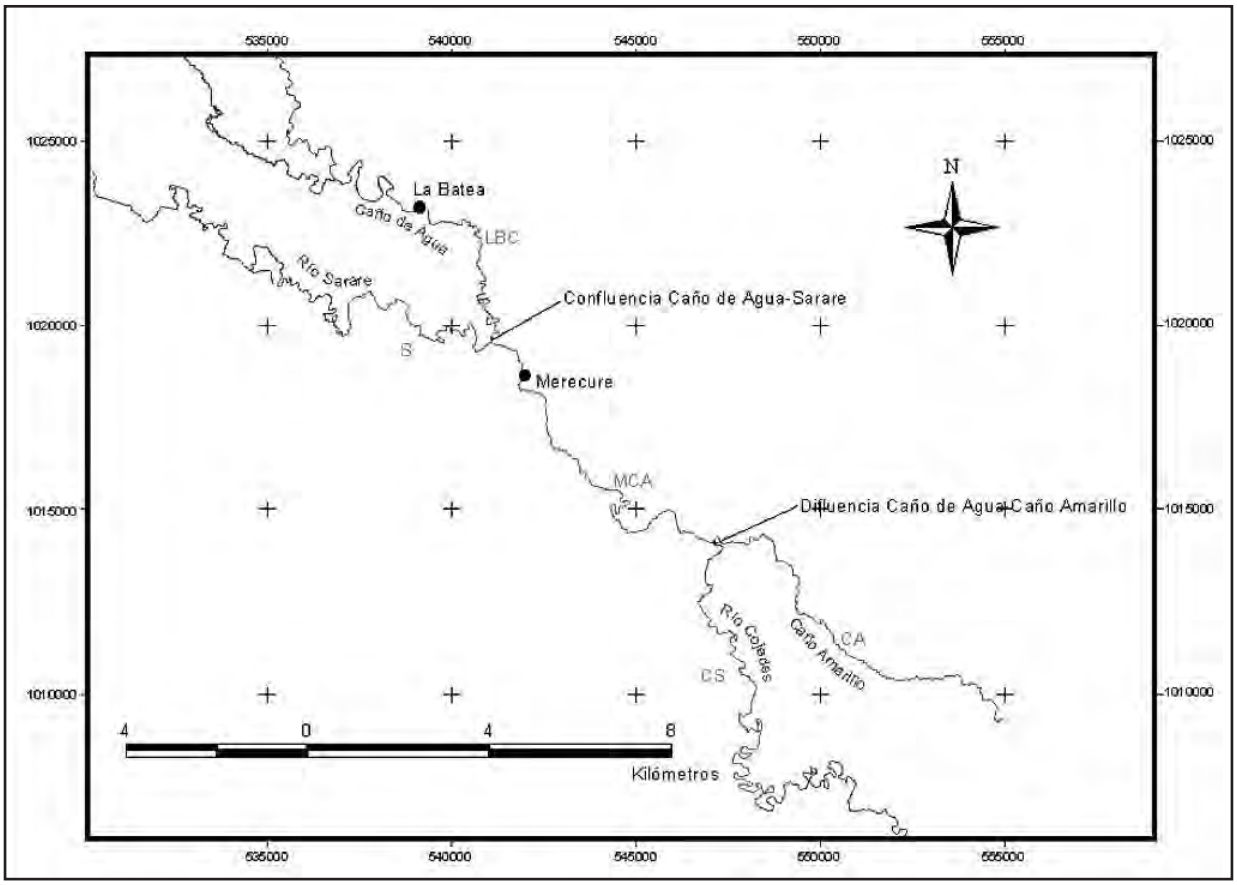



**Figure 2.** Study area. There appear the principal points of reference indicated in the text. The sampled sectors are indicated by initials: LBC, La Batea-Confluencia; MCA, Merecure-Caño Amarillo; S, río Sarare; CA, Caño Amarillo; CS, río Cojedes Sur.

### Crocodilian Counting

Nocturnal counts were performed to determine the population status of C. intermedius in the Cojedes river, since December 2005 to May 2006. Monthly repetitions were performed, sampling each night sections of about 15 km of river. The counting of caimans was made sampling both river beaches. Most of the samplings were made alongside the current flow.

In the counting, the maximal approach to each individual was intended in order to have a positive identification of the species (*Crocodylus intermedius, Caiman crocodilus*) and to estimate the size of the animal (total length, LT). In the field the size of the animals was estimated in intervals of 30 cm, but for the analysis intervals of size of 60 cm were used (Seijas 1998, Seijas y Chávez 2000):

- Class I: Individuals below 60 cm of LT
- Class II: Individuals between 60 to 119 cm of LT
- Class III: Individuals between 120 to 179 cm of LT.
- Class IV: Individuals between 180 to 239 cm of LT
- Class V: Individuals equal or above 240 cm of LT.

The neonate individuals (Class I or younger than 6 months of age) were counted but not considered for the abundance estimate. The individuals were also grouped in size categories, as described by Seijas (1998) and Seijas and Chávez (2000), the individuals between 60 cm to 180 cm of LT were considered young, the animals between 180 to 240 cm of LT were classified as sub-adults and those bigger than 240 cm as adults.

The caimans' index of abundance was calculated by dividing the number of animals registered by the kilometres of river crossed. The abundance values obtained in each sampling (inv/km) were expressed as percentages of the values obtained at the beginning of May (considered as 100 %) in the same section of the river, in order to analyze the abundance variation as the dry station pass by. A correlation analysis was made to describe the relationship between these percentages and the days after January 17$^{th}$ (the first day of sampling considered for the analysis), as an indirect measurement of the water level (Seijas 1998, Seijas y Chávez 2000).

The spatial variation of the abundance was analyzed by comparing the obtained values (ind/km) among the different sections of the river and among the different sections that compose each section, with a Kruskal-Wallis test.

The average values of the density combined with capture-recapture analysis and the individual dispersal for each studied section were used to calculate the minimal size of the *C. intermedus* population in the study area.

The caimans' population structure for all the river, and for each one of the sections of it, were calculated using the maximal number of individuals registered for a particular size class, independently the month of sampling, as the minimal number of animals of that class present in the river (Messel *et al*. 1981). The structure among sections was compared by a Chi-Square test ($X^2$) using contingency tables. The values for the Class I group were not considered in the analysis to avoid bias in the results, because the high death indexes the neonate individuals of this class have.

Meanwhile the samplings, young caiman captures were performed in different sections of the Cojedes river. The captures were made by hand or using metallic laces.

**Releasing program evaluation and performed captures**

In order to evaluate the reintroduction program of the year 2005, the study area's freed individual rate of residence was calculated, obtaining percentages for the number of freed animals captured from the total number of freed animals of the study area. For this purpose young animals no longer than 1,5 m of total length were captured.

For each captured animal the following data was registered: total length (LT) from the tip of the snout to the end of the tail, body length (LC) from the tip of the snout to the back part of the cloaca, head length (Lcab), snout length (Lhoc) from the tip of the nose to the front border of the eye's orbit, tail width (GC), the maximal width of the tail just after the cloaca, and weight (P) in grams for individuals less than 10 kg. The measurements of LT and LC were made with metric tape and a 5 millimetres precision; the Lcab and Lhoc measurements were made with a Vernier and a 1 millimeter precision. The weight was measured with a 5 g precision in individuals less than 1 kg, 50 g precision for individuals between 1 and 5 kg, and 100 g precision for individuals above 5 kg, with precision pesolas of 1, 5 and 10 kg.

In addition sex and scaly-related data was collected from the captured animals; disposition of the nape, post – occipital and dorsal scales, number of scales in the simple caudal crest and double caudal crest, and ventral scales. Besides this the geographical coordinates, habitat and animal disposition were recorded.

Each captured individual was carefully checked to detect wounds, scars or mutilations. The presence of marks of *Paratrichosoma*, a parasite that makes holes in the skin (King y Brazaitis 1971, Ashford y Muller 1978), and ecto-parasites (leeches), was in the same way registered.

The relative fatness of the captured individuals was established (physical condition measurement), condition indexes developed for Seijas (1998) for Cojedes river caimans were used, where length measurements are related with the weight of the animals. The length measurements that best fits for this caiman's condition index is the snout length (Lhoc) (Seijas 1998). The formula for calculating the condition index (IC) is:

$$IC = 441,3 * P * Lhoc^{-2,982}$$

According with the formula, the individuals with an IC below one are considered relatively thin, and those that have an IC above one are considered relatively fat, in addition the IC values obtained were compared with the ones found by Seijas (1998) in the Cojedes river, assuming that the values for the caimans would be around 1.

The recaptured young caiman growth rate was calculated. Because many of the individuals had part of the tail missing, the growth rate was calculated with the LT and LC (Seijas 1998). Only the data referring to recaptures of more than 60 days were used to calculate the rate of growth.

By the other hand, the growth of the captured caimans was compared with earlier data of the Cojedes river of the past years, by a model developed by Seijas (1998) starting with the

equation of the growth model of Von Bertalanffy, which represents the maximal grow of a species in a given time (t). In order to apply the model, the body length (LC) of the animals was used, in this way the bias from the animals that lose part of the tail was eliminated.

$$LC = 1{,}136 * (1 - 0{,}8768 * e^{-0{,}1407 * t})$$

In addition, as we have the reintroduced caiman freeing locations, the dispersal of the individuals was also analyzed with the Geographic Information System.

## RESULTS

Since December 2005 till May 2006, 25 nocturnal samplings were performed in the five defined sections of the Cojedes river, monthly repetitions were accomplished for most of the sections, except for Caño Amarillo and South Cojedes in which only one survey was possible. The results obtained during December were not included in the analysis due to the level of water of the river was still too high, therefore, the crocodilian populations would still remain dispersed because of the flood dynamics of the river and the adjacent savannah.

The sections with more number of surveys and therefore the most representative ones are La Batea – Confluencia and Merecure – Caño Amarillo.

### Abundance

The lowest abundance indexes (ind/km) of caimans, was found in the South Cojedes and Caño Amarillo sections with values of 0,35 and 0,66 ind/km, in the months of April and May respectively, is important to notice that there was lower sampling effort in these sections compared with sections with more repetitions. Meanwhile the higher indexes were reported in La Batea – Confluencia with values of 5 ind/km for February and 4,8 ind/km for May; in Merecure – Caño Amarillo 4 ind/km for February, and 3,9 and 3,6 ind/km for March and May respectively. (Table 1)

**Table 1.** Caiman abundance in different sections of the Cojedes river. The values are expressed in individuals by kilometer of river ridden (ind/km). CA, Caño Amarillo, CS, Cojedes Sur, LBC, La Batea-Confluencia, MCA, Merecure-Caño Amarillo and Sarare.

| FECHA | CA | CS | LBC | MCA | S |
|---|---|---|---|---|---|
| 17/01/06 | --- | --- | 2,6 | 2,4 | --- |
| 18/01/06 | --- | --- | --- | 2,3 | 0,9 |
| 01/02/06 | --- | --- | 5 | 2,3 | --- |
| 02/02/06 | --- | --- | --- | 3,4 | 2,4 |
| 20/02/06 | --- | --- | 3,15 | 4 | --- |
| 21/02/06 | --- | --- | --- | 3 | --- |
| 28/03/06 | --- | --- | 4,8 | 3,9 | --- |
| 29/03/06 | --- | --- | --- | 3,7 | 2,7 |
| 18/04/06 | --- | 0,35 | --- | --- | --- |
| 19/04/06 | --- | --- | 3,3 | 2,9 | --- |
| 02/05/06 | --- | --- | --- | 3,2 | --- |
| 03/05/06 | --- | --- | --- | 3,6 | --- |
| 16/05/06 | --- | --- | 3 | 2,3 | --- |
| 17/05/06 | 0,66 | --- | --- | --- | --- |

A tendency was observed related to the increase the number of observed individuals as the dry season get stronger, this by the analysis of the temporal change of the fraction individuals by kilometer of river during the sampled months (including young, sub-adults and adult caimans), even though the values remain relatively constant through all the samples. The resulting correlation between the fraction of observed individuals and the days passed since January 17th (determined as the first day of sampling) resulted positive but not statically significant (R = 0,317, P = 0,173). It must be noticed that for this analysis the values of abundance for May were excluded, because the rain season had already started.

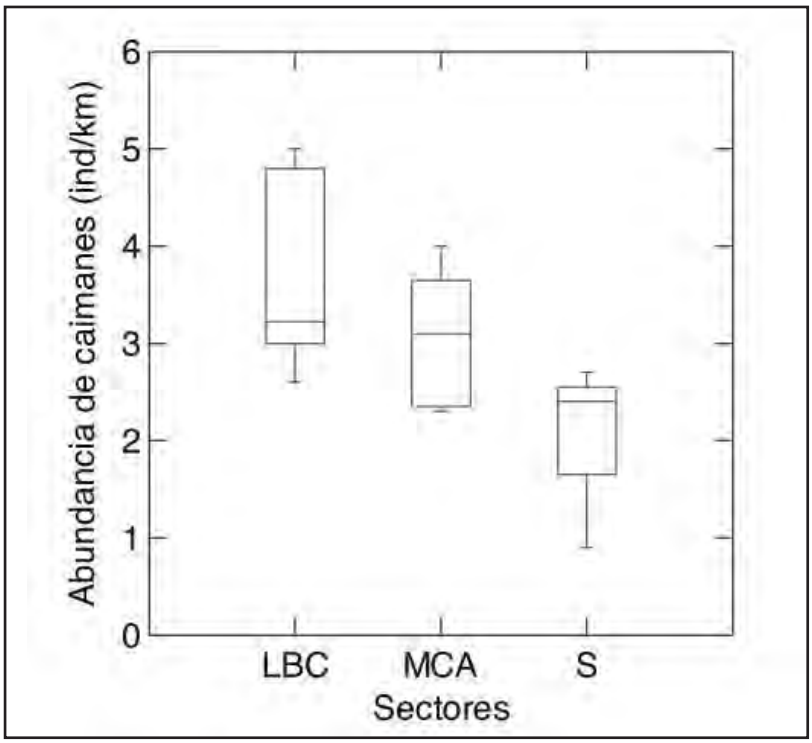



**Figure 3.** Caiman abundance values variation among the more representative sampled sections of the Cojedes river: LBC = La Batea - Confluencia, MCA = Merecure-Caño Amarillo and S = Sarare.

A Kruskal-Wallis test was made to compare the abundance values among the different sections of the river and no statically significant differences were found (H = 4.462, P =0,107), South Cojedes and Caño Amarillo were excluded from the analysis due to the small size of the sample (Figure 3).

In the study area a caiman population was estimated with a minimal size of 78 individuals without counting the neonate animals, using the average values of the abundance index for the most representative sectors and with the surveys in the South Cojedes and Caño Amarillo sections (Table 2). This number is based in abundance indexes below the maximal abundance indexes obtained in the different sections of the river. If we use the maximal values, the population estimation would be 100 individuals, that means a 22% higher population. It's important to notice that in the following paragraphs the minimal population estimate will be readjusted with the help of the capture-recapture and individuals' dispersal analysis.

**Table 2.** Minimal size of the caiman population based on the abundance indexes in the different sections of the Cojedes river.

| Section | Length (km) | Number of samples | Density (ind/km) | Estimated number | Maximal density (ind/km) | Estimated number |
|---|---|---|---|---|---|---|
| Caño Amarillo | 7,5 | 1 | 0,66 | 5 | 0,66 | 5 |
| Cojedes Sur | 14 | 1 | 0,35 | 5 | 0,35 | 5 |
| La Batea – Confluencia | 5,8 | 6 | 3,6 | 21 | 5 | 29 |
| Merecure – Caño Amarillo | 12,3 | 12 | 3,08 | 38 | 4 | 49 |
| Sarare | 4,4 | 3 | 2 | 9 | 2,7 | 12 |
| **Totals** | **44** | **23** | | **78** | | **100** |

The calculations were made for the Cojedes river from La Batea, through the mouth of Caño Amarillo to the South Cojedes, and in effluents as a section of Caño Amarillo and a section of the Sarare river.

Nevertheless, if we use the same criteria used by Ayarzagüeña(1987), Seijas (1998) and Seijas y Chávez (2000), for estimating the caiman populations in all the Cojedes river system we would also need to consider: 1) Caño de Agua Norte, 2) North Cojedes, 3) Caño Culebra, and 4) other parts of the sections of the study area not sampled. Besides -that, in this study we will only estimate the size of the caiman population in the sampled sections, because the characteristics of the not sampled Cojedes river system sections may be variable and thus the caiman abundance values.

Besides all, some comparisons were made related to the abundances found in the different sections of the Cojedes river of this study, and the previous studies performed with the Cojedes river caimans. (Ayarzagüena 1987, Seijas 1998 y Seijas y Chávez 2000, Chávez 2000).

The indexes of abundance were compared for the 1991-1996 seasons (Seijas 1998) and 1997-1999 (Chávez 2000), and the present study, by a Kruskal – Wallis test. The results show slight differences between the numbers, but these are not statically significant differences (H = 8, P = 0,127). (Table 3).

**Table 3.** Caiman abundance indexes through the years for the sampled sections of the Cojedes river.

| Section | Length in km | Abundance Ind/km (1991-1996) | Estimated number (*) | Abundance Ind/km (1997-1999) | Estimated number (*) | Abundance Ind/km 2006 | Estimated number 2006 |
|---|---|---|---|---|---|---|---|
| La Batea– Confluencia | 5,8 | 7,26 | 42,10 | 8 | 46,4 | 5 | 29 |
| Merecure– Caño Amarillo | 13,1 | 4,88* | 64 | 6 | 78,6 | 4 | 53 |
| **Total** | **19** | | **106** | | **125** | | **82** |

* The estimated number for each sector comes from the product of the abundance found for Seijas (1998) y Chávez (2000) and the length of the sections determined in the study.

**Population Structure**

In the most representative sampled sections, the caiman populations were integrated by a major proportion of adult individuals Class V: in the La Batea-Confluencia section there were a predominant presence of Class V individuals (52%), followed by Classes II, IV and III (20, 16, and 12 respectively); In the Merecure-Caño Amarillo section we found the same thing, a major percentage of individuals Class V (39,68%) followed by Class II (31,74%) and in a less proportion Classes III and IV (17,46 % and 11,11 % respectively).

In the Sarare section the larger proportion observed were Class II individuals, with 60 %, followed by Classes III and V individuals (27,27% each one of them). Finally in the section with lesser samples, Caño Amarillo, the major proportion of individuals belongs to Class V animals (60%) followed by 20% of each one of the Classes II and III. It must be noticed that South Cojedes was not considered for this analysis because there were not enough observations to infer the population structure in that section (Figure 4).

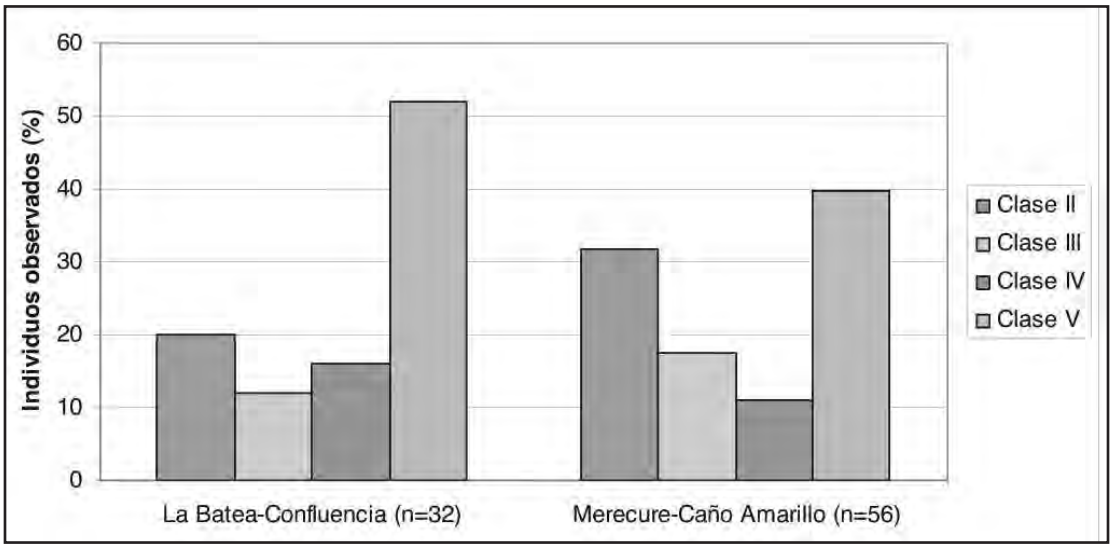



**Figure 4.** Caiman population structure for the section with more number of samples of the Cojedes river. The size classes are related to the total body length (LT).

The different size structures were compared through contingency tables, using the maximal observed values for each one the size classes in each one of the sampled sections, thus the obtained differences were not statically significant ($X^2$ = 12,454, P = 0,410). In addition only the two most representative sections, for the number of samples, were considered for the structures comparison, likewise no statically significant differences were found ($X^2$ = 2,176 P = 0,537).

As in the abundance, the caiman's size structure found in this study was compared with the population structure of previous studies. For this reason the two most sampled and representative sections of the study, La Batea-Confluencia and Merecure-Caño Amarillo, were compared.

Comparing the surveys performed by Seijas (1998) during 1996 and 1997, and the present study (2006), in La Batea-Merecure section, we notice that the population structure suffered variations, although these are not statically significant ($X^2$ = 15.656, P = 0,001). None the less, its important to point that in this lapse of time La Batea-Merecure section increase the proportion of sub-adult and adult individuals (Class IV and V) and the number of young Class II individuals reduced, by the other hand the proportion of Class III individuals remain relatively constant. In the Merecure–Caño Amarillo section, in this lapse of time the number of adult Class V individuals increase significantly, and the young Class III and II individuals reduce to a lower proportion (Figure 5 and 6).

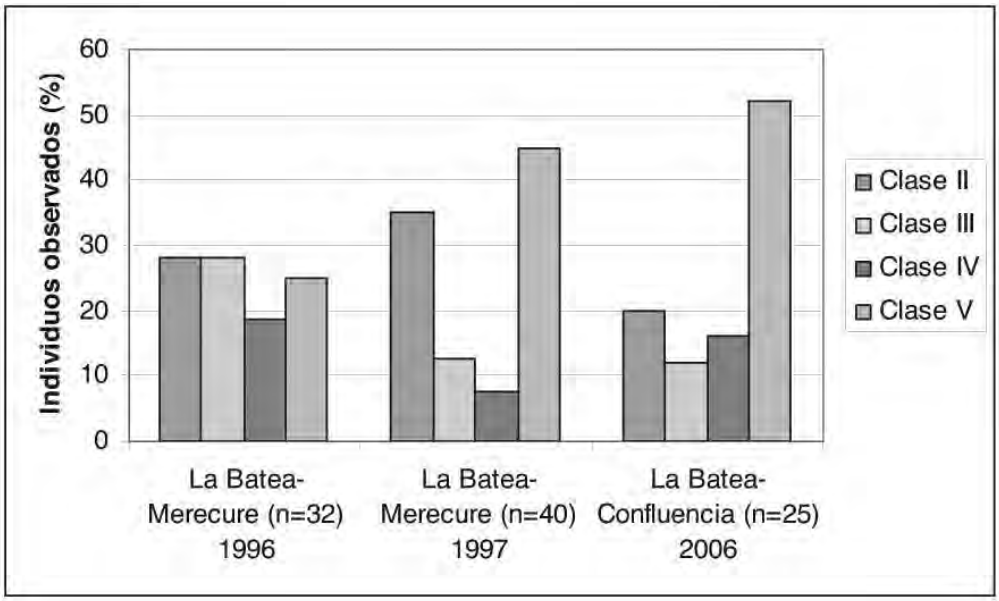



**Figure 5.** Caiman population structure variation in La Batea-Merecure section, since the surveys performed in 1996-1997 (Seijas 1998, Seijas y Chávez 2000) till the samples of the present study 2006.

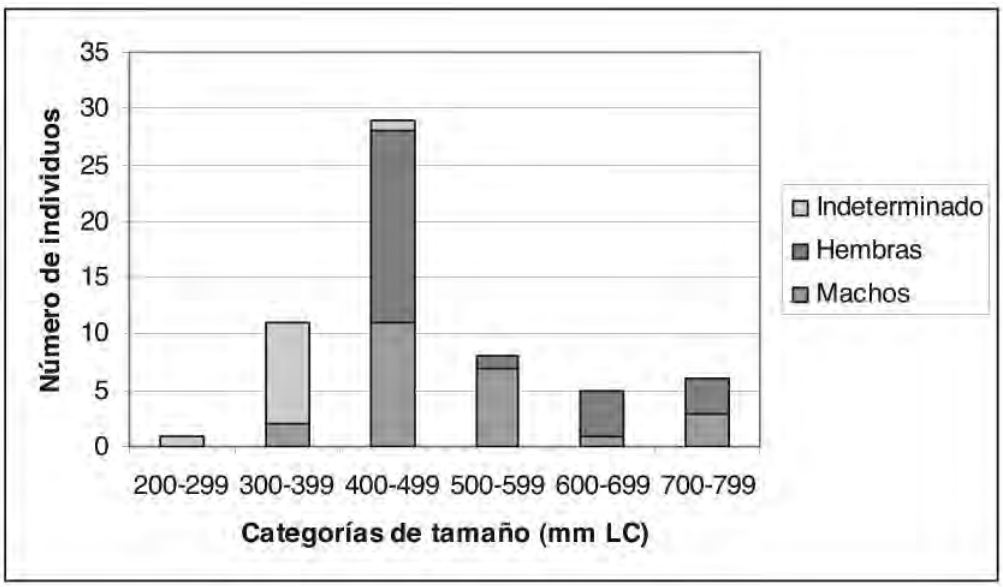



**Figure 6.** Caiman population structure variation in the Merecure-Caño Amarillo section, since the surverys performed in 1997 (Seijas 1998, Seijas y Chávez 2000) till the samples of the present study 2006.

**Cojedes river releasing program evaluation**

Since January to May 2006 81 young caiman were captured in the Cojedes river. There were captures in all the sampled sections, although, most of the samples (86,5%) came from the Merecure-Caño Amarillo section (75,3%) and La Batea-Confluencia section (11,11%) (Figure 7).

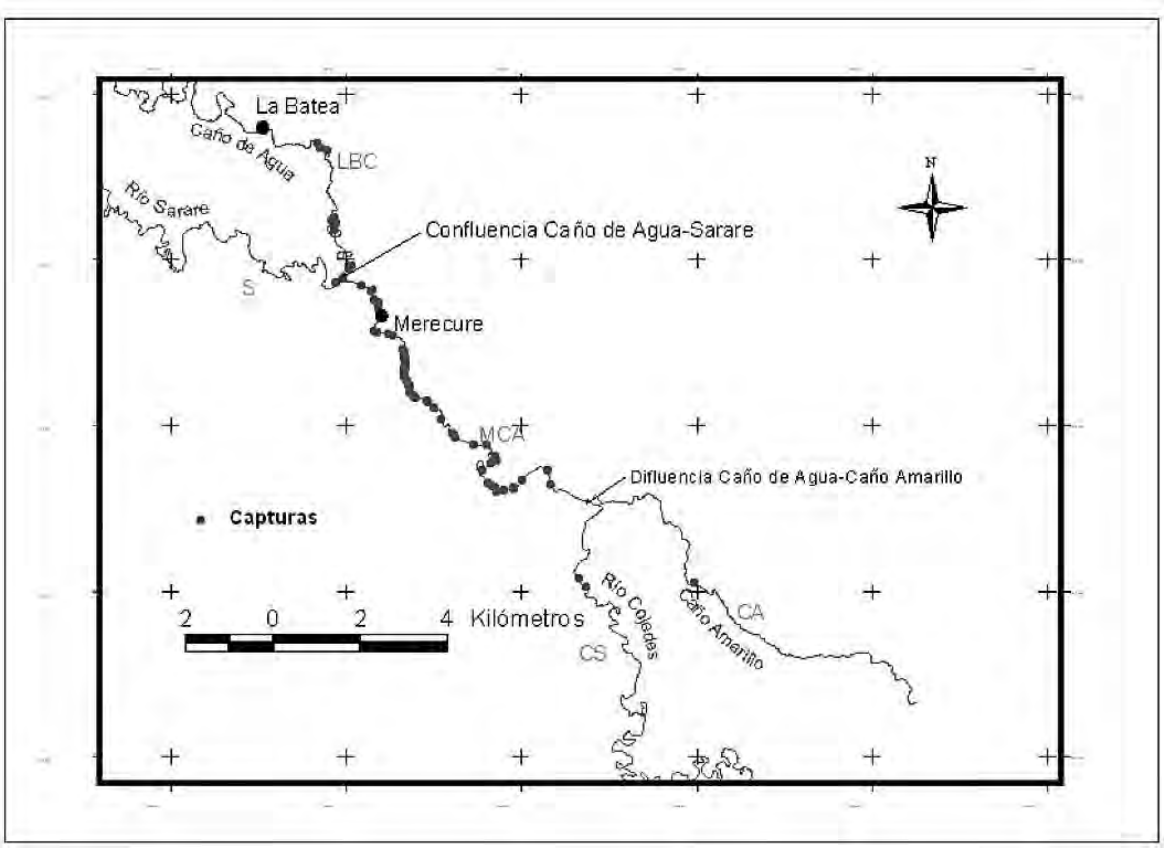



**Figure 7.** Detail of the study area showing the reference points that limit the river sections were most of the caimans were captured. LBC, La Batea-Confluencia, MCA, Merecure-Caño Amarillo, S, Sarare, CS, South Cojedes and CA, Caño Amarillo.

From the 81 captures, 61 individuals were captured at least one time, the rest of the animals were recaptured and in many cases a single animal was recaptured more than once. From the 61 different captures during the nocturnal surveys, 90% belong to wild individuals (55 animals), meanwhile the other 10% was composed by marked animals, that means wild animals recaptured from different earlier studies. Besides, only three of these captured animals (5%) belong to the releasing of 2005, and the other 3 individuals presented metallic plates of animals of other studies not related with 2005 releasing.

With base in these results the rate of residence of the 2005 released individuals was calculated, for the study area is 1,5% after one year of the release (in total 207 animals were released).

Most of the captured individuals (wild and released), 52 individuals were in sizes between 600 and 1200 mm of LT, and between 300 and 800 mm of LC. Seven individuals belong to Class III, and two to Class I. The data about the captured animals are presented in the annexes.

The captured individuals were grouped in size categories, with intervals of 100 mm of LC (Seijas 1998) (Fig. 8). From all the captured individuals, without considering Class I individuals, we found 24 male and 25 female, giving us a relationship of sexes of 1:1, we couldn't determine the sex of the other animals.

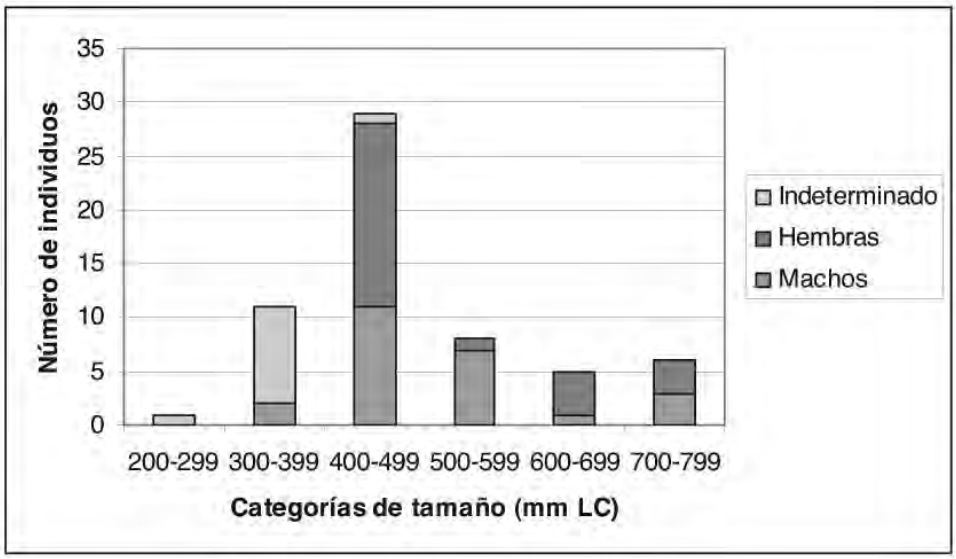



**Figure 8.** Size and sex of the Orinoco caimans of the Cojedes river.

Applying the model developed for Seijas (1998) for calculating the condition index of the captured caimans of the Cojedes river, we get values from 0,52 to 1,43, with an average value of 0,83 (± 0,15).. The correlation of the condition indexes and the different measurements found in the captures (LT, LC, Lcab, Lhoc and GC), give us in all cases positive highly significant correlations (Table 4). This situation suggest that the animals as bigger they get the fatter they are.

**Table 4.** Results of the correlation between the condition index and the measurements obtained in the caiman captures (LT, LC, Lcab, Lhoc, GC, weight).

| Variables | R of Pearson | Probability |
|---|---|---|
| Total length | 0,390 | 0,054 |
| Body length | 0,484 | 0,000 |
| Head length | 0,362 | 0,005 |
| Snout length | 0,430 | 0,001 |
| Tail width | 0,578 | 0,000 |
| Weight | 0,584 | 0,000 |

The condition index of the released individuals recaptured in the present study was between 0,67 and 0,97 and it was directly related with the size of the animal, individuals of higher size shown indexes closer to 1, meanwhile in the smallest ones the index was lower. The condition index of the captured animals in the different sections of the Cojedes river was relatively constant and no significant differences were found (H = 2,174, P = 0,825). Although, the higher condition indexes were found in Sarare and South Cojedes, this may be caused by the small size of the sample in these sections (Table 5).

**Table 5.** Caiman condition indexes (IC) from the different sections of the Cojedes river.

| Sections | Number of individuals | Average condition index | Standar deviation |
|---|---|---|---|
| Caño Amarillo | 1 | 0,808 | --- |
| South Cojedes | 2 | 0,864 | 0,19 |
| La Batea-Confluencia | 8 | 0,852 | 0,16 |
| Merecure-Caño Amarillo | 47 | 0,832 | 0,15 |
| Sarare | 2 | 0,907 | 0,11 |

A correlation was also calculated, between the condition indexes of all the captured animals and the days that passed since January 17th (the first day of sampling), as a indirect measurement of the effect of the dry season. A negative correlation was found (R = -0,089), but not significant (P = 0,497). We couldn't find neither differences between males and females (H = 0,192, P = 0,909). In addition the incidence of wounds, mutilations and parasites was analyzed. Forty of the captured animals show wounds (66,6%), the individuals with more incidence of wounds were between 300 and 500 mm of LC (Figure 9).

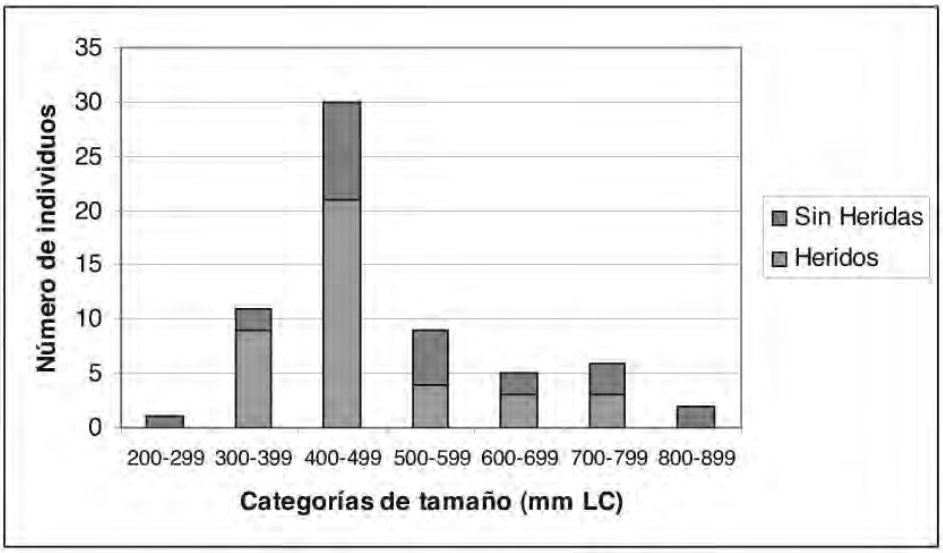



**Figure 9.** Frequency of animals with or without wounds in the captured samples of the Cojedes river.

The individuals with wounds or mutilations weren't distributed evenly between the samples of different sections, and were related with the number of captures performed in each section. Most of the wounded caimans (47,54%) came from Merecure-Caño Amarillo. All the captured animals from all the river sections show a high incidence of wounds (Table 6). The differences between sections were not significant ($X^2$= 5,135, P = 0,4).

**Table 6.** Proportion of wounded and healthy captured caimans in the different sections of the Cojedes river.

| | Captured caimans | | | | |
|---|---|---|---|---|---|
| **Sections** | **Healthy** | **Proportion (%)** | **Wounded mutilated** | **Proportion (%)** | **Total** |
| Caño Amarillo | 1 | 100 | 0 | 0 | 1 |
| South Cojedes | 0 | 0 | 3 | 100 | 3 |
| La Batea-Confluencia | 22 | 5 | 6 | 75 | 8 |
| Merecure-Caño Amarillo | 17 | 35,5 | 30 | 64,5 | 47 |
| Sarare | 0 | 0 | 2 | 100 | 2 |
| **Total** | **20** | | **41** | | **61** |

Among the wounded animals, nine (20%) had a limb missing; two (4,44%) had the jaw broken or out of place, 15 (33,3%) had bites and/or scars in the body, and 14 (31,1%) had lost some fingers. The 44,4% of the individuals presented more than one type of wound or mutilation. The most frequent wound, although, was represented for the lost of one part of the tail (44.4%). This fact is easily appreciated when the number of rows of the simple caudal crest (CCS) scales are counted, as shown in Figure 10. Two of the captured individuals also had parasites, one of them *Paratrichosoma* in the ventral scales and the second one leeches in the mouth. Two individuals were captured outside the water, one of them completely healthy (released), and the second one almost dead with very severe wounds in the neck, it possibly get out of the water to die. The average condition index of the recently wounded caimans was 0,7968 ± 0,1664 and some of them looked quite thin, this could be also produced for the presence of important mutilations that can affect their weight. Although, no statistical significant differences were found between healthy and mutilated animals. (Mann-Whitney Test = U = 391,5, P = 0,894).

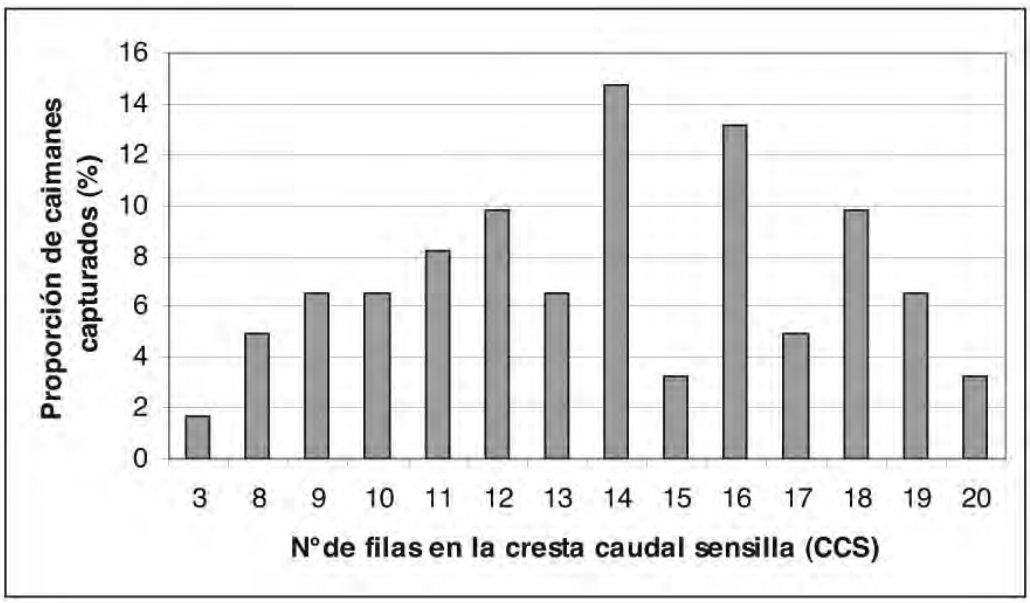



**Figure 10.** Percentage of captured caimans in the Cojedes river according with the number of scales rows in the simple caudal crest.

The incidence of wounds in the released caimans coming from the reintroduction program was 50%, three of them did not show wounds at the moment of the capture and were complete,

the other three show different types of wounds, one had parasites in the mouth (leeches), another one had a piece of the tail missing and the last one was about to lose the right hand, that was inflamed and losing parts of the skin.

The growth model developed by Seijas (1998) for the caimans of the Cojedes river, was applied only in four wild animals previously captured during this study, three that came from the releasing, and to three marked individuals from earlier studies. Although 15 individuals were captured more than once, most of them were recaptured in periods of time shorter than the required to perform the model of growth rate, only the animals that were recaptured with a lapse of time higher than 60 days were considered for the analysis.

The body length values (LC) obtained in the equation were compared with the values observed in the field through the Wilcoxon sings test; where significant differences were found (Z = 2,701, P = 0,007). In most of the cases the observed sizes were greater than the expected ones, this may suggest that the captured animals of the study show a greater growth than the animals captured by Seijas in 1998 in the same river (Table 7).

**Table 7.** LC values expected and observed in the captured and recaptured caimans, to which the growth rate was calculated.

| | LC Values | |
|---|---|---|
| **Individual** | **Expected** | **Observed** |
| ZOO-213140 | 252,31 | 341 |
| UZ-1648, 234408 | 265,85 | 400 |
| UZ-1687, ZOO213174 | 270,73 | 414 |
| UZ-1613 | 181,19 | 450 |
| UZ-1694 | 174,42 | 740 |
| UZ-1692 | 300,62 | 612 |
| 234353-234354 | 490,26 | 490 |
| UZ1171-UZ1172 | 375,72 | 425 |
| UZ1669 | 273,12 | 510 |
| UZ1547-UZ1548 | 385,18 | 432 |

The annual growth of the recaptured individuals in the Cojedes river show values between 5,2 cm/year and 36,46 cm/year, with a media value of 19,83 cm/year, and values between 4,33 mm/month and 30,38 mm/month, with a media value of 16,52 mm/month. These values show a growth tendency similar the ones reported for Caño Guaritico, and Capanaparo river, where Chávez (2002) and Llobet (2002), presented growth values between 19,4 and 27,1 cm/year, and values between 15,18 mm/month and 17,25 mm/month respectively.

The annual growth in the recaptured individuals of the Cojedes river show values between 5,44 cm/year and 19,42 cm/year, and values between 4,53 mm/month and 16,19 mm/month, using the body length measurement (Table 8). In the same way these values can reflect a similar growth tendency than the one shown by Seijas (1998), with values between 1,05 and 14,2 mm/month.

It's important to notice that in the Cojedes river as long as the captured animals become bigger and in consequence older, they show higher annual growth rates. To make sure of this, the annual growth rates and the lapse of time between the capture and recapture events were correlated, as a measurement of the survival in the environment. Also the annual growth rates and the total length (LT) of the last capture event were correlated. In both cases positive sadistically significant correlations were found: LT vs. Growth rate (R = 0,725, P = 0,018) and Lapse of time vs. Growth rate (R = 0,641, P = 0,046). This situation points that, possibly after a year of survival, the animals show a better adaptability to the environment.

With the existing data base of the Crocodilian Specialist Group of Venezuela, the releasing points of the released caimans of the Cojedes river were located. Then the dispersion distance of the three captured animals that came from those releasing processes was calculated, two of them were captured more than once. The individual ZOO 213140 released on May 21st 2005 moved about 2,3 km from the releasing point Via Caño Amarillo until the capture place, this animal was captured two more times, from about 250 m away from the first capturing point, in both occasions. The individuals UZ-1648-234408 and UZ-1646-ZOO 213174, released on May 3rd 2005, they moved 1,1 km and 3,7 km respectively from the releasing point Confluencia Sarare – Caño de Agua to the capture location, the individual UZ-1648-234408 was captured two more times, moving about 100 m from the first capturing point. (Figure 11).

**Table 8.** Body length, annual and monthly growth in the recaptured individuals of the Cojedes river. The initial LC of the wild animals is from the first capture event, and for the released animals the size when they were released.

| | | | Growth (LC) | | |
|---|---|---|---|---|---|
| **Individual** | **Initial LC** | **Final LC** | **Lapse of time(days)** | **cm/year** | **Mm/month** |
| ZOO-213140* | 341 | 390 | 311 | 5,44 | 4,53 |
| UZ-1648, 234408* | 400 | 455 | 350 | 5,73 | 4,77 |
| UZ-1687, ZOO213174* | 414 | 515 | 364 | 10,1 | 8,41 |
| UZ-1613 | 450 | 470 | 106 | 6,67 | 5,55 |
| UZ-1694 | 740 | 740 | 93 | 0 | 0 |
| UZ-1692 | 612 | 720 | 456 | 8,64 | 7,2 |
| 234353-234354** | 490 | 860 | 1126 | 12,01 | 10,01 |
| UZ1171-UZ1172** | 425 | 798 | 700 | 19,42 | 16,19 |
| UZ1669 | 510 | 565 | 372 | 5,39 | 4,49 |
| UZ1547-UZ1548** | 432 | 798 | 733 | 18,22 | 15,19 |

*Caimans from the reintroduction program

** Caimans marked in previous studies.

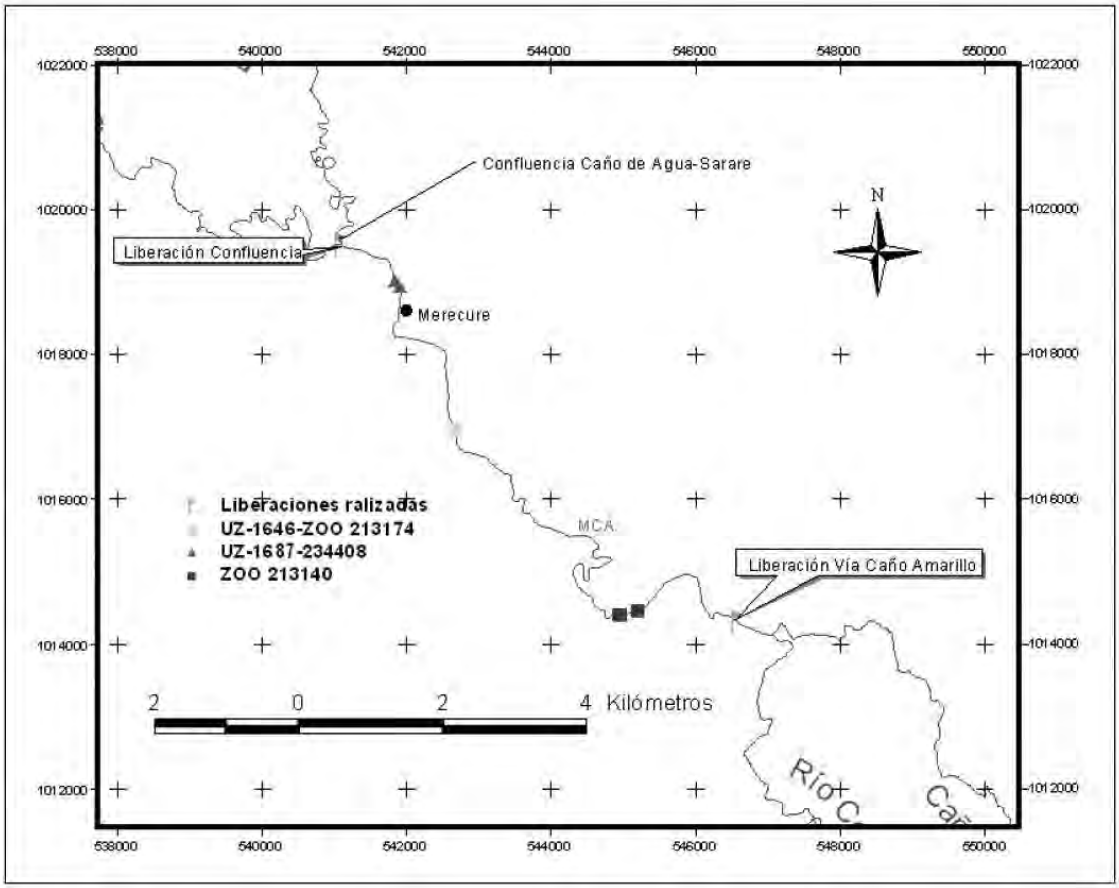



**Figure 11.** Dispersal of the released animals in Cojedes river in the year 2005 (Via Caño Amarillo 177 released individuals and Confluencia Caño de Agua-Sarare river 30 individuals released) and captured in the present study in 2006.

In addition, the dispersal distance of the wild captured and recaptured animals of the present study was calculated, taking as the initial point of the individual the first capture, two wild animals were recaptured more than once. It was observed that most of the individuals had a relatively small dispersal from the first point of capture to the recapture point (in a lapse of time of three months), two individuals moved between 1 and 1,2 km (UZ-1694 y UZ-1613), five of them moved between 15 to 100 m, and the individual UZ-1601 moved 210 m between captures, nevertheless, in a fifth capture the animal was in a very close position to its initial point. Besides that the dispersal distance of the marked individuals from previous studies was calculated, and from an individual that was captured in the present study and that was recaptured in the year 2007 by other study in the area, two of the three individuals marked in previous studies show a dispersal distance of 1,6 and 1,5 km (234353-234354, UZ-1547-UZ-1548) from its first capture to the recapture point (in a lapse of time of three years one month and two years respectively), the third individual moved only 9 m (UZ-1171-UZ-1172) between capture and recapture in a two years lapse. Finally the individual UZ-1669 marked in the present study moved 166 m in a lapse of one year (Figure 12).

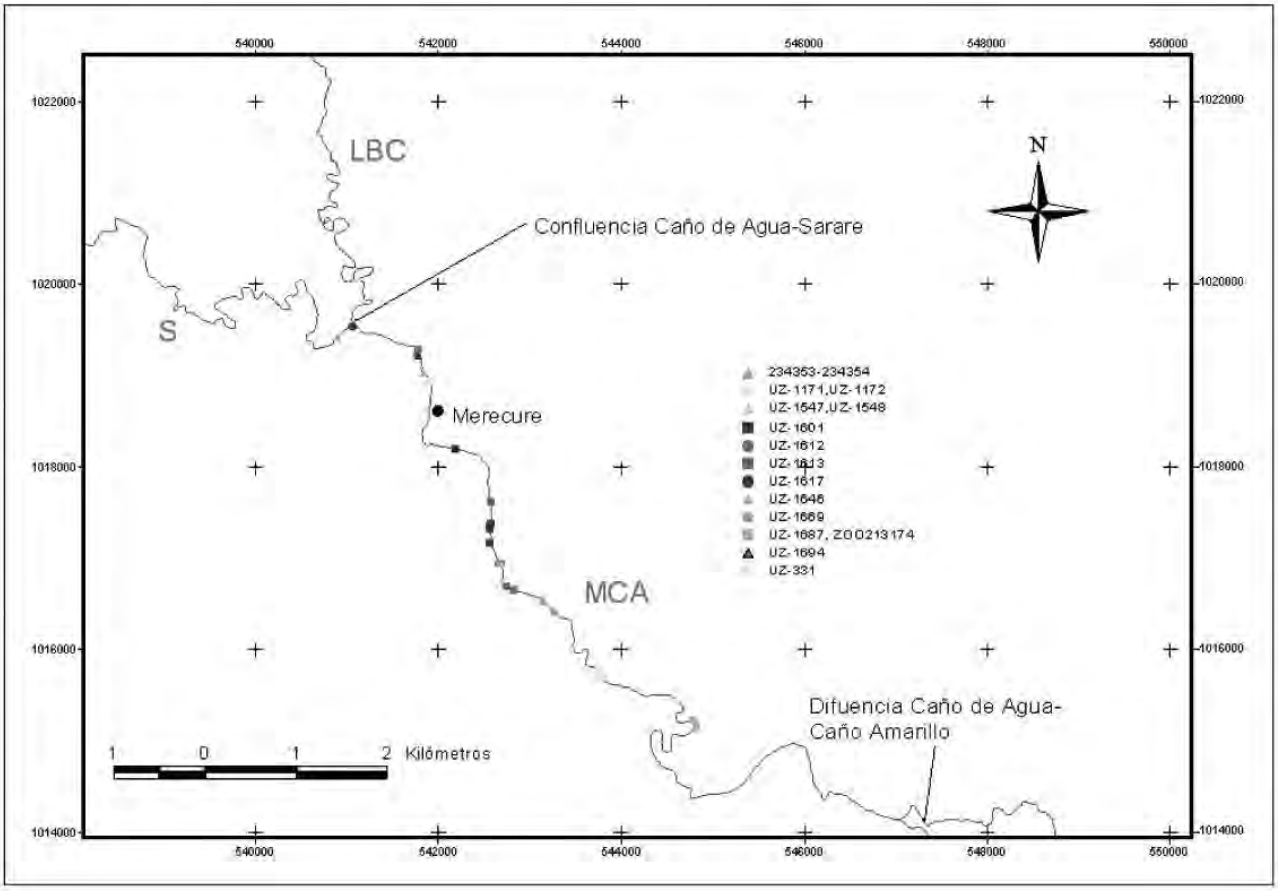



**Figure 12.** Dispersal of the wild individuals captured – recaptured in the present study.

## DISCUSSION

### Abundance

In previous studies performed in previous years, is pointed that the best populations of the Orinoco's caiman is found in the Cojedes river (Godshalk 1978, 1982, Ayarzagüena 1987). Seijas (1998) presented results of the population status of the Orinoco's caiman since 1991 to 1997, in the Cojedes river system, the river sections that can be properly compared with this study are La Batea-Confluencia and Merecure-Caño Amarillo. The number of animals estimated by Seijas (1998) in all the Cojedes river system was of 540 individuals. If we only take the two highly representative sections of this study, the estimated number would be 106 individuals, being the population found in the previous study (1991-1997) higher in a 23% than in the present one. Chavez (2000) presented population status results of the Cojedes river from the years 1997-1999, and found that in a general way the population size of the Cojedes rivers tends to be relatively stable between these years; 287 individuals (without neonates) in almost 50 km surveyed in year 1997; 228 individuals in 46,7 km surveyed in year 1998; and 289 individuals in 58,8 km surveyed in year 1999. In the same way, if we only compare the two similar sections of this study, in the 19 total km surveyed, the number estimated by Chávez (2000) would be of 125 individuals, that means that the population of the lapse 1997-1999 is higher in a 34% than the one found in the present study (82 individuals). It seems that the actual caiman population estimate in the sampled sections is below the previous year estimations.

An important factor to be considered is the fact that in 2005, 207 individuals of *C. intermedius* were released in the Cojedes river. And thus it was expected to find higher population indexes than those found in previous years, at least in the areas close to the releasing points, but in the other hand, the population indexes of the sections closer to the releasing points were below or relatively constant to the ones found in previous studies.

The monitoring of crocodilians represents some problems, specially controlling the biological variabilities as the carefulness of the animals and the population density (Pacheco 1994, 1996). In the same way, the tendency is to underestimate the populations when the population densities are too low. Finally, the relative abundance indexes are going to underestimate the real size of the population, because part of this population will be usually remain undetected and its very hard to establish a relationship between the relative abundance index and the real density of the area (Hutton y Woolhouse 1989). In the present study we evidenced that the abundance indexes can change from one nocturnal survey to the other.

Is important to consider the methodology used for estimating the caiman populations, because there are a lot of factors that can affect the visibility of the caimans during the nocturnal counting. The water level is the most important one (Seijas 1998). The differences in the visibility of the caimans and the water levels of the river, have important implications for the population status monitoring. Seijas in 2000, points that the best period to conduct the sampling is between November to January, because during this lapse not only a larger fraction of caimans can be observed, besides, the number of babas is low, which reduces the sampling time and avoids the investigators fatigue. Nevertheless, specially for the present study (2005-2006) the water level on these moths was too high and thus a lower fraction of caimans were visible, besides the chance of navigating in more sections of the river because of the high level of the water, this water level floods the near lowlands and savannahs to the study area and thus the caimans have more space to disperse. The major caiman fraction was observed in May (advanced drought period), but the low river level make the accessibility to some sections of the river difficult.

The caiman population in the Cojedes river was not evenly distributed. There were differences among sections as in abundance as in size structure. The higher densities were detected in the La Batea-Confluencia and Merecure-Caño Amarillo sections, river sections that keep the meanders and are still surrounded by forests. La Batea – Confluencia results are below the ones estimated by Ayarzagüena (1987), the same that are relatively constant to the ones reported by Seijas y Chávez (2000). The caiman density decreased down waters Merecure – Caño de Agua, values that were also below numbers found in previous studies, these places have been progressively affected by deforestation and human presence.

The results for the Sarare, Caño Amarillo and South Cojedes, was insufficiently sampled and compared to the results of previous studies they are also below the estimates, it is strongly recommended to focus more efforts in these sections in order to have better estimates for the caiman populations.

**Population structure**

The caiman population structure didn’t show significant statistic differences among the different sections of the river, in La Batea-Confluencia, Merecure-Caño Amarillo and Caño

Amarillo sections, the caiman population was structured by a higher proportion of adult Class V individuals (52%, 39,68%, 60% respectively). In the other hand, in the Sarare and South Cojedes sections there was a higher proportion of young Class II individuals, with values of 60% and 45,45% respectively.

In 1990 Sijas, found that the population of Caño de Agua Sur (La Batea-Confluencia) was particularly composed of sub-adult and adult individuals (>1,8 m LT), and the Merecure-Caño Amarillo section show an mixed population structure, with a higher proportion of young Class II and III individuals.

In La Batea-Confluencia and Merecure-Caño Amarillo sections, through the years, besides the populations seem to be relatively constant, the size structure show an increase in the proportion of Class V adult individuals and a decrease in the Class II and III young individuals. It seems that the population structure in this place in the last 9 years (1997 – 2006), could have been influenced by: 1) a replacement process from young individuals to adults; 2) a change in the distribution and dispersal of the individuals, responding to a change in the habitat quality among the river section, because the main nesting beaches of the Cojedes river are found in Caño Amarillo Sur (La Batea – Confluencia) (Seijas 1998); and 3) by differences in the death rates among the individuals of different sizes in the different sections of the river.

As Seijas pointed in 2001, maybe the most logical answer is that the differences shown in the abundance and population structure, are produced by the human activity over the caiman populations. This situation, added to the bad perception of the local people about the caimans, can clearly and definitely affect the population structure. Another factor that can influence the population size structure is the existing relationship between the animals carefulness and the human pressure in a given area (Pacheco 1996, Llobet 2002). In the river sections far from human activities, as Caño de Agua Sur (Seijas y Chávez 2000), the big caimans tend to have a higher chance of survival and establishment, making in this way, a higher fraction of the population.

**Releasing Program Evaluation**

There was no previous studies in the Cojedes river about the population restoration program before the present study. In the rest of the country, most of the releasing processes had had a continuous tracking, except for the study performed by Muñoz and Thorbjarnarson in 2000, where eight caimans where constantly radio-telemetry tracked for eleven months for experimental purposes. Besides that other two studies with a relatively high sampling effort were performed in the Refugio de Fauna Silvestre Caño Guaritico and in El Frio ranch surroundings (Lugo 1998, Chávez 2000).

Seijas (2003) pointed that the success indicators for the releasings can be obtained from the fraction of individuals that survive or stays in the releasing area, by the body growth rates that the individuals show in their new environment, and by the eventual reproduction of the released caimans.

Inside the population restoration program, in the RFS Caño Guaritico, in survival-residence terms, Lugo (1998), using diurnal and nocturnal countings, calculated residence rates for the released individuals between 8,9 and 12,2% for the number of released individuals of previous years, Chávez (2000), using a similar methodology, calculated a residence rate of 7 and 16% for the caimans released two years eight months and six years eight months previous to his study. In the Cojedes river, in the present study, the calculated residence rate is 1,5% from 207 released caimans in May 2005. Then, the residence rate for the released individuals of 2005, one year after the releasing, is far below the values found for Lugo (1998) and Chávez (2000) in the RFS Caño Guaritico. Its necessary to point that, we are talking about residence and not about survival, because it's possible that some of the released animals may have completely left the study area, or that besides they have stayed in the area they hadn't been captured (Seijas 2003). Some of the authors agree that the survival data are based in casual observations (Neill 1971) or in a small sample size (Modha 1967, Webb y Messel 1977). The residence values found in this study could be underestimated because of the crocodilian monitoring problems earlier mentioned.

It must be noticed that the nocturnal counting, and the capture-recapture method, are less efficient that the radio-telemetry one used by Muñoz y Thorbjarnarson (2000) and because of this it may probably show underestimates (Seijas 2003)

In the other hand, it is not discarded the chance that some of the released individuals could not survive in their new environment, possibly because intra or inter- specific interactions, as, for instance, the anecdotic data collected by Seijas (com pers) in other areas of the country where releasings take place, in which the just released caimans were predated by adult babas.

The body growth as al physical condition shown by the caimans in their new environment, constitutes another way to evaluate the releasing success, and at the same time it may show the habitat quality where the releasing took place. (Seijas 2003).

The media growth rate of the released caimans in the Capanaparo river (0,079 cm LT/day) was similar to the wild animals (Muñoz y Thorbjarnarson 2000). The growth data of the released animals in a new environment reported by Lugo (1998) and Chávez (2000) in the RFS Caño Guaritico, show values between 28,1 and 32,7 cm/year, and between 19,3 and 46,5 cm/year, respectively. In the present study, the caiman growth rate in cm/year for the released animals in the Cojedes river are between 2,8 and 11,14. In any way, compared to the growth rate of the caimans of previous studies, the growth rate of the caimans in the Cojedes river seems to be too slow, and clearly far below the potential shown by the species.

In the other hand, in the Cojedes river, the growth rate of the wild individuals measured in the study, was very similar to the calculated values of the released individuals that came from the releasing. But the wild marked animas from previous studies show growth rates highly superior to the wild and released individuals with lower sizes, therefore, the growth rate of the wild caimans in 2006, was relatively similar to the monthly growth reported by Seijas (1998), for wild caimans in the same study area.

In the Cojedes river system, massive fish deaths are frequently reported, at the end of the dry season and the beginning of the rain season, caused by the high levels of pollution in

the water, this pollution comes from discharges of effluents, agrochemicals and other specific pollutants that come from cities and towns that are in course of the river (Seijas 1998, 2001, Mendoza 2003, Elorga 2007 (en prensa)). This situation may slightly affect the abundance of food resources for the caimans, but, through the years this may represent an important decrease in the availability of food resources. The direct consequences these changes can have in the availability of food resources and in the growth of the caimans, cannot be determined by the available data.

Seijas (2003) points that in most of the localities where releasing processes took place till date (Caño Matiyure del hato Cedral and Caño Guaritico-Macanillal),the growth of the released caimans has been correct, with very close values to the ones obtained in the zoocriadero, this means that these localities are the most favorable for the development of the individuals of the specie. In contrary, the same author points that some environments are of low quality for *C. intermedius*, and that the releasing of individuals in this kind of localities should be revised; as in an specific place as Tucupido dam, where the growth rates registered were extremely low for the two released caimans (-0,2 and 12,7 cm/year), numbers relatively similar to the ones found in the present study.

The values of the physical condition index calculated for the Cojedes river caimans, in the present study were relatively lower that the ones obtained by Seijas (1998) and by Llobet (2002) in the Capanaparo river.

The released individuals captured show condition indexes similar to the wild captured animals. Although, several factors might affect the relationship between the seasonal changes and the physical condition index of the animals in natural conditions (lower IC as the dry season becomes stronger), as the availability of food resources and the temperature changes between seasons (Chabreck y Joanen 1979, Hutton 1987, Jacobsen y Kushlan 1989).

About the dispersal of the released animals, Muñoz y Thorbjarnarson (2000), reported a maximal distance of dispersion of 12 km for released animals in a lapse of one year after the releasing. Llobet (2002) found an even higher distance of dispersal, of 21,86 km for an individual, in a lapse of time higher than 7 years after the releasing until the recapture. This behavior matches with the one described by Messel et al. (1981) for *Crocodylus porosus*, which he names as an animal that travel long distances. Muñoz y Thorbjarnarson (2000) also point that the Orinoco's caiman tends to move moderately upwards the river (3 to 8 km) and just a little downwards the river (1 to 2 km). In the Cojedes river the dispersion of the individuals was lower than the reported by Llobet (2002), the maximal distance that was registered for the released animals of the place was of 3,7 km downward the river in a year lapse of time from the releasing, and the lowest dispersion distance was of 1,1 km downward the river. In addition, the dispersal of wild animals captured and recaptured in this study was lower (in a lapse of time of one and three months) being the highest dispersal distance of 1,2 km and the lowest of 15 m. As it was pointed earlier many individuals were captured more than once, in most of the situations the capture points were relatively close one to the other (about 100 m). It seems, that the young caimans of the Cojedes river tends to stay in a reduced action area.

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# Long-term population data of the Orinoco crocodile (*Crocodylus intermedius*) in the Cojedes River System, Venezuela

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**ABSTRACT:** Since 1991, at least 145 nocturnal spotlight counts of Orinoco crocodiles have been conducted in several segments of the Cojedes River System (CRS), which amounts to a total of 1396 km of river surveyed. The effort, however, has not been uniformly distributed; rivers segments such as Cojedes Norte (CON), 28 times, Confluencia-Caño Amarillo (CAM), 35 times and La Batea-Confluencia (LBC), 28 times, are the ones that have been visited more frequently, precisely those easier to visit for logistic reasons. Other river sections has been surveyed fewer times. These do not allow reliable analyses of population trends. In all river sections the abundance of crocodile population have decline. Population structures have also changed, but no clear pattern is apparent. The population decline is worrisome. The Ministry of Environment should be in charge of a systematic monitoring program in accordance with universities and ONGs involved in the conservation of the species.

**RESUMEN:** Desde el año 1991 se han realizado al menos 145 conteos nocturnos de caimanes del Orinoco en el Sistema del Río Cojedes (SRC) los cuales suman un total de 1396 km de río muestreados. El esfuerzo, sin embargo, no ha estado uniformemente distribuido. Algunos segmentos de río, como Cojedes-Norte (CON), 28 veces, Confluencia-Caño Amarillo (CAM), 35 veces y La Batea-Confluencia (LBC), 28 veces, son los que han sido visitados con mayor frecuencia, que son, precisamente aquellos más fáciles de visitar por razones logísticas. Otros segmentos han sido recorridos en muy pocas oportunidades como para permitir análisis de tendencias confiables. En todos los segmentos analizados ha quedado en evidencia un declive en la abundancia poblacional. La estructura de la población también ha cambiado, pero no se hace evidente la existencia de algún patrón. La disminución de la población es preocupante. El Ministerio del Ambiente debería responsabilizarse del desarrollo de un programa de monitoreo, en acuerdo con las universidades y ONG que trabajan por la conservación de esta especie.

## INTRODUCTION

The best known population of the Orinoco crocodile (*Crocodylus intermedius*) is found in the Cojedes River System (CRS, the Cojedes River plus its branches and tributaries), in central Venezuela. Several investigations have been conducted in the CRS in the last twenty years (Ayarzagüena 1987, Seijas y Chávez 2000, Chávez 2000, Mendoza 2003, Navarro 2007, Ávila-Manjón in prep.). All these studies, however, have been somehow disconnected or have covered different river sections. In this paper we try to put together the available information regarding population numbers and size-class structure to see if there is any

evidence of trends. This analysis will be useful for the design of a monitoring program for the species.

## MATERIALS AND METHODS

We analyzed published and unpublished results of nocturnal spotlight counts of crocodiles in the CSR. All these surveys were conducted in similar way, i.e., from a 3.7 m boat powered by 10 or 15 hp outboard engines. Crocodilians sighted were approached as close as possible to allow positive identification of the species (the spectacled caiman is also present in the river) and to estimate total length (TL). In this paper we only deal with the results for *C. intermedius*. For the present analysis four size categories were recognized (Seijas and Chávez 2000):

| Category | Regarded as |
|---|---|
| Less than 1,2 m TL (excluding hatchlings) | Smalls |
| TL ≥ 1,2 y <1,8 m | Juveniles |
| TL ≥ 1,8 y <2,4 m | Sub-adults |
| TL ≥ 2,4 m | Adults |

In surveys prior to 1996, the length of river sections were calculated on the maps, or estimated according to the time taken in travelling them. After that year, the use of Global Possitioning Systems (GPS) permitted a much more accurate determination of the length of each sections. The Index of relative population abundance (PI) was expressed as number of individulas observed per kilometer (Ind/km). For river sections, PI were plotted against survey date to see if any population trend emerged.

For a particular year, population structure of crocodiles for sections with more than one survey was calculated using the maximum number of individuals in a size category, regardless of the survey in which they were observed. That was assumed to be the best estimate for that particular size class and for that year (Messel *et al*. 1981, Seijas y Chávez 2000). Comparisons of population structure among river sections were made using contingency tables.

## RESULTS AND DISCUSSION

Population abundance: Since 1991, at least 133 nocturnal spotlight counts have been conducted in several sections of the CRS (Fig. 1). The total effort amounts to 1396 km of river surveyed. The effort, however, has not been uniformly distributed. Some rivers sections have been surveyed 15 or more times, whereas others have been studied in only a few occasions (Table 1). For the analyses, surveys conducted under conditions that compromise their reliability (for example, after a heavy rain or in dates too advanced into the rainy season) were excluded for analyses.

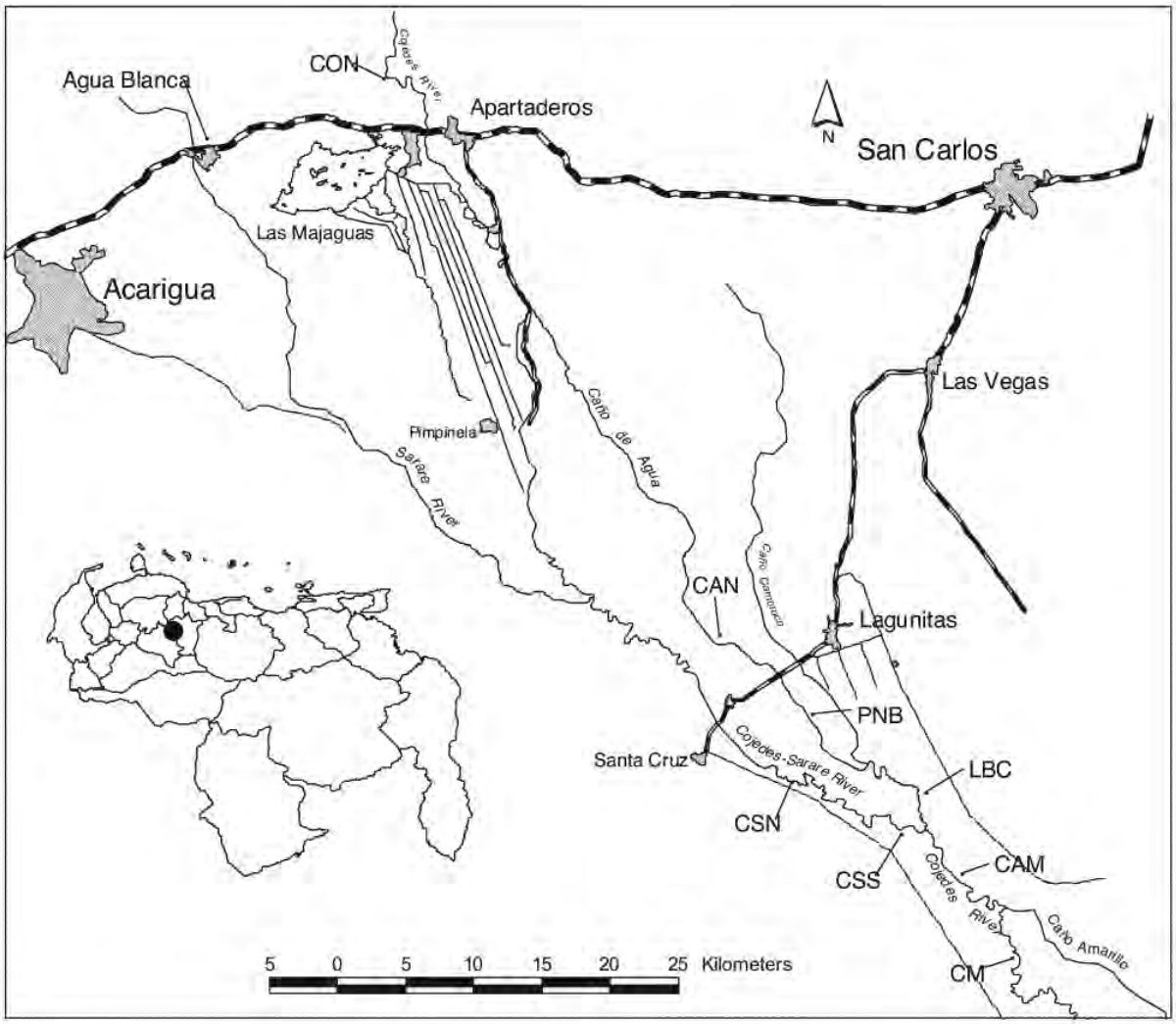



**Figure 1.** Rivers sections of the CRS: Cojedes Norte (CON), Caño de Agua Norte (CAN), Puente Nuevo-La Batea (PNB), La Batea-Confluencia (LBC), Confluencia-Caño Amarillo, Cojedes Medio (CM), Cojedes-Sarare Sur (CSS), and Cojedes Sarare Norte (CSN). Two others river sections, Cojedes Sur and La Culebra are not shown in the map.

**Table 1.** Number of times each river section has been surveyed.

| River segment | N° of surveys | First date | Last date | Elapsed time (years) |
|---|---|---|---|---|
| Cojedes Norte (CON) | 28 | 19-Jan-93 | 23-Feb-02 | 9,10 |
| Caño de Agua Norte (CAN) | 15 | 03-Jul-93 | 07-Feb-08 | 14,61 |
| CA-Puente Nuevo-La Batea (PNB) | 19 | 12-Feb-92 | 16-May-02 | 10,26 |
| CA- La Batea-Confluencia (LBC) | 28 | 14-Jun-91 | 16-May-06 | 14,93 |
| Confluencia-Caño Amarillo (CAM) | 35 | 13-Jun-91 | 28-Feb-08 | 16,71 |
| Cojedes medio (CM) | 5 | 25-Feb-98 | 18-Apr-06 | 8,15 |
| Cojedes Sur (CS) | 4 | 19-Mar-94 | 18-Apr-97 | 3,08 |
| La Culebra | 3 | 11-Apr-96 | 17-Aprr-97 | 1,02 |
| Cojedes-Sarare Norte (CSN) | 4 | 04-May-93 | 29-Apr-99 | 5,99 |
| Cojedes- Sarare Sur (CSS) | 4 | 03-May-01 | 29-Mar-06 | 4,91 |

Cojedes Norte (CON), river section located north to the road “troncal 5”, has been surveyed 28 times. A plot of population index vs date of surveys clearly indicates a sustained decline of the number of crocodiles seen (Fig. 2). From 1993 to 1997, PIs were around 3 ind/km. In the latest surveys (Mendoza 2003) PIs have not reached 1 ind/km. The low variability of surveys for 2001 and 2002 assign a high confidence to these results. This river section is the most deteriorated of all considered in this study, due to its proximity to agriculture and other human related activities (Mendoza y Seijas 2007).

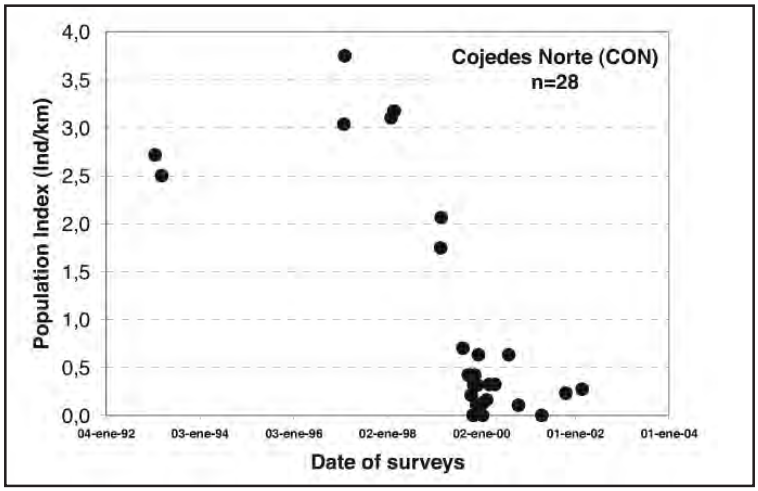



**Figure 2.** Plot of population indices (PI) vs date of surveys for Orinoco crocodiles in CON. Population abundance has clearly declined well below the levels of 3 ind/km or more shown in years 1997 and 1998.

The last survey in Caño de Agua Norte (CAN), conducted in February 2008, produced a PI of 2.6 ind/km, which is the lowest ever obtained in that river section (Fig. 3). That figure is very low if compared with PIs obtained from 1993 to 1999 which were around 4.6 ind/km. However, it is necessary to conduct new evaluations in the short run to determine if the most recent figure is truly an indicative of a declining tendency.

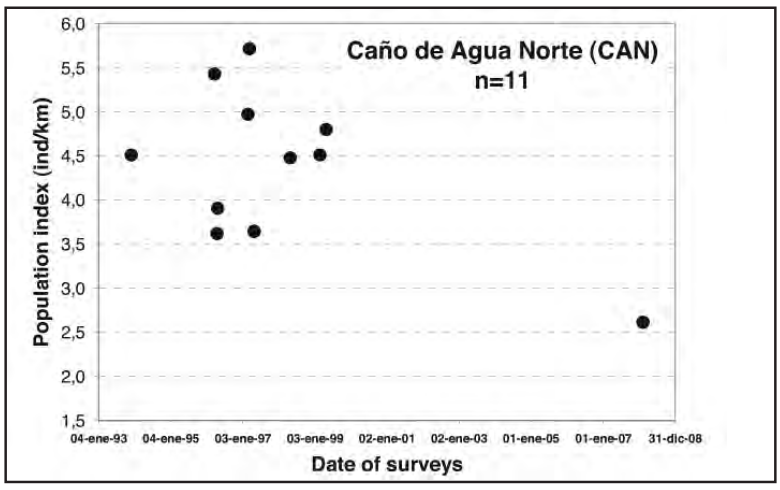



**Figure 3.** Plot of population index (PI) vs date of surveys for Orinoco crocodiles in CAN. According to the latest survey, population abundance has clearly decline from the levels above 3 ind/km shown in 1997-1998.

The decline of the Orinoco crocodile population is also apparent in La Batea-Confluencia (LBC) (Fig. 4). The PIs observed there during 1996 and 1997 varied from 3.3 to 8.7 ind/km,

with an average of 5.5 ind/km. More recently, and particularly after 2002, PIs have never reached 3 ind/km, with an average of 2.3 ind/km. The differences between the PIs for these two groups of years is significant (Wilcoxon 1 way test, $X^2$=10.6; *P*=0.001).

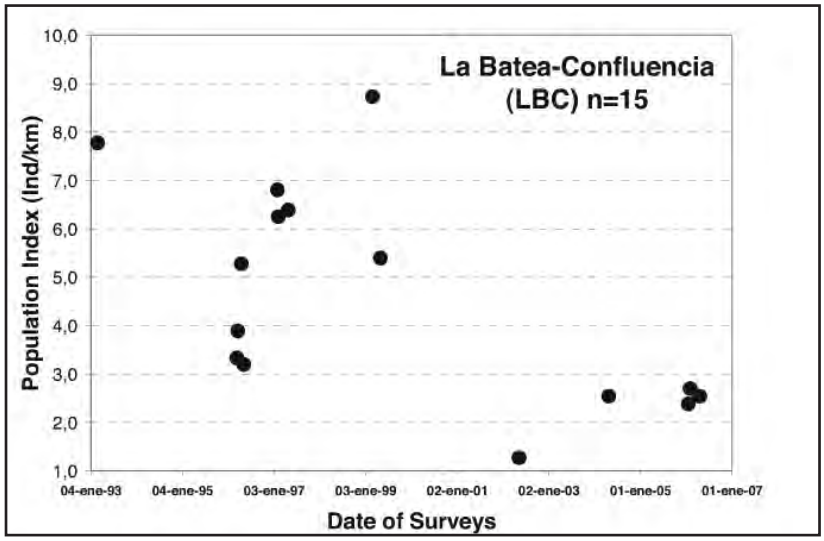



**Figure 4.** Plot of population indices (PI) vs date of surveys for Orinoco crocodiles in La Batea-Confluencia (LBC). Although data show a high dispersion, population decline is apparent.

The situation in CAM is a little bit more complex (Fig. 5). As occurred in river sections already analyzed, PIs show relatively high values from 1996 to 1999. The three surveys for 2003 and 2004 indicate a substantial decline of PIs, with values less than half the average of previous years. More recently, however, population data indicate an increase, probably due to the fact that 360 captive reared juvenile crocodiles have been released in this river section or in its proximities.

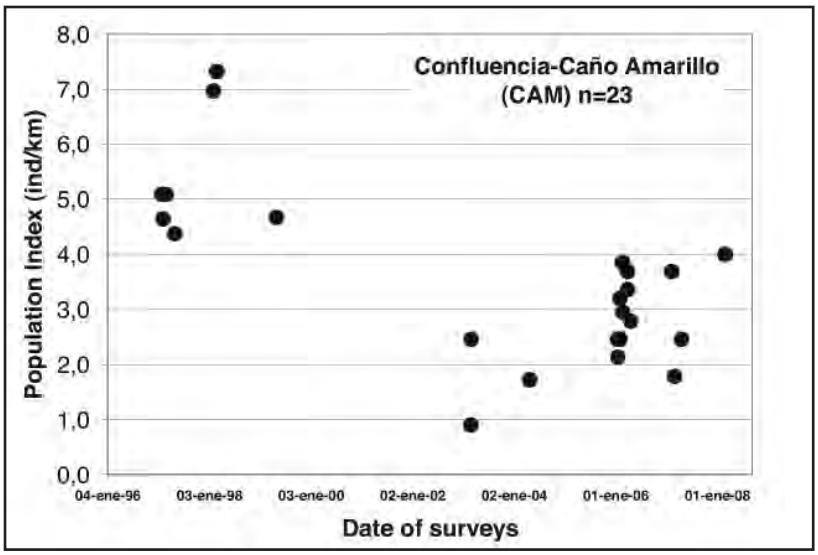



**Figure 5.** Plot of population indices (PI) vs date of surveys for Orinoco crocodiles in CAM. Two trends are shown: a declining one from 1993 to 2004, and an increasing one after that year.

**Population structure:** In some river sections of the CRS population structure of crocodiles has also changed, but in some cases is difficult to distinguish trends. The following graphs show only population structures for those river sections that have enough data to attempt comparative analyses.

There are data for five non continuous years in CAN. The first survey is for 1993. In that year, even though the population was dominated by small crocodiles, adults represented an important fraction (32%) of it. Population structure for 1996, 1997, and 1999 were essentially the same. If we take data from 1996 as the most representative of those years (based in higher number of surveys), and compare it to the one from 1993, the differences are significant ($X^2$=7,7; *P*=0,05). Results for year 2008 are based on one survey that covered 8 of the 17 km of the segment (Fig. 6).

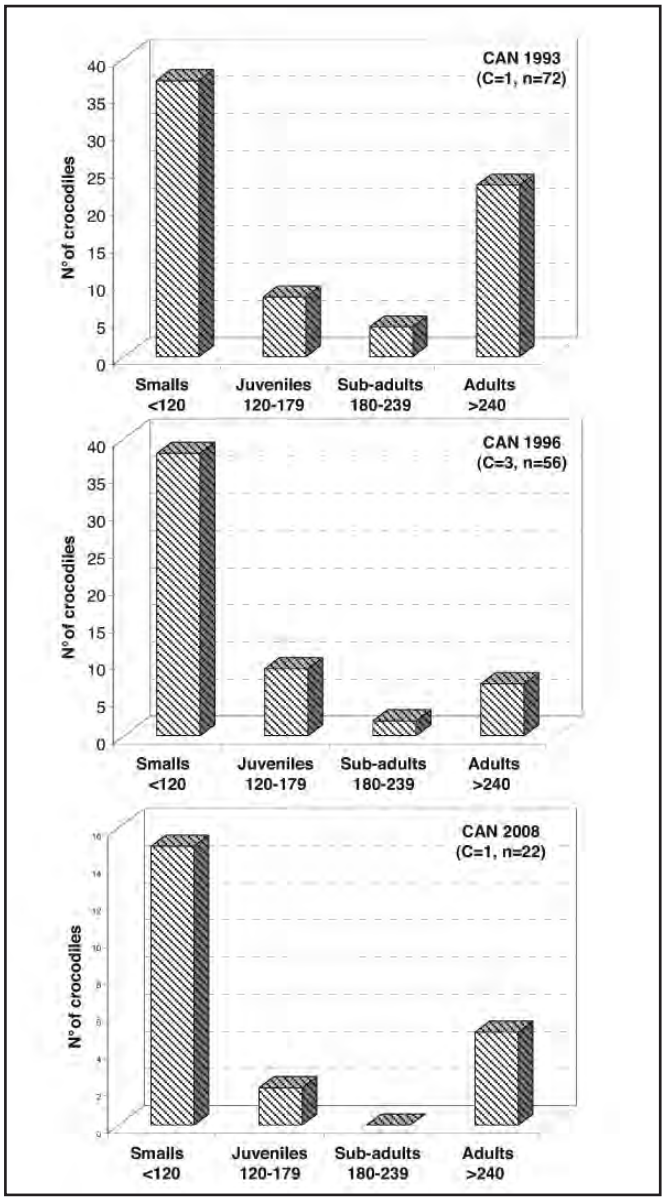



**Figure 6.** Population structure of crocodiles from CAN. Results for 1997 and 1999 (not shown) are essentially equal to the ones for 1996. C, number of counts; n, number of crocodiles. Size categories in cm.

In the case of LBC, population structures have shown a high variability, but no trend is apparent (Fig. 7). The proportion of adults in this section has always been important, to the point that this class was the dominant one for 1997 and 2006. LBC is a river segment of Caño de Agua where every year takes place the majority of nesting events in the entire CRS (Ayarzagüena 1987, Seijas and Chávez 2002). Conditions for nesting in Caño de Agua may change from one year to another depending on the severity of the dry season and the

availability of deep pools and nesting beaches, which may cause that adults enter or leave this river section. This could partially explain variations in population structure from year to year. Another source of variation is that visibility of different size classes of crocodiles varies according to changes in the river as the dry season progresses (Seijas y Chávez 2000) adding noise to the results.

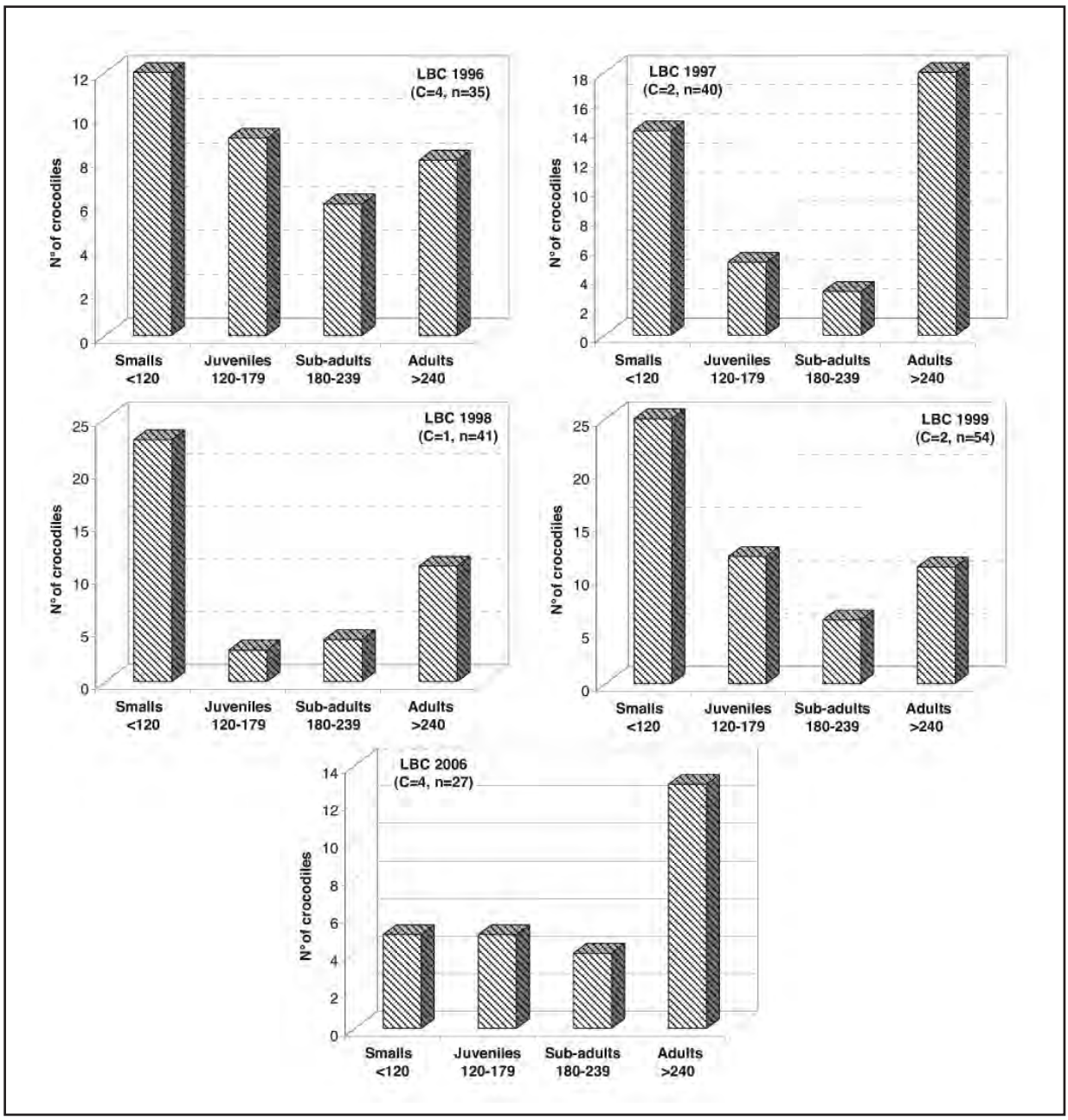



**Figure 7**. Population structure of crocodiles in LBC. C, number of counts; n, number of crocodiles. Size categories in cm.

In CAM population structure has also changed, somehow, in an erratic form (Fig. 8). The importance of juveniles seems to have declined, whereas the proportion of adults has increased. Changes in proportion of small crocodiles from 2006 to 2007 could be explained by the release into the river of captive reared individuals.

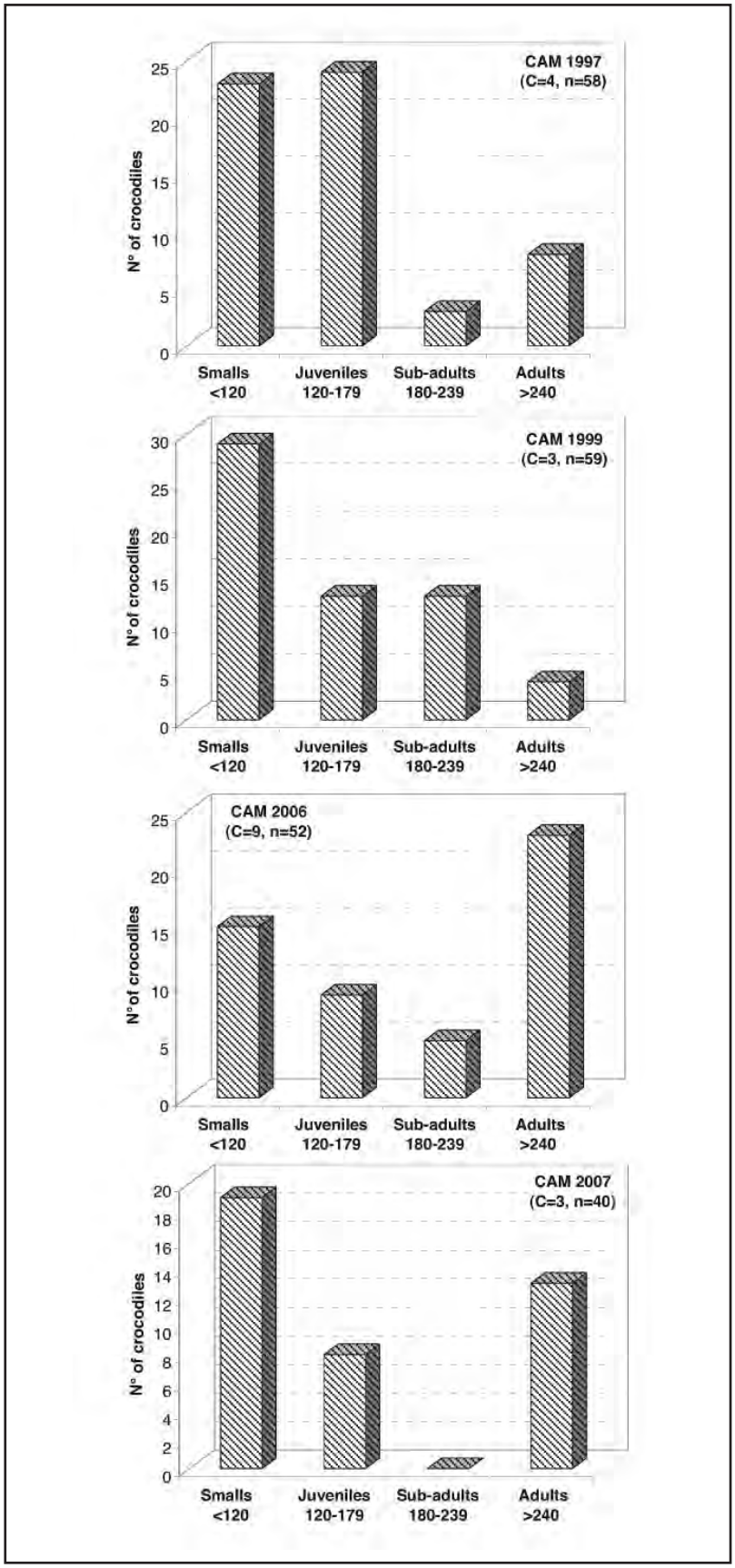



**Figure 8.** Population structure of crocodiles in Confluencia-Caño Amarillo (CAM). C, number of counts; n, number of crocodiles. Size categories in cm.

## CONCLUSIONS AND RECOMMENDATIONS

There are unequivocal signals that the population of Orinoco crocodiles, in different river sections of the CRS, has declined in recent years. The reasons of the decline have not been carefully investigated, but our observations indicate that human presence in the river has increase in recent years. The CRS is not legally protected, but there is a proposal to declare a portion of it as a Wildlife Reserve. Legal protection is not enough, and a rigorous law enforcement plan to protect against illegal killing of this protected species must be implemented immediately. Monitoring of crocodile population in the CRS should continue, but sources of variations should be reduced to increase the accuracy of results. The establishment of a precise monitoring program is a priority, such as it has been recognized in the "National Strategy for the Conservation of the Orinoco Crocodile and its Action Plan" (ENCCOPA 2007). This program must be under the responsibility of the Ministry of the Environment, in coordination with universities and ONGs involved in the conservation of the species.

## ACKNOWLEDGEMENTS

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# Double clutching in a captive female Orinoco Crocodile *Crocodylus intermedius* at The Dallas World Aquarium, TX.

**Luis Sigler**

**ABSTRACT:** The Dallas World Aquarium imported a pair of adult Orinoco crocodiles *Crocodylus intermedius* from Venezuela in 1998. The 25 year old female had previously laid three clutches in captivity. The pair mated in Dallas and laid an unproductive clutch December, 1998. The female was inactive until 2002 when modifications were made to the nesting area. In April, 2003 the first Orinoco crocodiles born outside their natural distribution, hatched. Each year, from 2003-2007, a successful clutch has been laid producing 83 hatchlings. In April 2007, 40 days following the hatching of a clutch laid in December 2006, the female laid a second clutch, which appeared to contain only two eggs, found in the water. It was later discovered that another 29 had been laid in the nesting area. These were not detected until December 2007 when they were uncovered during another nesting event. Of two eggs discovered in the water, one was fertile and incubated artificially. It developed an embryo that died at day 60 due to low humidity in the incubator. The 29 eggs left in the nest were infertile despite the fact that conditions were optimal. Further studies must be done to understand this unusual behavior. American alligators have been known to retain sperm. It is suspected that this may also be true in crocodiles. Sperm may be retained from one mating and used to fertilize a second clutch of eggs. This double clutching event may be the first of its kind reported in the Americas. It has been reported previously for two Asian species- the Mugger crocodile, *Crocodylus palustris*, in India, and the Siamese crocodile, *Crocodylus siamensis,* in Cambodia.

## INTRODUCTION

The Dallas World aquarium (DWA) located in downtown Dallas, TX, USA, imported a pair of Orinoco crocodiles *Crocodylus intermedius* from Venezuela in 1997 for display in an indoor exhibit at the second level of the Rainforest building. The male was 18 years of age and the female 25 years old. The exhibit consists of 64,000 liter fresh water divided in two ponds and with a land-water ratio of 40:60. To provide heat we installed two 60 watts Flood UV lamps (Mega ray Zoo Reptile UV®) and four heat stripes (1500 watts) over the nesting area. The water is maintained at 80° F (26.6 °C) and is constantly filtered into the system.

The crocodiles are fed every Saturday with eviscerated rainbow trout *Oncorhynchus mykiss* + vitamins (Mazuri® Zu Bird tablets) at an approximate amount of 10 pounds a week. Some days they are trained and rewarded with chicken drummets (no more than 10 pieces a week). They shared their exhibit with Black Pacus *Piractus brachypomus* and Red belly piranhas *Pygocentrus nattereri* and with free flight birds upon which they occasionally prey.

Orinoco crocodile's courtship and breed in the wild during September and October and females deposit eggs in early January to early February (Medem 1981). The breeding activities from 2003 to 2007 were closely followed at the DWA, and despite the latitude difference between Colombia-Venezuela and Dallas, TX, the breeding season for our Orinoco crocodiles under artificial/captive conditions is quite similar (October – November). Nesting was

recorded in December and January, and hatching time under artificial incubation in March and April after an average of 92 days (Sigler 2007).

Double clutching must be defined as an additional clutch of well developed eggs between two normal nesting events; with enough time to realize it is different in the ovo genesis from the first one and not affecting the characteristics of the third one.

The first clutch from the 2007 season was laid on December 20th 2006, and was composed of 45 eggs buried on the sand bank where the female usually nests. After 90 - 95 days of artificial incubation, 30 crocodiles hatched between March 18th and 23rd 2007.

The second clutch was composed of 31 eggs: two were laid in the shallow pond of the exhibit around 12:00 pm on April 26th 2007, and the remaining 29 were buried in the nesting area (Fig. 1) but were not discovered until December 26th 2007. The two eggs laid in the pond were collected in less than an hour with an extendible net (Fig. 2) and moved to the incubation area in a plastic bucket with sand and leaves.

The incubator was set at the normal temperature and humidity (30.0 °C and 96% RH). After that, the eggs were measured (Table 1) and the mucus coats were removed. The mucus coats were clear and appeared normal. When the incubator was stabilized, a plastic container filled with vermiculite saturated with water was used for the eggs (Fig. 3). A thermo – hygrometer was placed inside the container to make the parameters visible from the outside. The two eggs showed a kind of band the following days after the incubation started, but only one appeared normal.

When the humidity was lower than 92% a misting bottle was used to spray water over the eggs in the vermiculite. The eggs were candled with a flashlight and only one showed a kind of yellow reddish content and the other showed an air bubble instead (Fig. 4).

In early June, after 41 days of incubation, the rains were so heavy over Dallas, and the DWA showed some leaking areas close to the incubator. We supposed the humidity will be kept high but it dropped some days to lower 80's %. Many days the egg container inside the incubator had been misted heavily but after 3 days the humidity decreased again.

On June 30th (65 days of incubation), I opened both eggs after candled and noticed a dead embryo 70 mm long in the fertile egg (Fig. 5), and a whitish yolk in the other egg witch has an air bubble (Fig. 6). The embryo showed a little autolysis which suggested it died tree weeks before.

The other part of the clutch was found on December 26th while the female was digging for her usually December clutch (2008 season). We saw on the TV closed circuit when she dug out some eggs from the underneath of the nesting area. When we approached the nest, the odor of decomposing tissues suggested those eggs were not from this season and must be part of the clutch deposited on April 26th 2007. The eggs were together and buried in 3 layers 30 cm into the sand, as a normal clutch (Fig. 7). All but four of the eggs were broken and their content revealed no evidence of fertility. The unbroken eggs were measured and opened, they had whitish yolks and the albumin had no blood vessels (Fig. 8).

Double clutching was only reported in Asia for two species of crocodiles. The Mugger (*Crocodylus palustris*) held at the Madras Crocodile Bank in India, and it is showed in some females from different generations (MCBTCH 2008). Double clutching has also been seen in Siamese crocodiles (*Crocodylus siamensis*) in Cambodia in three different facilities in at least four females longer than 2.25 meters and around 25 years old. 80% of the time the first clutch was larger than the second. In at least 20% of the cases the second clutch produced hatchlings but the success rate of the other clutches is unknown, as the eggs (possibly fertile) were not collected for artificial incubation (Nao 1995).

It is possible that the combination of enough food offered to the crocodiles at the DWA (Mc Mahan *pers. comm*.) and the lack of environmental signals as water level and ambient temperature confused the reproductive system of the crocodiles (Thorbjarnarson *pers. comm.*)

American alligators have been known to storage sperm (Gist *et al* 2007). It is suspected that this may also be true in crocodiles. Sperm may be retained from one mating and used to fertilize a second clutch of eggs.

To my knowledge this is the first report of double clutching in *Crocodylus intermedius* where a clutch of 31 eggs was laid on April 26, in between two normal clutches laid on December 20th 2006 and December 27th 2007. One egg of the second (April) clutch was fertile and developed normally but died from incubation problems on an estimated age of 45 days. Main comparison data among first (n=45) and second clutch (n=6) are showed on Table 2.

**ACKNOWLEDGES**

The comments of John Thorbjarnarson and William Mc Mahan were really appreciated. Special thanks to Dr. Ruth Elsey for all the suggestions to the manuscript. Paula Carlson also helped with proofreading.

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**Table 1:** Egg measurements from the second clutch.

| Egg # | Fertility | Length | Width | Weight |
|---|---|---|---|---|
| 1 | Positive | 86 mm | 52 mm | 125.3 g |
| 2 | Negative | 82 mm | 47 mm | 119.4 g |
| 3 | Negative | 80 mm | 48 mm | Non registered |
| 4 | Negative | 81 mm | 50 mm | Non registered |
| 5 | Negative | 81 mm | 48 mm | Non registered |
| 6 | Negative | 80 mm | 48 mm | Non registered |

**Table 2:** Resumed information about the reproductive events cited in this document.

| | Normal clutch 2006 – 2007 | Double clutch 2007 | Normal clutch 2007 – 2008 |
|---|---|---|---|
| Date of laying | Dec 20th 2006 | April 26th2007 | Dec 27th 2007 |
| Clutch size | 45 | 31 | 47 |
| Egg mass | 5 243.4 g | 3 792.5 g | 5 228.7 g |
| Egg size (average) | 79.9 x 49.4 mm | 81.6 x 48.8 mm | 79.4 x 48.7 mm |
| Fertility | 100 % | 3 % | 72.34 % |
| Incubation time | 90 – 95 days | Failure at day 45 | 89 – 92 days |
| Date of hatching | March 18 – 23 2007 | ---- | March 22 – 25 2008 |
| Hatchlings produced | 30 | 1 dead embryo | 31 |



**Figure 1.** Female Orinoco crocodile *Crocodylus intermedius* digging a hole in the sand. Two hours after, she laid two eggs in the exhibit pond.



**Figure 2.** Position of the two eggs laid in the water and before removed with an extensible net.

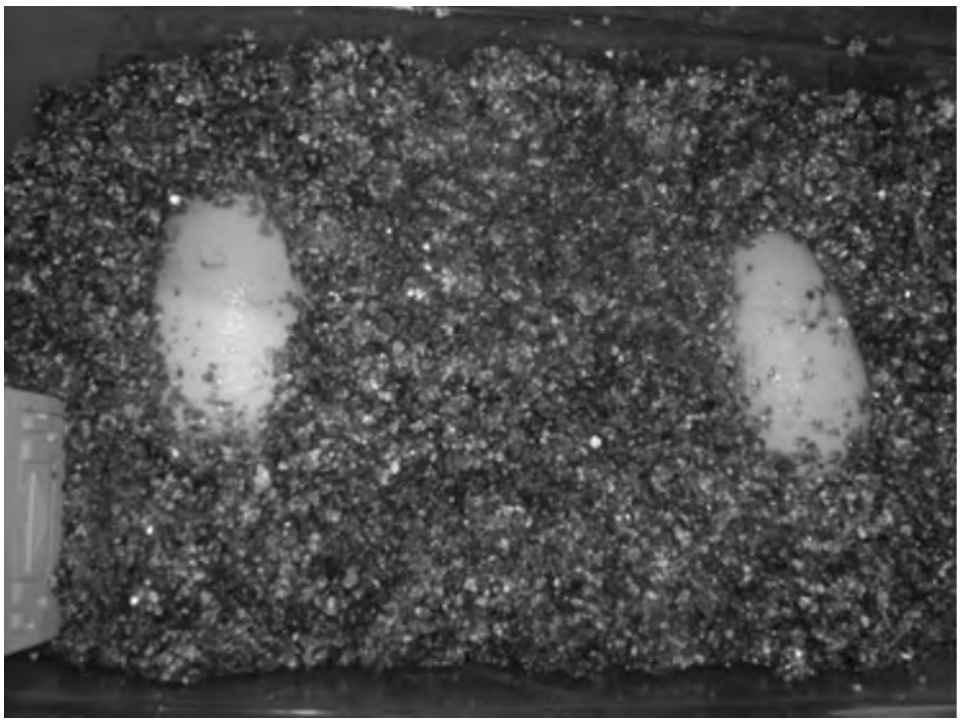


**Figure 3.** The two eggs were deposited in a plastic container with vermiculite saturated with water.

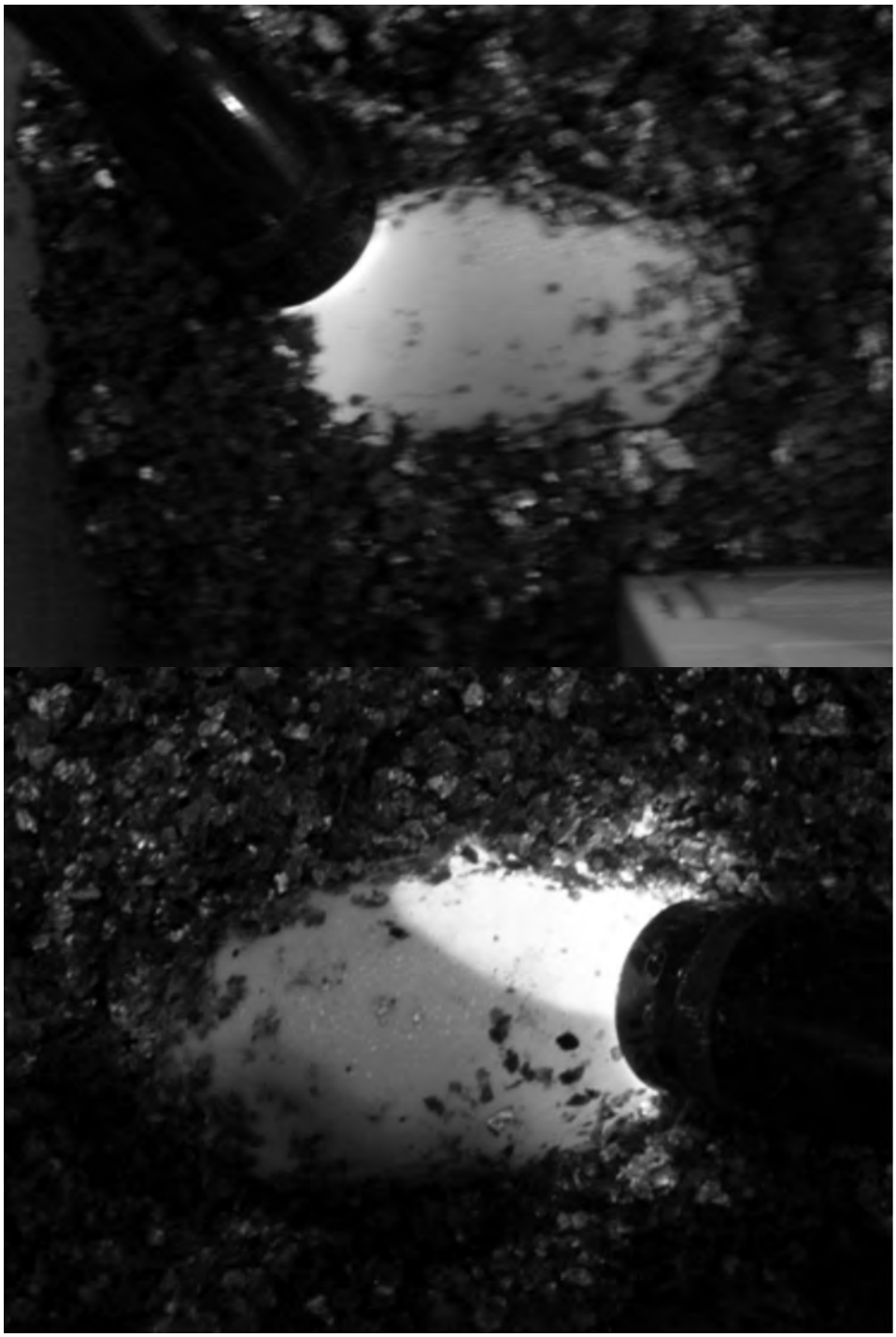


**Figure 4.** Candling the eggs to check their status (top is fertile and bottom is not).




**Figure 5.** Embryo development in the fertile egg.



**Figure 6.** Infertile egg content.



**Figure 7.** Disposition of the buried eggs.



**Figure 8.** Four of the non broken eggs buried in the nest.

# *Tomistoma schlegelii*: results of a Workshop, and development of an Action Plan for its Conservation.

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**ABSTRACT:** We present the results of the first International Workshop dedicated to the conservation of *Tomistoma schlegelii*, held in Thailand in March 2008. The primary aim of the Workshop was to develop a Conservation Action Plan for the species. The current status of Tomistoma throughout its range is described, along with major threats and management issues. This leads into the key issues within the Action Plan. We also briefly discuss recent work in Tomistoma biology and management (including captive breeding), as well as TTF activities that have enabled and supported this work.

# Born to be wild: conservation and re-enforcement of Philippine crocodile populations in San Mariano, Isabela, Philippines

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**ABSTRACT:** A reproducing population of the critically endangered Philippine crocodile *Crocodylus mindorensis* was discovered in the municipality of San Mariano, Isabela Province, in 1999. A community-based conservation strategy here has addressed the direct threats to crocodiles in the wild: hunting, habitat conversion and destructive fishing practices have largely stopped in San Mariano. There is broad societal support for *in-situ* crocodile conservation and sustainable wetland management. Three core crocodile home-ranges are actively protected by communities and the local government. The crocodile population in San Mariano is slowly increasing but remains critically small. A full recovery of the crocodile population to viable levels depends on successful reproduction and survival of juvenile crocodiles. Crocodile nests are often lost to predators. Hatchling mortality in the wild is high, mainly because optimal wetland habitat for juvenile crocodiles is scarce. In 2005 a head-start program was set up aiming to reduce hatchling mortality. Crocodile nests are guarded. Hatchlings are collected from the wild and kept in controlled conditions. After 18 months the captive-raised juveniles are soft-released in artificial small ponds. A trial release in 2007 of 4 captive-raised juveniles has been successful: after one year all have survived and adapted to wild conditions. In 2008, 30 captive-raised juveniles have been released. The positive experiences with community-based crocodile conservation in San Mariano and the results of the re-enforcement program could serve as a basis to reintroduce captive-raised Philippine crocodiles in former parts of their range.

# Actions for Gharial Conservation and Management in Teraï, Nepal

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**ABSTRACT:** Since 1978, thanks to Dr Tirtha Maskey a Conservation program has started in Nepal with the gharial breeding centre construction in Chitwan National Park, Teraï. Few years after, the first released sessions have started in the main Teraï Rivers. In spite of these actions, Nepalese gharial population decreased. In 2000, Nepalese government, via Dr Maskey in collaboration with Luc Fougeirol team, starts to increase the effort for gharial conservation. During 6 years, French biologists were gone in Nepal to support Department of National Park and Wildlife Conservation (DNPWC) in gharial survey and breeding centre development. But in September 2006, the program was affected by the death of Dr Maskey in tragic helicopter crash. WWF, WWG and Awely (French NGO) have decided to initiate a partnership and follow the mission with an ambitious project. With the agreement of DNPWC, the project argues for 5 mains goals: 1/continue increase in scientific knowledge on gharial and its habitats; 2/ integer favourable measures for gharial conservation in protected areas; 3/ Develop the ex situ conservation measures (breeding centres); 4/ continue population awareness on the protection of their environment through gharial conservation; 5/ contribute with Gharial Task Force in the reinforcement of gharial conservation for world biodiversity. With the strong affect of the Chambal sanctuary gharial population, (mass death in the end of 2007, more than 100 individuals), gharial conservation in Nepal gets more and more a priority because of the upstream situation of those population.

## INTRODUCTION

Two species of the family Crocodylidae are found in Nepal: The Marsh Mugger, *Crocodylus palustris* belongs to the subfamily crocodilinae and the Gharial *Gavialis gangeticus* Gmelin 1789, belongs to the subfamily gavilianae is only survivor of the Gavialidae family (Maskey and Percival 1994). The gharial is the most aquatic of all the crocodiles, and its hydrodynamic body allows it to be an excellent swimmer. The peculiarity of the gharial morphology is striking. It has a large extremely slender snout and adult males grow around their nostrils a bulbous nasal appendages called “ghara”, which is absent among other crocodilians

### Context

### Global context

Historically present in North part of Indian sub-continent, gharial is now mainly present in

Nepal and India. In spite of its wide distribution and abundance in the past, it is the least known of the 23 species in the world (Whitaker and Basu 1983). During the 2006 CSG meeting organized in France, conclusions have been alarming: it had remains only 200 wild adult gharials.

This crocodile is the most endangered, it is why that it is classified in Annex 1 of CITES Convention and since April 2007, Critically Endangered Specie on IUCN red list.

**Nepalese context**

In Nepal we can find the second most important population in the world with approximately 60 adults. These populations are still living in Narayani and Rapti Rivers in Chitwan National Park, Babaï and Karnali Rivers in Bardia National Park and Koshi River in Koshi Tappu Wildlife Reserve.

There are numerous causes to the decline of the gharials populations. For a long time, the gharials have been victims of an intensive poaching because of different parts of their body were thought to have medicinal properties or to be linked to mystical practices. The big males, who are the only ones to have a ghara, were thus the favourite preys of the poachers. The setting up of National Parks and Wildlife Reserves in Teraï has considerably reduced this threat.

Today other disruptions have appeared:

- Human disturbance: there are too many fishermen who create important basking troubles and their fishing practices are very destructive.
- Deterioration of habitat quality: there are riverbank extraction and mainly water pollution. (factories upstream from the park rivers).
- Dams built down-river from Chitwan rivers constitute today an obstacle that cannot be crossed by the aquatic species (fishes, crocodiles, dolphins…) when they swim upstream. The gharials swept along by the strength of the current during the monsoon, and when they try to swim upstream when the low waters come back, they find themselves in front of a wall, and they cannot go back to the population of the park. The same problem applies to fishes, their preys.

**Tragedies for gharial conservation**

World gharial conservation has known the two last years, tragedies which have increased the difficulty and the emergency of the situation.

In September 2006, a tragic helicopter crash had caused Dr Thirtha Man Maskey’s death. Passionate by this reptile, he had made his thesis on Gharial Conservation and he has always been dedicated on gharial conservation (first during his work for DNPWC and finally for WWF Nepal). His death had implied a new start with different partners so reorganization for conservation project in Nepal.

In December 2007, Mass death of gharial had started in Chambal Sanctuary Rivers. Since that time more than hundred adults and subadults are dead. Starting the last week of January, specialists from India and all other the world have investigated the deaths. It is suspected

that a toxin caused the kidney damage and failure, though a toxin but its source has not yet been identified (GCA website).

**Conservation initiatives in Nepal**

In Nepal, within the Chitwan National Park, the specie has been prevented from extinction thanks to a captive rearing programme initiated in 1978: the Gharial Conservation Project in Kasara. It aims to breed gharials from a captive population but also from wild eggs (collected on Chitwan park river banks). Dr Maskey was the initiator of gharial released in the beginning of 80's when he was Director General of DNPWC. Since 1981, about 700 young gharials from the rearing centre, the Gharial Conservation Project, have been released. Dr TM Maskey was the first and the most committed person on gharial conservation in Nepal.

In this context, in 1999, Dr Maskey had requested help from French NGO to make survey of released gharials and increase breeding techniques and sanitary survey in Kasara Centre. Each year since 2001, French volunteers from SOS Crocodiles and now Awely come to make survey in National Parks Rivers and increase breeding centre capacity (Cadi *& al.* 2002, Cadi *& al.* 2005). The last action was the creation of a new nursery in 2006.

**A new project for Nepalese conservation**

Even if the increasing of ex situ conservation conditions (with breeding centre) in Nepal seems to be in favour of gharial, the future actions are numerous. Now with the partnership of WWF Nepal, WWG (Wildlife Watch Group) and the support of DNPWC (Department of National Parks and Wildlife Conservation) we wish to put into place a project to increase gharial conservation in Nepal. With the mass death in India, Nepalese conservation takes priority and legitimacy for populations upstream from Gangetic basin. Moreover, it is the responsibility of Nepal which possesses 30% of world population and finally because of our experiences permits us to have good analysis of the situation.

So we can and we have to save one of the last gharial populations, as it did for rhinoceros or tiger several years before.

To be effective this project has to be global. For that we have identified 5 components:

1. Continue increase in scientific knowledge on gharial and its habitats which is, for instance, too low,
2. Integer favourable measures for gharial conservation in the management of protected areas (Chitwan NP, Bardia NP, Koshi Tappu WR…),
3. Develop the ex situ conservation measures (breeding centres of Chitwan and Bardia) to increase gharial population in Nepal,
4. Continue population awareness on the protection of their environment through gharial conservation,
5. Contribute in the international context of Gharial Conservation Alliance, in reinforcement of gharial conservation for world biodiversity.

**Increase scientific knowledge**

As we made for 6 years in Chitwan NP Rivers, we have to continue survey in these rivers but also in Bardia NP and Koshi Tappu Wildlife Reserve. First to permit us data collections on individual number and population type and secondly to determinate favourable habitats.

Gharial is a good sign for river quality so more than gharial habitats; this survey will underline habitat quality of many aquatic species like dolphin and otter.

For released survey, our reports made during the 4 last years underline the necessity to release gharial in February and more upstream from river than possible. Moreover, long net fishing has to be banned, protected areas created and prospecting launched.

To make prospecting, we will use cattle mark for identification and telemetric system to know gharial behaviours. We know that wild population depend of released gharial so we have to increase survival rate.

**Studies to develop conservation measures in protected areas**

But gharial survey will not be useful for specie conservation if we do not study actual rivers management in protected areas. We have to study threats which press on rivers. Chemical pollution which implies bioaccumulation risks for aquatic species especially for gharial in top of aquatic ecosystem. Human disturbance which destructs favourable habitats for gharial: rivers level decreases each year because of intensive agriculture and Fishermen press fish stock in using long net, electricity or poison. All these threats will be responsible of aquatic ecosystem destruction without actions.

Make studies and after an action plan with strict rules in protected areas will permit to save gharials, aquatic ecosystem and also the only resource of numerous local people as Bothe or Musher (fishermen ethnos).

Gharial is one of the best indicators of rivers quality and working. So it will be useful to make rivers studies through gharial with the Freshwater program or our partner WWF Nepal.

**Develop ex-situ conservation measures**

But before favourable habitats come back, we have to protect and develop rearing population. In 2006, we have built (with the support of La Ferme aux Crocodiles, France) a new nursery in Kasara Breeding centre which has permitted increase survival rate of babies' gharial. Now, we have to increase reception capacity with new pools and food quality with a fishing farm to give its live fishes with good size.

To avoid extinction of breed specimens in case of diseases, we will create a second breed pole as productive as Chitwan pole in Bardia National Park. Chitwan will be a model; we have already made plans and estimation of these new constructions.

The results in Kasara breeding centre permit us to think about international breeding sites. A partnership with zoos which can provide adaptive structures will give us a solution to:

- increase world breed effective,
- find financial support for in-situ conservation,
- increase people awareness all other the world.

Finally to have efficient breeding centre, we will make rearing gharial survey with growth and sanitary check-up.

**Increase people awareness**

To take into place this entire program, we cannot work alone. Population awareness and participation will be very important for this project.

To have regular update on gharial status, we will work with Park rangers who will be able to make this work during their patrol. But for that we will teach them on gharial specie and prospecting techniques.

Buffer Zone Community participation is important. In one hand the community will work with us during construction as we had mad during for nursery and to manage these structures like fishing farm. In other hand they will be the mediator with villagers. Our survey had showed that villagers are afraid by gharials because they do not know that gharial is only fish eater and so non aggressive crocodile. Community will make easier our communication with villagers.

Children are Nepal future, it is important to involve them in our awareness program. We wish to work with school and orphanage to teach children on gharial but also rivers conservation. To increase their involvement, we will take into place expositions with children creation like draws, paints, poetries in public place but also hotels.

Using events will be an advantage. Events like local festival, Wildlife weeks or released sessions.

Even if they are in part responsible of bad gharial situation, fishermen have to be included in this project. With them with will create an alert network (for us and Parks authorities) if they see gharials in bad situation. They are always on rivers; they will be our best partners to check gharials regularly. Moreover they already help us for egg collection.

As we will make for rangers, we will teach guides on gharials and prospecting methods to update gharial status. More than this updating, hotels and guides will help us on international awareness in providing for example “Gharial boat safari”.

The partnership with WWG for Dr Maskey resources centre will permit us to propose report and give conferences on gharial and rivers conservation to Nepalese students who wish to work on Conservation and Environment.

Develop international awareness through international volunteers will permit to increase our work in Nepal during this project.

**Gharial international survey**

Created in June 2006 during CSG meeting, Gharial Conservation Alliance is the best way to develop international survey. Near 30 persons forms this group: specialist from CSG, gharial country representative or zoo curators.

But Nepal has another responsibility. With India, it is the only country which possesses real population and efficient breeding structures. To avoid genetic problems, we have to launch an exchange program with India. In Kasara, gharials are breaded since 1978 with the same group: 2 males and 11 females. So there is a risk of genetic impoverishment.

**Project structure**

It will be a four years project with an important partnership:

- Local, with rangers, community, villagers, children, fishermen, tourism actor,
- National, with WWF Nepal, WWG – Dr Maskey resources centre,
- International, with Gharial Conservation Alliance, international volunteers, zoos.

The project will be managed by Awely, WWG and WWF Nepal in partnership with the Department of National Parks and Wildlife Conservation. It will be Awely program officer who will apply action program in partnership with Nepalese local coordinator who will be resource person for gharial conservation in Nepal.

## CONCLUSION

In conclusion, it is important to underline that gharial conservation is an international input in biodiversity conservation and Nepal is one of the last chances to save this species. But more than save the gharial, this project will imply local people with participative actions. As it had been made for tiger or rhino, it is time to push the gharial as an emblem for Nepal.

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# Report on the investigation into gharial mortality in the Chambal river in India.

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**ABSTRACT:** In mid-December 2007 sub-adult and adult gharial (*Gavialis gangeticus*) started dying in the lower parts of the Chambal river in India and at first a protozoan infection or lead poisoning were suspected as causes. During an on site investigations by an international group of CSG veterinarians a number of postmortems were out in the field. The findings were visceral and particular gout with primary kidney failure. These findings could be reconciled with previously done postmortems and confirmed by histopathological examination. A toxic agent responsible for causing this mortality, still ongoing at the time of writing, has not yet been identified.

A puzzling feature of this event is that mugger (*Crocodylus palustris*) and river dolphins (*Platanista gangetica gangetica*) in the same river did not appear to be affected. Toxicological, ichthyological and hydrological investigations are ongoing at a local level. They fall outside the competency of the investigating CSG team.

# An update on the *Crocodylus porosus* and *Crocodylus novaeguineae* Conservation and Management in Papua New Guinea (1982 – 2008)

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**ABSTRACT:** This paper presents the results from the ongoing monitoring program in the Sepik River System of PNG and provides an updated analysis from the 2006 France meeting. The *C. porosus* surveys are being conducted annually whilst the *C. novaeguineae* surveys are conducted biannually. Although there are notable missing data in the preceding years for both species it is assumed that this will not greatly affect the analysis of the data in any way. The data from all sets were updated with the recent March 2008 results indicating that the *C. porosus* population for N = 12 sites which has been surveyed consistently since 1982 showing 20% reduction from 2007 observations. This reflected an annual mean percentage reduction of .9% annually. Similarly the nesting effort for N = 29 sites also represented a reduction of nest observation of 6.2% nesting effort from 2007. Again this also reflected a mean percentage reduction of .4%. Generally it can be suggested that the unusual decline in nesting effort showed no significant difference in the trend for all sets of data. The raw data for the prior years indicated similar nesting effort with the only increases notable in 2007. The *C. novaeguineae* depicted an increase in nesting effort from the 2007 counts. N = 21 reflected 33% increase in nest numbers whilst the N = 45 reflected a 9.8% increase. Both sets indicated an increase of 1.8% annually. It can be suggested that the wild population densities are healthy rather than declining for both species. It can be suggested for both PNG species that the wild population densities are healthy rather than declining. This is consistent with our skin export figures from PNG which reflected the wild and ranched skins exports from Mainland Holdings for the last 10 years since 1997 at approximately 25 000 - 30 000 skins annually. Thus, the status of the PNG population level in terms of wild harvests is considered viable for the current dispensation to enable commercial exploitation to continue.

## INTRODUCTION

This is about the 10th appearance from the Papua New Guinea (PNG) delegates that constitute the industry and the government since the 7th Meeting at Caracas – Venezuela in which the initial paper was presented (Hollands 1986). The current report provides an update to the results for the *Crocodylus porosus* for March 2007 and 2008 and *Crocodylus novaeguineae* for October 2007. As in the previous reports, the data used in this report have been consistently maintained over the years by the Department of Environment and Conservation (DEC) with additional data being added after surveys from each peak nesting seasons. This report is intended to update the recent survey results from the 2006 report presented at the 18th Working Meeting of the Crocodile Specialist Group (CSG) in Montelimar, France.

We have attempted to put together a budget that will facilitate for the expansion of the aerial survey program to other areas of PNG; unfortunately we were not able to get the support for the expansion program. The industry in itself although sustainable is very small and the costs involved carrying out the expansion together with the capacity within the current establishment of the Department is under staffed. A representative ground survey work has been carried out to three provinces. The surveys were conducted primarily to assess the trends in community use of the species from the wild and the hunting methods. These surveys are recommended to be conducted biannually to each area to enable some consistency especially for the long-term data usage and interpretation.

Hence, this paper will present in brief the information on the enforcement work and the skin trade in PNG. It will not discuss in detail the significance to implementation of the monitoring program, management and the commercial skin trade. This has been described in Solmu (2004) a report presented at the 2004, 17th CSG Working Meeting in Darwin, Australia.

## METHODS

The application of the survey techniques for this highly technical component of the PNG program has been described in various reports (Hollands 1987, Cox *et al* 2006, Cox *et al* 1994b, Manolis 1995, Solmu & Kula 1996, Solmu 2003 & 2004) and is being consistently maintained throughout the years. The survey covers an area of approximately 70 – 100km in length of both sides of the Sepik (Figure. 1) over all the floating herbaceous vegetation including lake fringes, oxbows and overgrown channels.

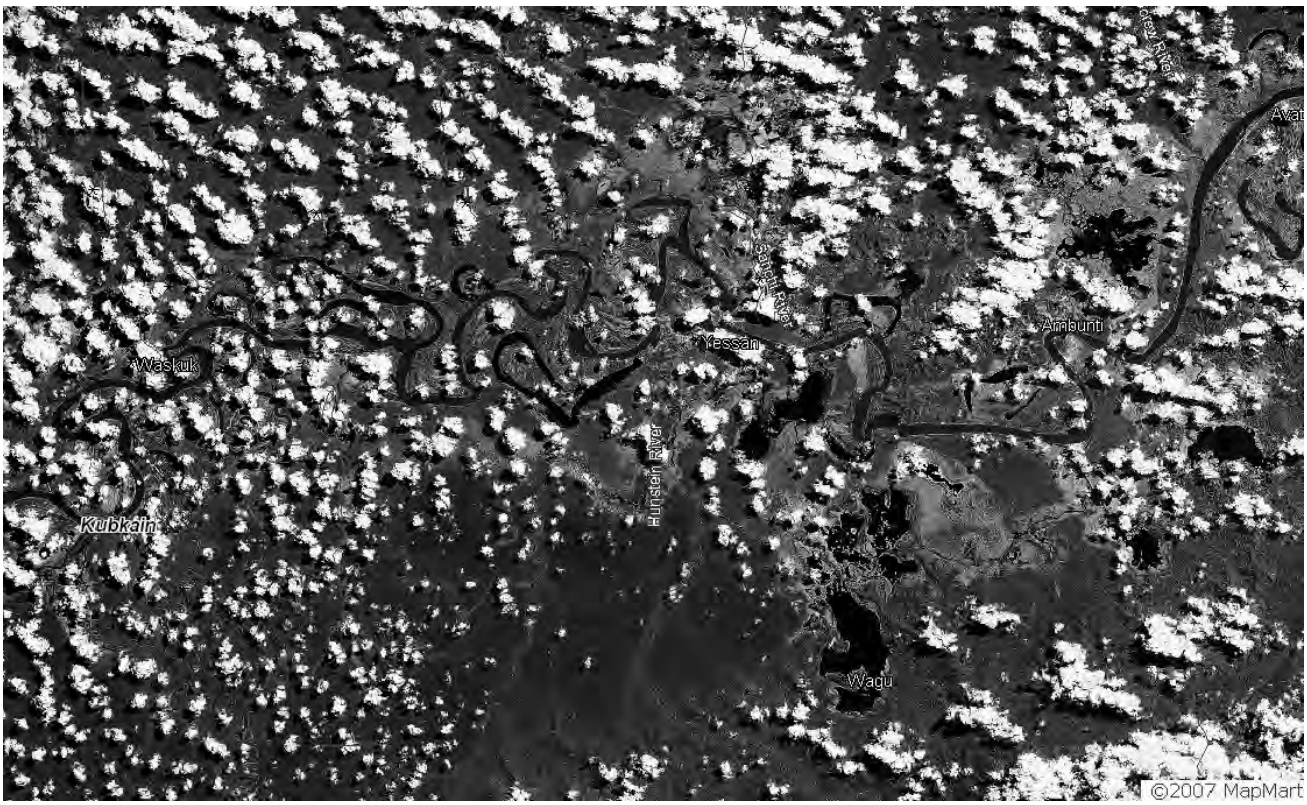



**Figure 1.** Sepik River wetlands and the survey areas.

The wetlands and the survey area covers about five (5) – ten (10) kilometers wide belt of active meanders that has created a active floodplain of up to 70 kilometers wide with extensive black water swamps (WWF 1999). Survey normally commences at around 8.00am and concludes at around 5.00pm depending on the weather and shadows, which are very important variables that affect visibility from the helicopter. Same dates are being maintained over the years for the conduct of this survey. The combined data especially from the N = 41 sites provides a wider coverage of all sites from the system and provides an index of adult abundance (Table 1).

## RESULTS

### *Crocodylus porosus*

The following tables presented the summarized data for the nesting efforts for each set of data. These data sets showed all survey years in which surveys are being conducted. The principle site N = 12, nests counts for 2008 (75) reflected a drop in nest counts from 2007 (90). This is evident through out all the sets e.g. N = 15, N = 29 and N = 41 (Table. 1).

With eye observations across Table 1 for the raw data, generally it can be assumed that there is an increase in nests observations from years 2003 – 2008 for sets N = 15, N = 29 and N = 41.

**Table 1.** The nesting index for all sets for the Sepik River System that reflects PNG.

| SITES | N = 12 | N = 15 | N = 29 | N = 41 |
|---|---|---|---|---|
| 1982 | 45 | | | |
| 1983 | 30 | 38 | | |
| 1984 | 43 | 49 | | |
| 1985 | 49 | 57 | | |
| 1986 | 51 | 57 | | |
| 1987 | - | - | - | |
| 1988 | 50 | 57 | 82 | |
| 1989 | 52 | 65 | 92 | |
| 1990 | 54 | 63 | 92 | |
| 1991 | 57 | 69 | 101 | 119 |
| 1992 | 63 | 75 | 103 | 123 |
| 1993 | 62 | 73 | 112 | 132 |
| 1994 | 46 | 57 | 97 | 111 |
| 1995 | 78 | 92 | 124 | 145 |
| 1996 | 61 | 73 | 115 | 137 |
| 1997 | 70 | 81 | 125 | 154 |
| 1998 | 37 | 45 | 63 | 77 |
| 2003 | 74 | 85 | 131 | 182 |
| 2004 | 72 | 82 | 129 | 161 |
| 2005 | 76 | 84 | 136 | 167 |
| 2006 | 74 | 87 | 142 | 184 |
| 2007 | 90 | 105 | 172 | 219 |
| 2008 | 75 | 89 | 162 | 209 |

The increases basically is assumed to be related to the community driven awareness for the value added incentives to the egg harvests program where adult females are being protected, not to say that we are also mindful of the current management plan for the legal size limits.

However, in observation of the data over the 23 year survey period, the trend indicates significant increases in the nests counts in 2007 counts. The increase is assumed to be partial in response to the egg harvest program; however that cannot be scientifically concluded. Generally the variability in the nests counts over the years is considered to be consistent with the exception of the 2007 where there is a higher nests observation.

The nesting effort for all years up to 2006 report at the 18th CSG is being updated with the recent March 2008 results, continued to reflect significant trends, (Figures. 3a and b), although there is a decline in the nest observations in 2008 which is considered insignificant.

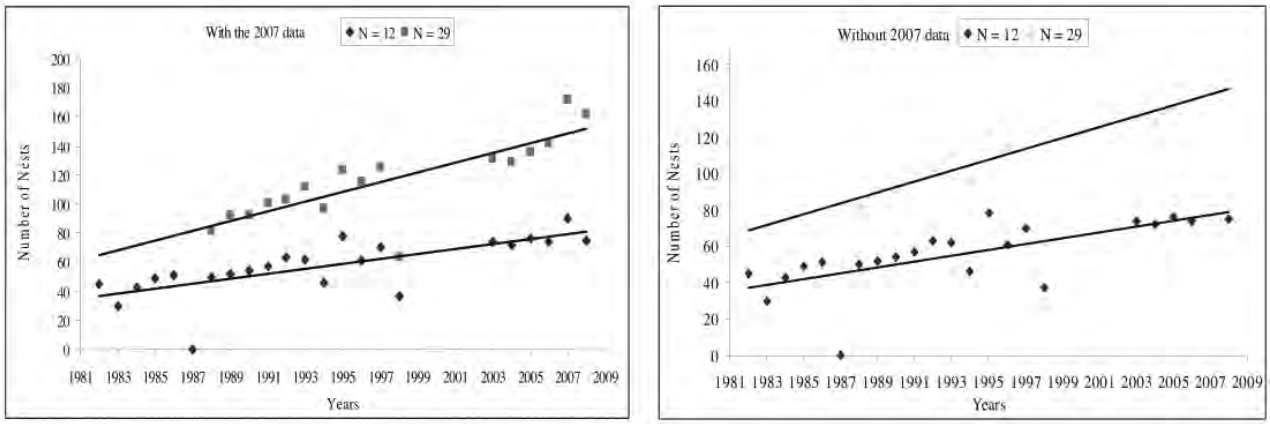



**Figure 3.** (a). With the 2007 data and, (b). Without the 2007 data. Total nests numbers observed during the survey as an index representing the PNG wild populations level for N = 12 and N = 29 for *Crocodylus porosus* survey years (1982 – 2008).

The equation for Figures 3. (a) With the 2007 data, being regressed with number of nests against years: N = 12 (-3349.6 + 1.7804Y), $r^2$ = 0.509, p = 0.0002; and N = 29 (-6589.22 + 3.357Y), $r^2$ = 0.6487, p = 0.0001. (**b**) Without the 2007 data, N = 12 (-3125.3 + 1.5956Y), $r^2$ = 0.469, p = 0.0005; and N = 29 (-5840.89 + 2.982Y), $r^2$ = 0.598, p = 0.005. Both sets maintained increases in nesting effort with or without the 2007 data despite the decline in 2008 observations.

***Crocodylus novaeguineae.***

**Table 2.** *Crocodylus novaeguineae* data sets indicating the index for PNG.

| SITES | N = 21 | N = 3 | N = 7 | N = 5 | N = 12 | N = 48 |
|---|---|---|---|---|---|---|
| 1981 | 75 | 5 | | | | 80 |
| 1982 | 102 | 8 | | | | 110 |
| 1983 | 107 | 14 | | | | 121 |
| 1984 | 75 | 11 | | | | 86 |
| 1985 | 110 | 7 | | | | 117 |
| 1987 | 73 | 11 | 21 | | | 105 |
| 1988 | 103 | 10 | 26 | 16 | | 155 |
| 1989 | 87 | 7 | 31 | 19 | 11 | 155 |
| 1990 | 89 | 4 | 14 | 16 | 12 | 155 |
| 1991 | 83 | 8 | 15 | 15 | 12 | 133 |
| 1992 | 71 | 3 | 12 | 6 | 8 | 100 |
| 1993 | 72 | 11 | 10 | 12 | 9 | 114 |
| 1995 | 91 | 12 | 15 | 11 | 19 | 148 |
| 1996 | 72 | 11 | 13 | 19 | 12 | 127 |
| 1999 | 74 | 12 | 5 | 9 | 4 | 104 |
| 2003 | 98 | 15 | 17 | 22 | 14 | 166 |
| 2005 | 99 | 21 | 19 | 31 | 14 | 184 |
| 2007 | 131 | 20 | 34 | 18 | 17 | 220 |

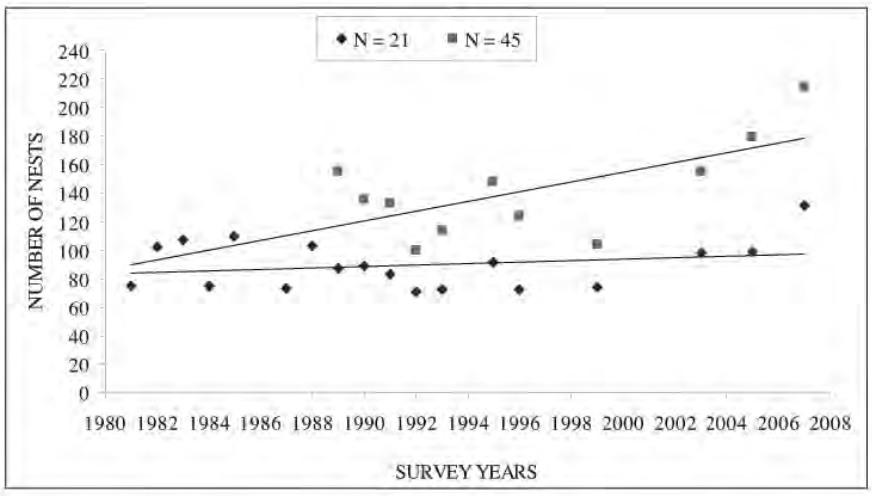
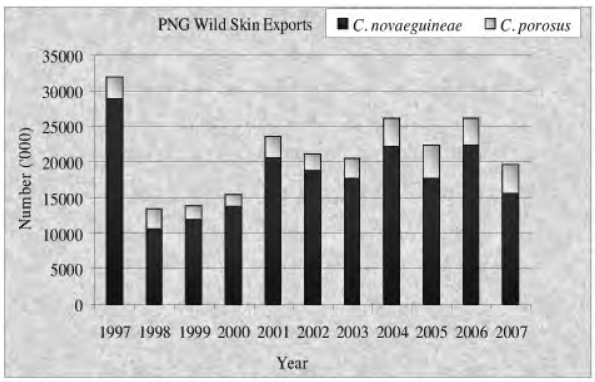



**Figure 3.** (c)**.** Results of the *C. novaeguineae* nesting trend from 1981 – 2007.

The regression equation for the *C.novaeguineae* populations (Figure 3c); N = 21 (-929.5 + 0.5117Y), $r^2$ = 0.056, p = 0.38; N = 45 (-6660.45 + 3.4074), $r^2$ = 0.403, p = 0.04. Both sets reflectd from the primary site N = 21 and the secondary set N = 45 had significant observations of nest counts and the regression line is considered significant however but stable for N = 21.

In general the overall results for the PNG population are considered stable and increasing although the is a significant threat in the habitat degradation from the exotic fish species.

**The Country Export**



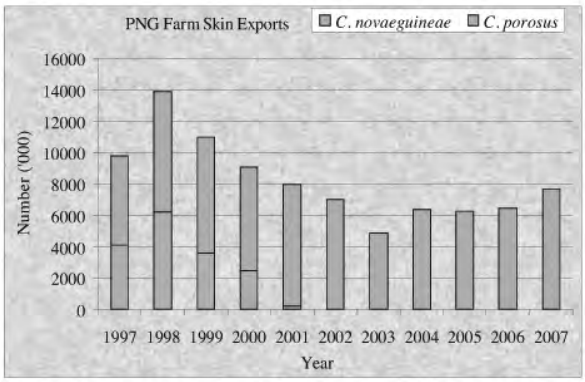




**Figure 4a.** Indication of wild skin exports. **Figure 4b.** Indication of farm skins exports.

The graphs in (Figures 4a and 4b) export from PNG reflects the annual shipment of whole wet salted skins. Most of the shipment of skins are exported to various small buyers around Europe, Asia and the Australian region. The component of the wild skins reflected a large number of *C. novaeguineae* and the balance of shipment the *C. porosus.*

This reflects that the *C. novaeguineae* is the most hunted wild population from all regions in PNG. It should also be noted that Figure 4c, reflected mostly the ranched whole skins from the Mainland Holdings. From the graph it can be assumed that the company has maintained its annual export figures at 6 – 7000 of *C. porosus* farmed skins since 1997, however has decided not to ranch the *C. novaeguineae* populations since 2001.

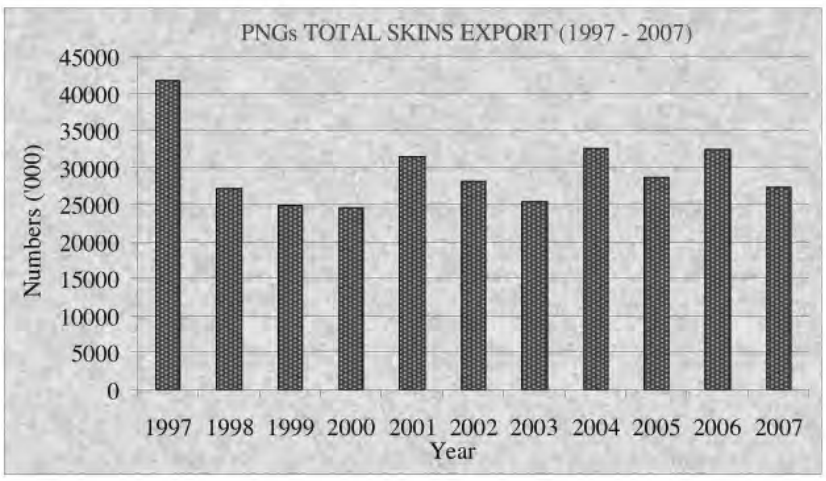



**Figure 4c.** The total skins exports both farm and wild from PNG.

Figure 4c, reflects the total aggregate skin exports from PNG that includes farm and ranched skins of both species. The graph reflects a consistent export market of raw hides from PNG by various exporters at 35 – 40000 skins annually. The export figures is consistent with average annual exports of total skins for the previous 10 years (1982 – 1993) (Solmu 1994) and (1997 – 2007).

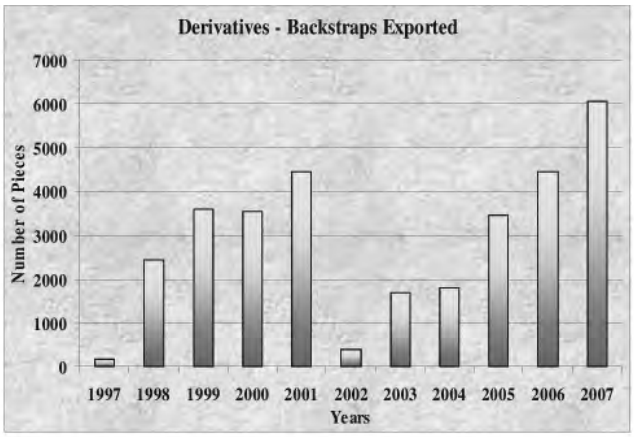
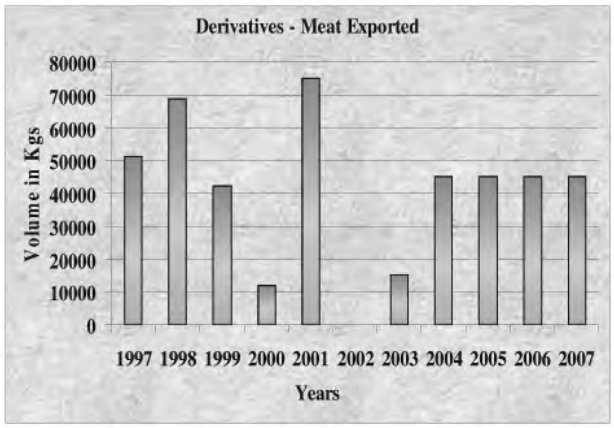



**Figure 4d.** Back-straps exports.




**F**

Figures 4d and e, reflects value added incentives on the resource apart from the whole skin market. Note that the export of by-products is allowed under the current management plan from established and registered operation in PNG. Currently the only farm allowed to export by-products from its establishment in large volumes is Mainland Holdings Limited of Lae. With the exception of Mainland other small holding facilities are restricted from trade in by-product exports. This is basically to restrict uncontrollable harvesting of wild animals for by-products which is difficult to control and in contravention of the existing management plan.

**Summary**

The crocodile industry in PNG continues to strive successfully and play a major role in the conservation of both its species in the country. This is through community awareness and value added incentives directed towards the major land owners. Although there are set backs in many areas for instance logistics to support government implementation of many of its programs the industry has supported where need arises. One of the important issues is the consistent government funding for the major component of the aerial survey, which is considered the heart of the industry in extrapolation of the index from the Sepik. The funding has been consistent for the last five years including this year to enable consistent data collection for this important program. It is hoped that funding will continue to be maintained for the long term and in the near future so that increases can be requested for implementing supporting programs and logistical requirements bought.

An interesting occurrence in the northern population now is the introduction of the exotic fish species that requires interventions for the prevention of habitat degradation. Biological control for the species is not possible due to the extensiveness of the wetlands and investments for control per se…. is unviable. Community support and consultative arrangements is very much needed to support major initiatives for this program.

Thus the industry at the current stage is stable and increasing for both species as evident from our regression analysis results and wild skins export figures. Its is hoped that with collective support from the private industry and partners PNG can continue to be the major role model for sustainable use of endangered species, especially crocodiles for other countries to learn.

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# Community Participation in Conservation and Management of Crocodiles through the Egg Harvest Program in the Sepik Region of Papua New Guinea

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**ABSTRACT:** The core aspect of the crocodile management program in Papua New Guinea depends heavily on sustainable utilization of wild resources. Over the last 40 years the program has progressed through four distinct phases. Initially from direct wild harvest to controlled harvest of size limits, then came the capture of small juveniles for ranching operations, and lately the harvest of wild eggs. Therefore the wild harvest of skins, juveniles and egg increases the benefit to resources owners and add value to facilitate and strengthen the approaches to habitat conservation and management of wild crocodile resources. The current egg harvest program implemented by Mainland Holdings, Department of Environment and Conservation, and the Sepik Wetlands Management Initiative provides direct benefits to resource owners who realize the importance of crocodile habitat conservation.

## INTRODUCTION

The vast wetland areas of Papua New Guinea is inhabited by two species of crocodiles, the New Guinea fresh water crocodile, *Crocodylus novaeguineae*, and *Crocodylus porosus* commonly known as the saltwater crocodile. *C. novaeguineae* is endemic to the mainland of New Guinea including Irian Jaya while *C. porosus* is found on both the mainland and the offshore surrounding island of Papua New Guinea. (Figure 1).

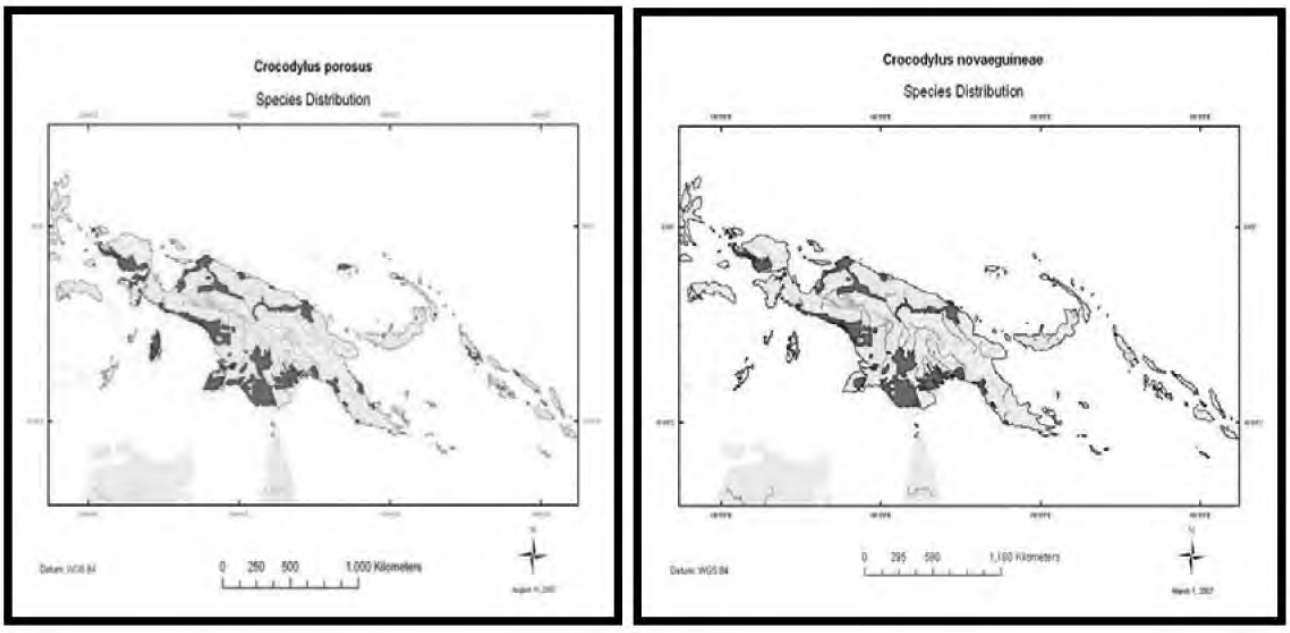



**Figure 1.** Distribution of *C.porosus* and *C.novaeguineae*

**Background to Egg Harvest**

The egg harvest program was launched in 1985 for *C.porosus* and 1988 for *C.novaeguineae* initially as a way to salvage eggs prone to flooding and human predation. Most of the eggs collected from wild in the Sepik all go to the Mainland Holdings hatchery in Lae, Morobe Province.

Eggs are collected during the aerial survey by the Department of Environment and Conservation - National Crocodile Monitoring Unit (NCMU) personnel due to the difficulties involved in handling of eggs thus ensuring good hatching success rate.

The program was suspended temporarily in 1994 after the *C.porosus* harvest due to mixed reactions by some land owners although with no biological evidence that the program was detrimental to recuperating of juvenile population in the wild.

After some consultation with the land owners, local politicians and the management authority it was agreed that the harvest should be conducted bi-annually for both species. Landowners were initially paid K 1.00 per viable egg, taken from the nest on their land, and in order to avoid loss in protein, a chicken egg was given to supplement the loss of eggs as payment. The price for the 1994 egg harvest increased to K 2.50 for *C.porosus* and K 2.00 for *C.novaeguineae*, including chicken eggs.

In 1996 *C.porosus* egg harvest saw an improvement in the prices of K3.00 for every single (viable) egg that includes giving away 2 chicken eggs (a 20% increase) an added value to the potential of saving eggs from human predation. Aerial surveys in 1996 confirmed a marked decline in human predation of nests.

From 1999-2001 no harvest was conducted because of funding problems the helicopter surveys were not been able to be carried out. Therefore, the canoe harvest started in 2002 and continued annually up to 2008. With the canoe harvest came the formation of the Sepik Wetlands Management Initiative (SWMI), a Community Based Organization (CBO) operating as the middle person between DEC, Mainland Holdings and the landowners. At the same time the price of eggs increased significantly from K7.00 in 2002-2004 to K10.00 in 2005 and there on, with the potential to increase further.

The egg harvest has been going on for 16 years now in Sepik Region with significant contributions to the socio-economic well being of the rural community whose land are usually inundated by floods and unfit for any other agricultural crops as source of income to sustain their livelihood. Both crocodile species are commercially utilized for their skins and this trade has been progressing for the past six decades. This long-term source of revenue and foreign earnings is significance and attention is drawn from both sectors of the Government and Industry to develop strategies for the long-term conservation and sustainable use of crocodiles.

The PNG crocodile management model has been considered a successful example of ‘sustainable utilization’ worldwide where mechanisms for both crocodilian species are utilized and developed through farming, ranching and captive breeding programs are combined with wild skin harvest.

Both populations are currently listed on Appendix II of CITES allowing a controlled export trade. The Management Program in Papua New Guinea has acquired from the start, a dual commitment for wildlife conservation and improvement of the well being of local communities in major wetland areas.

**Sustainable Crocodile Resource Management**

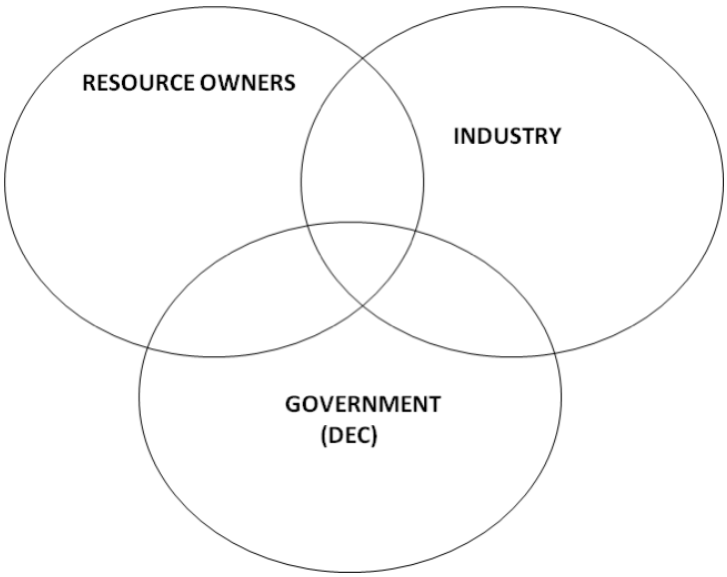



**Figure.2.** A simple model of sustainable use of crocodile resources in PNG.

The current practice in PNG is an indicated in Figure 2 where the link between the three important stakeholders is working effectively in term of corporation, capacity building, enforcement, regulation, monitoring and habitat protection

The governments role towards the Industry is more of a regulatory approach where the Department of Environment and Conservation (DEC) is the mandated agency in dealing with commercial use of wildlife resources regulates the industry and enforces wildlife laws.

To the local resource owners the government encourages them to use their resources sustainably. To achieve that the DEC carry out regular monitoring checks on the wild population and advises the local resources on the current status of wild population. In some cases quotas have imposed on egg harvest in some villages.

**EGG HARVEST AREA**

The egg harvest area covers the Middle-Upper Sepik (Figure 1) regions of Ambunti ($04^0$ 10. 445 E, $142^0$ 40.445 S), in the East Sepik Province. Most of the sites fall inside the aerial survey route. The harvest area covers approximately 75-80 km of floating vegetation along both sides of the Sepik River.

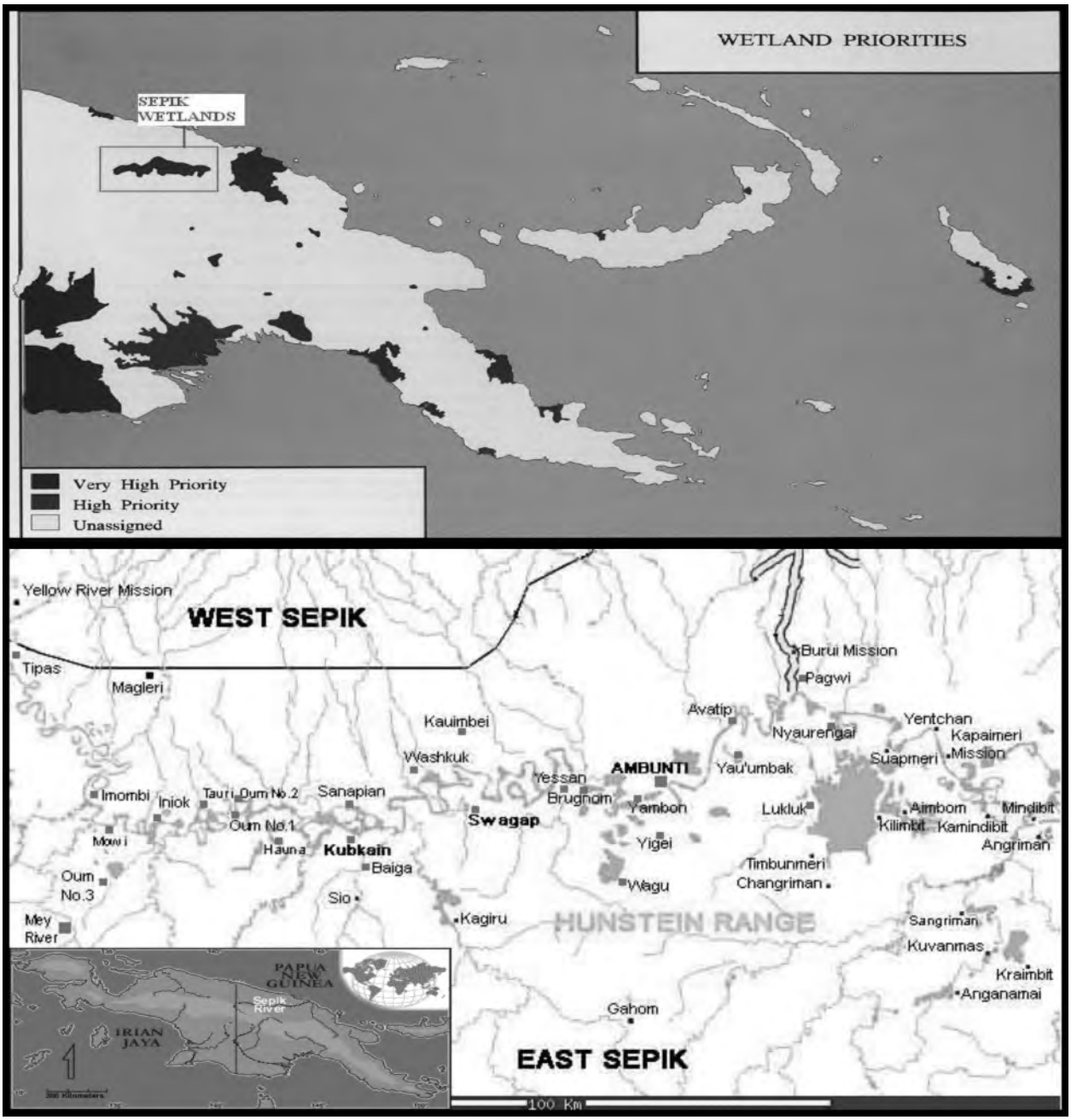



**Figure 2.** Map of Egg Harvest area.

The Upper Sepik river harvests sites include Hauna Levels, Oum Scrolls, Keipi, Bowami Lakes, North Bowami, Biaga scrolls and Kubkain Oxbows and scrolls. The Middle Sepik region includes sites in the vicinity of Ambunti, ranging upriver to sites in the vicinity of Hauna village and downriver to the Japandai village area and further down to Chambri Lakes. The harvest route stretches over a distance of 120 km. Crocodile nesting habitat identified includes: overgrown oxbows, fringing vegetation along lake margins, overgrown channels and scrolls.

In the harvest area, most villages are scattered along the Sepik riverbanks on dry ground however many of them are flooded during high water levels. Some villages are on higher grounds up in the Hunstein range and on the smaller hills where the river provides the only means of transport in these areas.

## EGG HARVEST METHODS

Previous harvests (1985-1998) were carried out using the helicopter drop and retrieval method in which two personnel were dropped off at the nest and picked later when the eggs were collected (Hollands 1985, Cox *et.al* 1989, Genolgani et.al 1991, Cox and Genolagani 1992, 1994). This harvest method was replaced with ground harvest using canoes to in 2002. Nests located are opened up very carefully; all nesting materials are placed in a carton box. The eggs are placed in the cartons and carefully covered by the original nesting material and brought back to the camp.

Clutches are then unpacked and the data recorded on clutch size and viability (live, dead, and infertile). Non-viable eggs are discarded; remaining good eggs are marked with permanent ink to indicate the top position, and then repacked in boxes of decaying grass. Temperatures are monitored every few hours and the eggs ventilated as necessary to maintain 32$^{o}$C until the harvest can be air freighted to the incubator.

## RESULTS OF EGG HARVEST

| Year | # of Nests | Total # of Eggs | Viable Eggs | Infertile & Dead Eggs | % of Viable Eggs | Average Clutch Size |
|---|---|---|---|---|---|---|
| 1985 | 14 | 795 | 661 | 134 | 83.1 | 56.8 (n=14 nests) |
| 1986 | 17 | 1061 | 859 | 202 | 81 | 62.4 (n=17 nests) |
| 1987 | No Harvest | | | | | |
| 1988 | 13 | 13 | 793 | 647 | 146 | 81.6 |
| 1989 | 20 | 20 | 1329 | 1198 | 131 | 90.1 |
| 1990 | 29 | 1613 | 1324 | 289 | 82.1 | 57.6 |
| 1991 | No Harvest | | | | | |
| 1992 | 35 | 2066 | 1656 | 410 | 80.2 | 59 |
| 1993 | No Harvest | | | | | |
| 1994 | 29 | 1726 | 1545 | 181 | 89.5 | 59.4 |
| 1995 | No Harvest | | | | | |
| 1996 | 47 | 2722 | 2145 | 577 | 78.8 | 57.9 |
| 1997 | No Harvest | | | | | |
| 1998 | 36 | 1983 | 1591 | 392 | 80.2 | 55.08 |
| 1999 | No Harvest | | | | | |
| 2000 | No Harvest | | | | | |
| 2001 | No Harvest | | | | | |
| 2002 | 62 | 3542 | 2772 | 770 | 78.3 | 57.13 |
| 2003 | 138 | 9518 | 7817 | 1701 | 81.4 | 60.61 |
| 2004 | 215 | 12756 | 10261 | 2495 | 80.4 | 59.3 |
| 2005 | 202 | 12140 | 9604 | 2536 | 79.1 | 60.1 |
| 2006 | 285 | 17006 | 13491 | 3515 | 79.3 | 59.7 |
| 2007 | 215 | 13390 | 10946 | 2444 | 81.7 | 62.3 |
| 2008 | | | | | | |

## NESTING EFFORT OF PRIMARY SITES

| Village | Name of Lagoon (Location) | Number of Nests (Year 2005) |
|---|---|---|
| SWAGAP | WABOGWA | 4 |
| | KAMSI | 3 |
| | NINGYUM | 6 |
| | LAU | 1 |
| | PREMBET | 5 |
| | KIAGUA | 1 |
| | PASMAIWA | 1 |
| | BOSWAL | 2 |
| | WEDOGBO | 2 |
| | SOAD | 2 |
| | DUABLO | 4 |
| | MUMBARA | 1 |
| | NOMBARA | 1 |
| | WARIG | 3 |
| | KAIYA | 1 |
| | NILUM | 2 |
| | KOKOBAGUWA | 1 |
| | **Total** | **40** |

| Village | Name of Lagoon (Location) | Number of Nests (Year 2005) |
|---|---|---|
| KUBKAIN | NANYANGWA | 1 |
| | YAHULUS | 3 |
| | LEGBAGTU | 1 |
| | GULHUA | 1 |
| | NEBGUBAG | 8 |
| | NEBGUBOS | 1 |
| | HUGNO | 2 |
| | GANGUEL | 3 |
| | WIDIHUK | 1 |
| | BALUWE | 3 |
| | **Total** | **24** |

| Village | Name of Lagoon (Location) | Number of Nests (Year 2005) |
|---|---|---|
| BAKU | PERLIT | 2 |
| | GUMAMO | 2 |
| | KERIBESIMO | 7 |
| | YARAK | 4 |
| | YANUK | 1 |
| | KARKOS | 1 |
| | SIP | 2 |
| | **Total** | **19** |

## MODEL OF EGG HARVEST PROGRAM

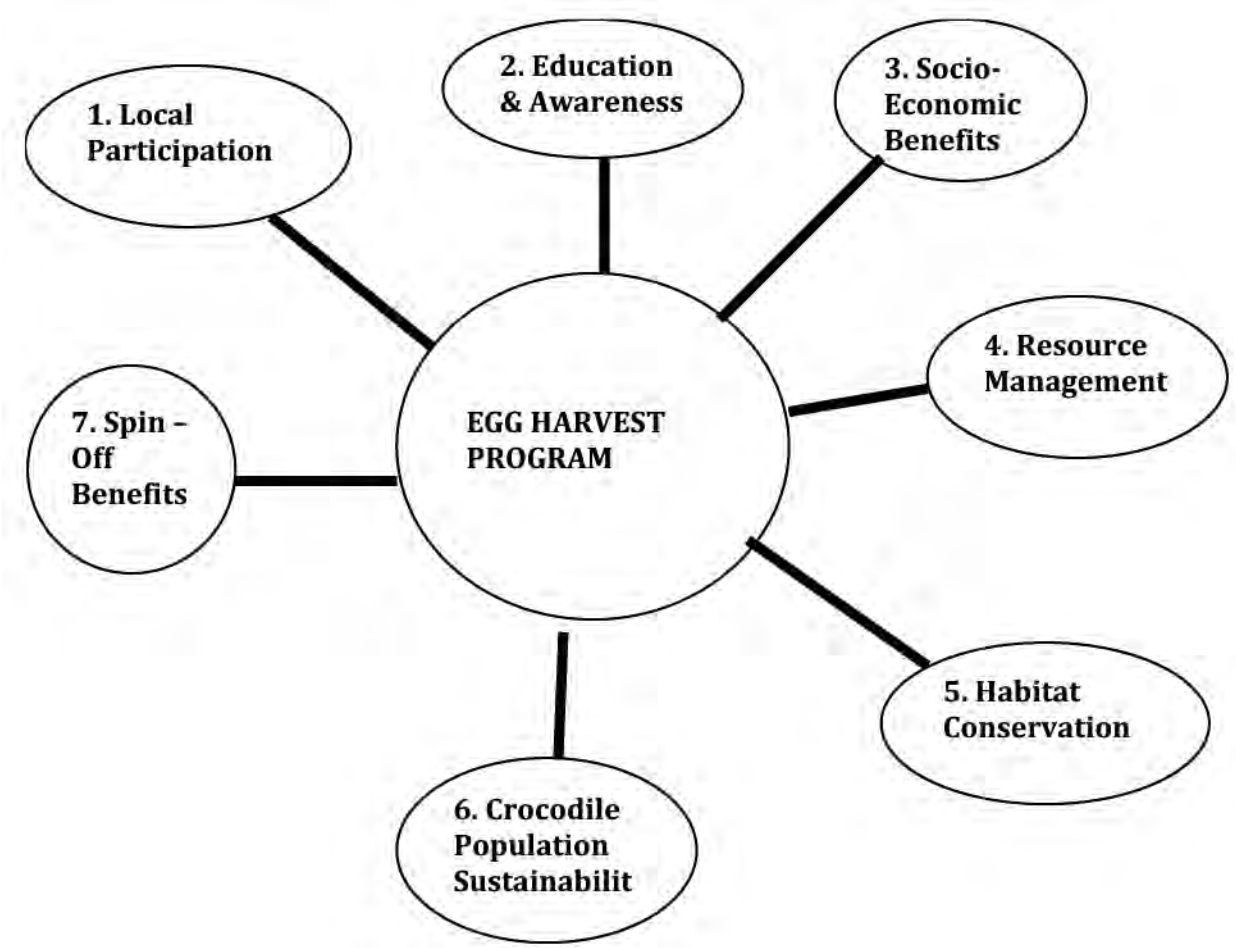



**Figure 1.** Model of egg harvest program.

## ACHIEVEMENTS FROM THE EGG HARVEST PROGRAM

**1**. Local communities
**2.** Habitat rehabilitation
**3.** Population enhancement
**4.** SWMI – United Nation's Equatorial award

## PNG GOVERNMENT STRATEGIC DIRECTIONS

- MTDS
- Amendment of International Trade Act

## INTERNATIONAL OBLIGATIONS

- CITES
- CSG/IUCN

## CURRENT EFFORTS TOWARDS WETLANDS HABITAT CONSERVATION

### Sepik Wetlands Management Initiative (SWMI)

The current efforts by the Sepik Wetlands Management Initiative (SWMI) a Community Based Organization in Ambunti, to carry out public awareness campaigns with the major objective of protecting wetland habitats. A clear example is the construction signboards at important primary survey sites to promote awareness on the habitat protection program is very helpful and complements the current DEC crocodile monitoring program

The SWMI in particular has emphasized and ensured the reduction in burning of nesting habitat in the survey areas. Burning is a cultural practice by locals in order to gain access to fishing and hunting areas. The awareness programme by SWMI, in recent years has resulted in reduction of burning in many of the primary nesting sites surveyed. It is evident the public awareness carried out has resulted in an increase in nesting activity in some sites

### Egg Harvest Program – A Conservation Tool

During the past two years, the nest owner's conservation program of egg collection has being continued to be carried out by Mainland Holdings (Cox *et. al* 2006). As part of Mainland Holdings captive breeding program, the egg harvest program is particularly aimed at salvaging crocodile nests that are prone to flooding and other natural predation, which accounts for over 40-45 % of egg losses in the wild (Cox and Solmu 1996).

The benefits of this program have played a significant role in the socio-economic development in these communities over the years. The egg harvest program provides financial incentives for the resource owners to protect nesting habitats from practices such as burning and crocodile nests from predation.

A primary aim of the egg harvest program is to promote habitat conservation, which offers nest owners an incentive for not burning nesting habitats thus substantially increasing the benefit derived from their resources. This over time has enabled resource owners to link habitat protection and the incentive through cash payments.

### Future Approach - Community Development

- Infrastructure
- Tax Credit Scheme
- Prov & LLG support

## CONCLUSION

The current management program for both CITES protected species (*C.porosus* and *C.novaeguineae*) although is effective however, needs to be reviewed to look at a long term management plan, to cater for the growing demands of the export commodity taking into consideration the sustainability of the wild population.

Therefore, Papua New Guinea has a viable and probably increasing wild *C. porosus* population,

as indicated by the increasing nesting trend from the analysis of the survey results. Meanwhile, *C. novaeguineae* indicates positive signs of improved nesting effort.

The primary aim of the crocodile monitoring program is to determine if population trends are increasing, decreasing, or are stable. This should be achieved through analyzing raw nest counts from the same sites surveyed consistently over the same years. Maintaining the 41 sites surveyed since 1982 for *C.porosus* and 48 sites since 1981 for *C. novaeguineae* is crucial in determining this. It should be noted the additional sites surveyed between 1989 and 2005 should be maintained as these data could be useful in future analysis of other nesting trends.

The increase in nesting activity over the past two years is attributed to habitat conservation initiatives and protection of crocodile nesting habitat in most of the primary nesting sites.

**Recommendations**

- Although increasing to stable nesting trends are depicted for both crocodile species, it is critical the long term monitoring programme is maintained by DEC and ensure sufficient funds are available on an annual to biannual basis.
- Capacity building is critical to ensure new scientists and community based organizations are adequately trained through hands on experience to ensure sustainability of the program
- Support existing programs such SWMI and WWF etc.
- GIS of survey sites and production of aerial survey photographs as sites have changed over time (long term)
- Crocodile harvest data needs to be continued to be collated as a secondary monitoring exercise to the aerial surveys to indicate harvest levels and trends by provinces and size limits..
- Continue to stream line surveys through carrying out surveys on a biannual basis and erasing surveys routes which indicate zero nesting over time
- Promote preliminary research and surveys with international organizations, government agencies regarding impact of introduced fish on crocodile nesting habitats.

**ACKNOWLEDGEMENTS**

Firstly our thanks to the Department of Environment and Conservation, especially Dr. Wari Iamo, Secretary of the Department, for organizing funding for the conduct of the aerial surveys. We extend our appreciation to Benny Gowep for been part of the survey team for nearly two decades.

The support of the Crocodile Industry, Greg Mitch of Bush Development Corporation and Mainland Holdings is acknowledged. Without their continuous support this report would not have been presented at the CSG meeting in France.

All these persons and organizations have in one way or the other been instrumental in the crocodile program over the many years; John M. Genolagani, Godfrid C. Solmu, Jack Cox, David Wilkins, Juda Nundima, SWMI, and SIL Aviation,

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# Report on the II National Workshop "Cuban Crocodile Status and Conservation Priorities"

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**ABSTRACT:** This will be a report on the forthcoming Workshop on Cuban Crocodile Status and Conservation Priorities (to be held May 12-17, 2008), an update of the one held in June 2006. This time, the discussions will include a more broad range of participants, including government representatives and decision makers at different levels, community members and guest specialists from different countries, who will discuss all issues related to Cuban crocodile status, present impacts and conservation strategy. Among the novelties that will be subject of discussion, are the preliminary results of ongoing research on Population Genetics and their implications for Cuban crocodile conservation. This report will be like hot, just baked bread. Obviously, we cannot forward an Abstract in advance.

# Distribution Pattern of Mugger crocodile population in Iran

**Asghar Mobaraki1, Siamak Yousefi[2], Amir M. Elmi[1], Elham Abtin[3], Ashraf A. Hosseini[3]**

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**ABSTRACT:** The existing small population of Mugger crocodile inhabit in southeastern part of Iran near Pakistan Border. The small but scattered population occupies vast type of fresh water habitats in the area. The main habitats could be classified in two main natural and artificial habitats. The main natural habitats are the small and large ponds along the main rivers, Kaju, sarbaz and Bahukalat. Most of these ponds have similar characteristics providing suitable habitats for the crocodiles. Generally, crocodiles avoid from shallow and running parts of the rivers and prefer fairly deep and calm parts of the rivers with suitable vegetation and sandy banks. As the other main habitat type, artificial water bodies also play essential support for the crocodile population too. Small and large ponds nearby villages constructed for the rain water storage as well as the dams constructed along the rivers supposed to be important habitats for the crocodiles too (Mobaraki 2002). The main part of the crocodile range due to its importance as crocodile habitat designated as “Protected area” named “Gandou” (local name for the crocodiles), more over some parts of the area also have designated as 19th Ramsar site of the country which annually host large numbers of migratory birds. The movement of crocodiles between the habitats is a quite usual recorded behavior in the area. In most habitats the crocodiles have close contact with local people. Different fish, amphibian and bird species are the main food resources for the crocodiles in these habitats. Some ponds in border area are supposed to be as crocodile habitats too, and some reports from local people indicating movement between the habitats of Iran and Pakistan have reported. Constructed dams on the main rivers had important effects on the habitats too.

# Recent Finds on mugger Crocodile study in Iran

**Asghar Mobaraki[1], Elham Abtin[2], Amir M. Elmi[1], Ashraf A. Hosseini[2] and Siamak Yousefi[3].**

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**ABSTRACT:** Mugger population was quite unstudied species in the country before. The main existing information was for the far years reported by Minton 1966, Kinunen 1970, Tuck 1975 and Anderson 1979. So in the past years field work and studies in order to collect information on biology and other different aspects of crocodile life cycle have been as a main activity. Reproduction of crocodiles was one of the important subjects that the gained information was as the first records in the country. Nesting season, location of the nests, eggs, hatchlings and their mortality rate were studied in this relation. Nesting season for Mugger crocodiles in Iran starts in April- May and the eggs hatch in June-July. We have records of 24 and 26 eggs with 8 cm length, 4.7 widths and 80-90 gr. Weight (Mobaraki 1998, 2002). The location of the different found nests indicates that in normal situation the crocodiles use fixed specific areas for the nesting. The results gained from collected hatchlings from 3 nursery sites show average length of 30.25 cm and 87.35 g body weight, N= 8 hatchlings, (Mobaraki and et all 2006). Relative to the number of eggs, the number of surviving hatchlings indicates a high mortality. The main feeding behaviors and resources of crocodiles through fecal sample collections also was identified which shows the crocodiles use different sources as food like insects, birds and fish species, but they are mainly dependant on fishes. Burrowing and movement between different habitats and water bodies were the most common recorded behaviors of crocodiles. The movement behavior seems to be a potential threat for the crocodiles too (Mobaraki and Abtin 2007).

# Eggs harvest records versus night counts in *Caiman latirostris*: What do they really mean?

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**ABSTRACT:** Information on the situation of crocodilians population under management (i.e. ranching), is crucial in order to verify the sustainability of the program, but it seems very difficult to be achieved with the standard night counts monitoring techniques in species with cryptic behavior like *Caiman latirostris*; so we present and discuss the value of the information on 15 years of eggs harvesting in one of the most studied populations of the species, in a location where harvest effort was similar among years since the beginning of the project, and where the majority of the nests (90% to 95%) were always available for the eggs collection. We compared this information with our monitoring records in the same period and location. We found that nevertheless night counts showed an average recovery of the population of 50%, the number of nests and eggs harvested increased in 750%. On the other hand, we found that negative environmental conditions like droughts, affected positively the results of the night counts, with more animals counted per km. (9.2), but clearly, those years the egg harvest was lower than the ones with average environmental conditions, which produces 5 or 6 times more eggs, at the same time night counts were of 5 to 6 animals per km. Finally, we also found that the major increase in eggs harvesting, happened after the ninth year from the first releasing, which coincide with the published information on the age of sexual maturity of the species. We are proposing here to utilize the information on eggs harvest, combined with the night counts results, in order to be able to evaluate the population status of species with habitat preferences such as *C. latirostris.*

## INTRODUCTION

Information on the situation of crocodilians population under management (i.e. ranching), is crucial in order to verify the sustainability of the program, but it seems very difficult to be achieved with the standard night counts monitoring techniques in species with cryptic behavior such as *Caiman latirostris.*

The Broad-Souted Caiman is one of the species that mostly uses heavily vegetated and shallow water environments, this makes really difficult to obtain reliable information on the populational situation because those places are often almost inaccessible for vehicles and people.



## METHODOLOGY

Here we present information of 15 years of eggs harvests in one of the most studied populations of the species, in a location where harvest effort was similar among years since the beginning of the project, and where the majority of the nests (90% to 95%) were always available for the eggs collection, called Laguna El Fisco. We compared this information with our monitoring records in the same period and location.

## RESULTS AND DISCUSSION

We found that night counts showed an average recovery of the population from less than 1 caiman per kilometer, up to almost 10 caimans per kilometer in 2000, then decreased to 4 caimans per kilometer in 2006 and 2007 (Fig nº 1).

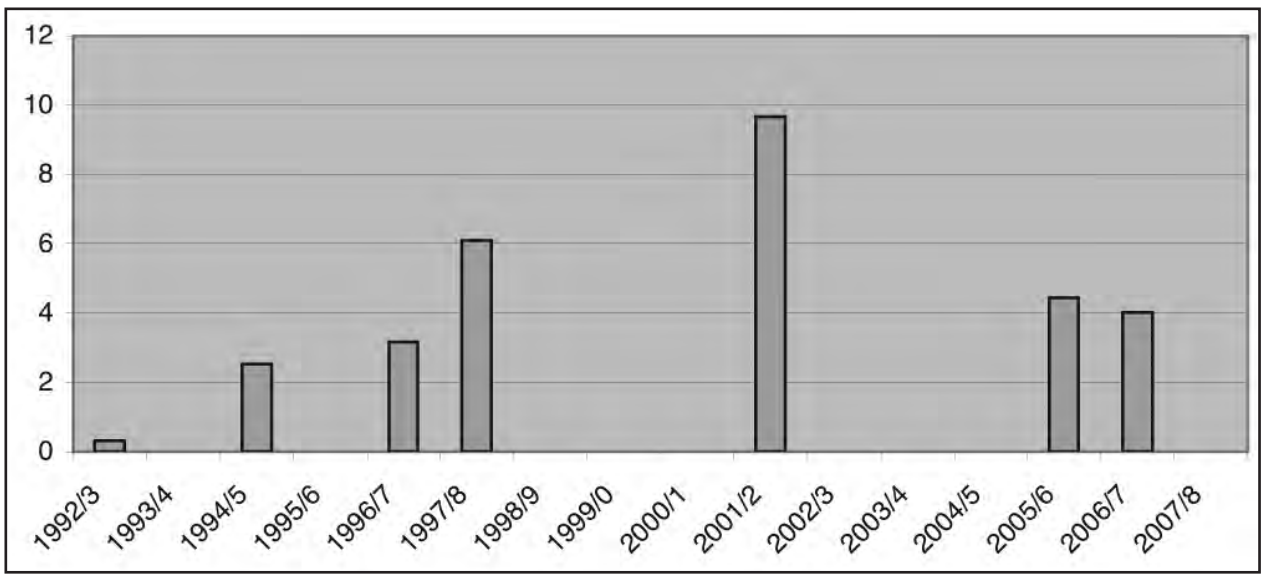



**Figure 1.** Night counts from 1992 to 2007.

Meantime, the number of nests and eggs harvested increased in 750%. (Fig. nº 2).

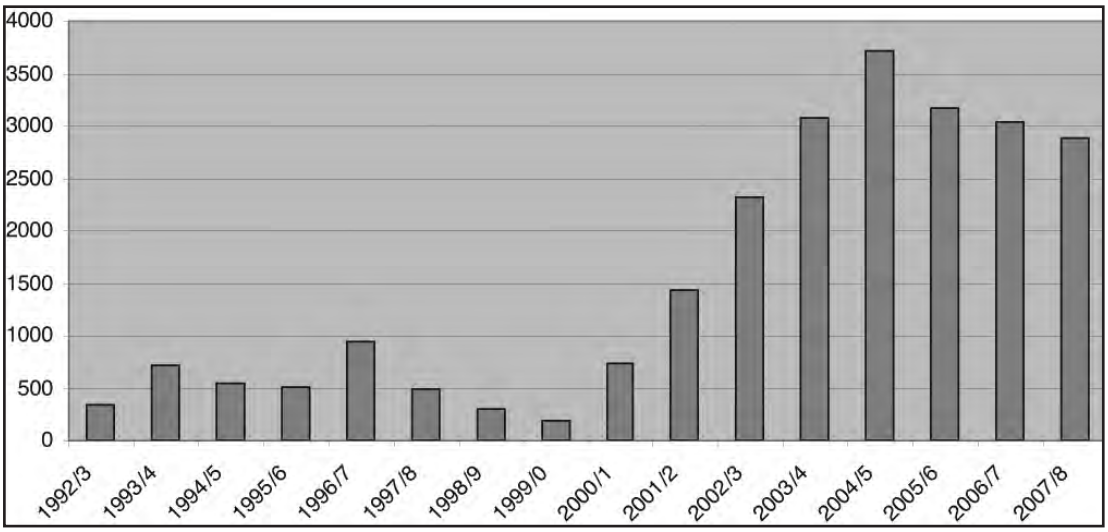



**Figure 2.** Eggs Harvest from 1992 to 2007.

Even thought that night counts seems to stabilize after year 2006 and 2007 at a level similar or even lower than in 1997 and 2000, the eggs harvest continued increasing, mostly after year 2001. This is actually coherent with the fact that at that time, most of the released caimans at the beginning of the 90's, supposedly must start to breed, accordingly with the published information on the age of sexual maturity for the species (Larriera *et al*., 2006). On the other hand, we found that negative environmental conditions like the drought in 2000, affected positively the results of the night counts, with more animals counted (9.2 ind. $Km^{-1}$), but clearly, those years the egg harvest was lower than the ones with average environmental conditions, which produced 5 or 6 times more eggs. We are proposing here to utilize the information on eggs harvest when possible, combined with the night counts results, in order to improve accuracy on the evaluation of the population status of species with habitat preferences such as *C. latirostris*.

# Hatching success of black caiman (*Melanosuchus niger*) nests and spatial relations on egg collection by humans in the Mamirauá Sustainable Development Reserve, Brazil

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**ABSTRACT:** Predation and flooding have been identified as the main causes of egg mortality for many crocodilians species. Mortality could be due to egg consumption by humans, a common practice in the Mamirauá Sustainable Development Reserve (MSDR). This study is aimed to answer the question: is the egg collection by people related to nest proximity to human communities? We searched for black caiman nests walking on the shore of 41 water bodies. We visited all nests twice to determine nest fate. We found 148 black caiman nests in 21 of the 41 studied water bodies. The proportion of hatched nests was 15%, flooding was responsible for egg mortality in 13% of nests; 70% of nests were predated. Humans were responsible for 36% of all predated nests (about 1140 eggs). Tegu lizard (*Tupinambis* sp), jaguar (*Panthera onca*) and probably brown-capuchin monkey (*Cebus apella*) were responsible for 63.7% of all predated nests.
We developed a cost-distance model to find out if the egg collection is limited by the distance to the lakes. We related the cost-distance values of each lake with the proportions of eggs taken, through regression models. We did not find influences of the cost-distance values on the proportions of nests collected by people. This suggests that there are economic incentives that justify travel efforts to the interior lakes. Pirarucu (*Arapaima gigas*) fishing, which coincides spatially and temporally with black caiman nesting, could be the incentive indirectly associated with egg collection. Since black caiman sustainable harvesting programs are being developed in the MSDR, it is necessary to establish limits on the human consumption of eggs in order not to compromise potential earnings from managed caiman hunting and population dynamics.

**RESUMO:** Predação e inundação de ninhos têm sido identificadas como as principais causas de mortalidade de ovos de muitas espécies de crocodilianos. A mortalidade pode se - dar por consumo humano de ovos, uma prática comum na RDS Mamirauá. Esse estudo pretende responder a pergunta: a coleta humana dos ovos de jacaré-açu está relacionada com a proximidade dos ninhos com as comunidades humanas locais? Percorremos a pé a margem de 41 corpos hídricos na procura de ninhos de jacaré-açu. Todos os ninhos foram visitados duas vezes para determinar seu destino. Encontramos 148 ninhos de jacaré-açu em 21 dos 41 corpos hídricos estudados. A proporção de ninhos eclodidos foi de 15%, inundações foram responsáveis pela mortalidade de ovos em 13% dos ninhos. 70% dos ninhos foram predados. O ser humano foi responsável pela predação de 36% dos ninhos (aproximadamente 1140 ovos). O jacurarú (*Tupinambis* sp.), a onça-pintada (*Panthera onca*) e provavelmente o macaco-prego (*Cebus apella*) foram responsáveis por 63.7% dos ninhos predados. Desenvolvemos um modelo custo-distância para saber se a coleta de ovos de jacaré-açu é

limitada pela distância e a dificuldade para chegar aos lagos. Relacionamos os valores custo-distância de cada lago com as proporções de ninhos consumidos, a través de modelos de regressões. Não houve influência do valor custo-distância dos lagos na proporção de ninhos coletados por ribeirinhos. Isso sugere que existem incentivos econômicos que justifiquem o esforço de deslocamento até os lagos. A pesca de pirarucú (*Arapaima gigas*), que coincide espacial e temporalmente com a nidificação do jacaré-açu, poderia ser o incentivo associado indiretamente à coleta de ovos de jacarés. Sendo que programas de aproveitamento sustentável de jacaré-açu estão sendo desenvolvidos na RDSM, é necessário estabelecer limites no consumo humano de ovos de jacarés através de discussões com as comunidades locais para não comprometer a dinâmica populacional e os potenciais ingressos econômicos do programa de manejo.

## INTRODUCTION

The development of crocodilian sustainable-use programs requires the estimation of reproductive and survivorship parameters as well as age and sexual structure of the population (Webb and Smith 1987). Egg, hatchling and juvenile survival rates are considered to be among the most important factors affecting population growth of *Crocodylus niloticus* and *C. johnsoni* (Hutton 1984; Smith and Webb 1985 in Webb and Smith 1987). Predation (Deitz and Hines 1980; Hussain 1999) and flooding (Webb *et al*. 1977; Kushlan and Jacobsen 1990; Campos 1993; Allsteadt 1994) have been identified as major causes of egg mortality for many crocodilian species.

The black caiman (*Melanosuchus niger*) is the largest alligatorid in South America (Ross 1998) and has the second heaviest average clutch mass in the order Crocodylia (Thorbjarnarson 1996). Although individual crocodilian eggs contain a small proportion of proteins and lipids (Manolis et al. 1987), total clutch masses average 0.67 and 6.2 kg (Thorbjarnarson 1996), and likely represent an important source of energy for nest predators. Although Amazonian turtle eggs are frequently traded (Klemens and Thorbjarnarson 1995; Moll and Moll 2004), there is no similar information on human use of crocodilian eggs. In the Mamiraua Sustainable Development Reserve (MSDR) it is common to find caiman eggs being collected by local people. Thus, the present study is aimed to determine if egg collection pressure is related with the proximity to human local villages. The objectives of this study were to: 1. quantify hatching success and mortality, 2. identify the causes of egg mortality and 3. examine the spatial relationships of egg collection by humans in the Jarauá Sector of the MSDR, during the 2007 nesting season.

## METHODOLOGY

### Study Area – The Mamiraua Sustainable Development Reserve (MDSR)

The MDSR is the largest protected *Varzea* (subject to flooding by high sediment load waters) forest in the Amazon Basin, with an area of 1.124.000 ha. It is located at the confluence of the Japurá and Solimões rivers, about 600 km west of Manaus, in the Central Amazon, Brazil. This study was conducted in an area of 25,500 ha (about one half of the total area of the Jarauá sector), located in the southeastern portion of the MSDR (Figure 1). The human population of the MSDR consists of 5,087 inhabitants and users (Data: Demographic Survey Mamirauá, 2006-MSDI), locally referred to as "*ribeirinhos*". The MSDR is a protected area

where black caiman sustainable harvesting programs are being undertaken. Varzea forests in the Central Amazon are characterized by periodic flooding with mean amplitudes of 10 m (Junk *et al*. 1989). In the MSDR, the highest water levels occur between May and June, and the lowest water levels between September and November (Ayres 1993). Black caiman nesting season occurs during low water levels (from September to December).

During the low water period, large volumes of water leave a great portion of the MSDR and many interior water bodies lose physical connection with the main water course. These water bodies are referred to as interior lakes, and have been identified as preferred black caiman nesting areas (Da Silveira and Thorbjarnarson 1997). These lakes are also home to the commercially valuable pirarucu fish (*Arapaima gigas*) (Castello 2008) which is traditionally captured during low-water periods.

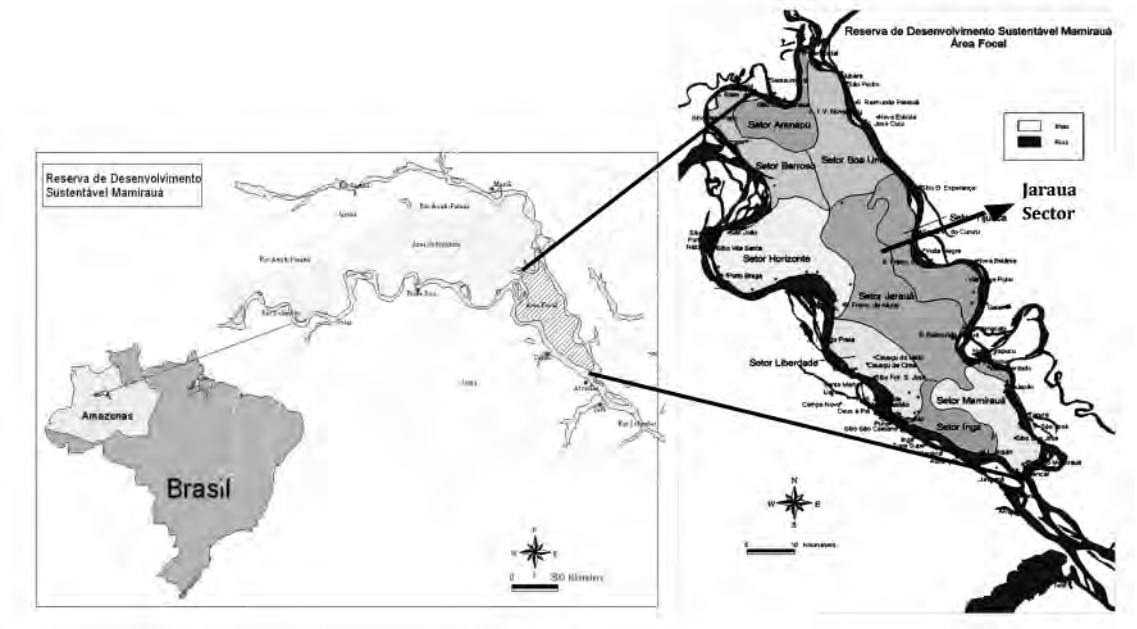



**Figure 1.** Location of the Study area. Source: Mamirauá Sustainable Development Institute.

**Data Collection** – We studied black caiman nesting in 41 interior water bodies between September 2007 and January 2008. We located the nests walking on the shores around the perimeters of water bodies. For this study, the lake margins were defined as the terrestrial area up to 20 meters away from the edge of the water. To determine nest fate, we visited all the nests found at the beginning and at the end of the incubation period, with a 30 to 60 day-interval between each visit. Nest fate was determined observing marks and egg shells left around the nests. In about one third of the studied nests we collected data of clutch and egg size.

**Spatial Modelling** – We developed a cost-distance model using the IDRISI Kilimanjaro program to find spatial relationships of egg collection by the people from the São Raimundo de Jarauá community, the principal users of lakes in the study area, this methodology was adapted from Seijas (2001). The cost-distance model is a tool that integrates remote sensing data (Landsat TM5 satellite images) with geographic-information-system information, in order to quantify how difficult (*i.e.* costly) it is for the *ribeirinhos* to get from their community to any one of the studied lakes, moving through surfaces with different degrees of locomotion

difficulties (*i.e.* friction values). This was accomplished by creating a map of land covers with different values of friction to represent the relative cost of moving through each land cover type. The main criterion used to calculate the friction values was the mean velocity in which a *ribeirinho* travels on water using a 5 hp boat (between 5 to 10 km/h) or walks on land through different types of várzea forests (between 0.2 to 1 km/h) (See Table 1 for details of this calculation). Using these friction values and the distance between the community and the lakes, the model calculates a cost-distance value for each lake. High cost-distance values represent lakes located far from the community with difficult access (lakes in which the *ribeirinho* have to walk great distances on land), whereas low cost-distance values represent more accessible lakes located close to the community. We excluded from the analyses lakes where nests were absent. Finally, cost-distance values were related through regression models to the proportion of nests collected by ribeirinhos in each lake.

**Table 1**. Criteria used to calculate friction values for each land cover type.

| Land cover | Mean velocity (km/h) | Friction value | Explanation |
|---|---|---|---|
| Open water | 10 | 1 | The base cost, the easiest and most utilized way to travel by ribeirinhos in the varzea |
| Water with exposed soils and floating meadows | 5 | 2 | Floating meadows impose higher difficulties to go trough |
| High varzea forest | 1 | 10 | Relatively easy to walk but ribeirinhos have to carry heavy things as paddle canoes and fish |
| Low varzea forest | 1 | 10 | Relatively easy to walk but ribeirinhos have to carry heavy things as paddle canoes and fish |
| Chavascal forest | 0.2 | 50 | Very difficult to walk, virtually a barrier. Ribeirinhos avoid to walk on these poorly-drained topographical depressions |

## RESULTS

We located 148 *M. niger* nests in 21of the 41 water bodies. We documented successful hatching in only 15% of the nests. Flooding was responsible for egg mortality in 13% of the nests, and in 3.4% of nests it was not possible to determine the nest fate (the nests were not found on the second visit). Predation was the main cause of egg mortality, totaling 69% of all studied nests.

*Ribeirinhos* were responsible for 36.3% of all nest predation (37 nests with mean clutch size of 30.8 eggs, about 1140 eggs). Other predators as tegu lizards (*Tupinambis* sp.), jaguar (*Panthera onca*), and probably brown capuchin monkeys (*Cebus apella*) were responsible for 63.7% of nest predation.

The cost-distance model identified the “Urucuraninha do cedro grande I” lake as the most costly for the community members to reach, as opposed to “Cobra” lake, which was the least

costly (Figure 2). Egg collection was found in both most and least costly to reach lakes, the regression models did not find a relationship between the cost-distance value and the proportion of nests collected by *ribeirinhos* ($R^2$= 0.002, P= 0.852). (Figure 3).

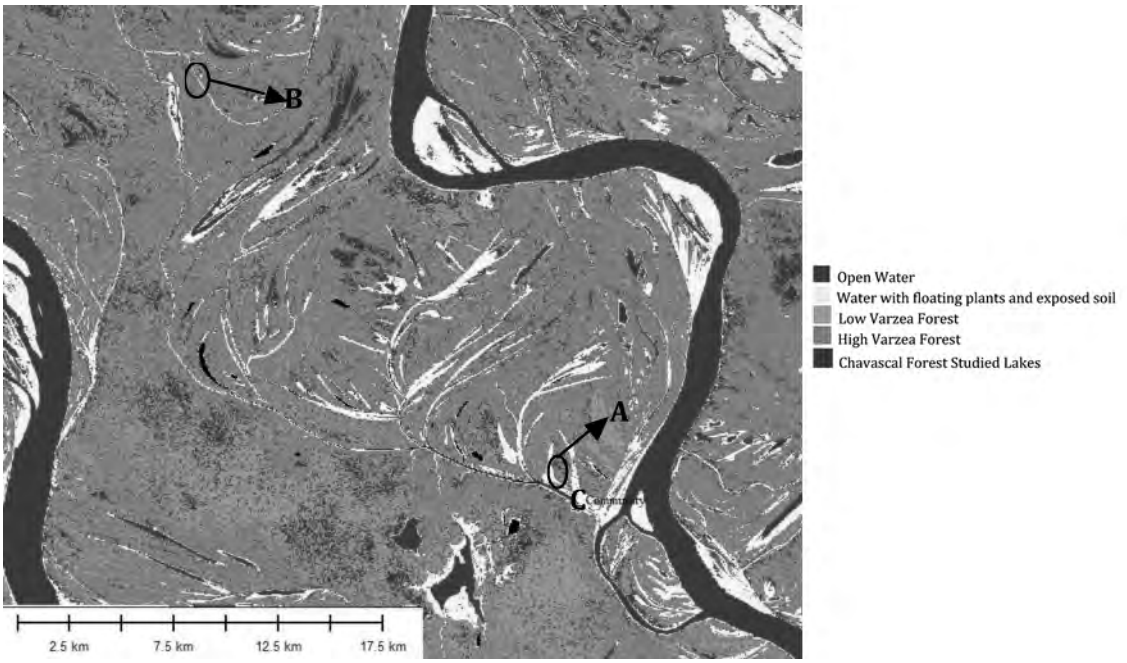



**Figure 2.** Land cover types, studied lakes, and Jarauá community in the Jaraua sector, RSDM A= Cobra lake (the least costly); B= Urucuraninha do Cedro Grande lake (the most costly); and C= Jarauá community.

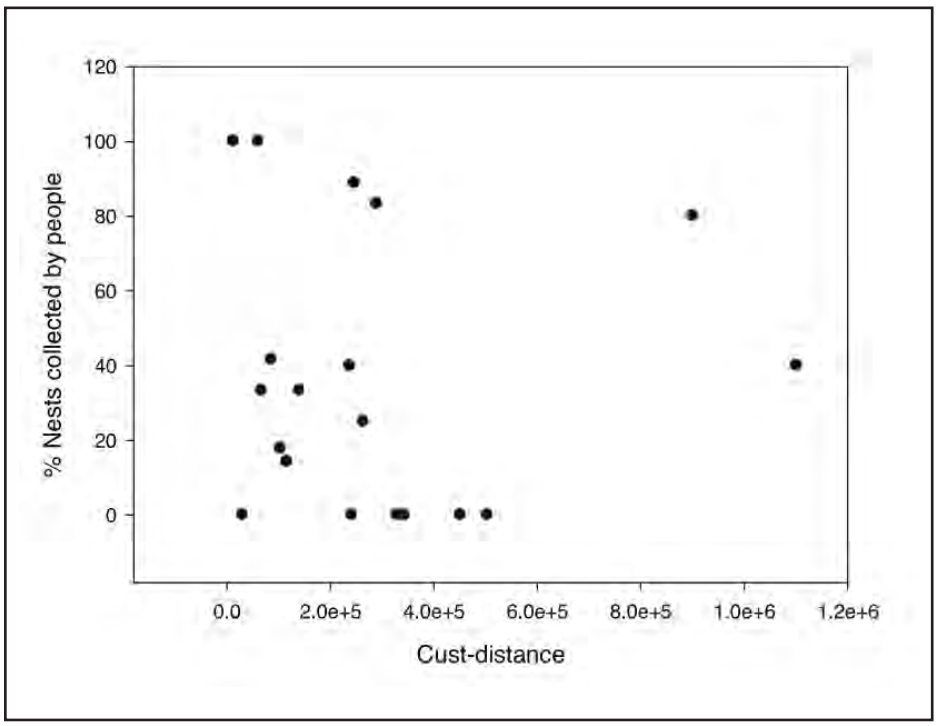



**Figure 3.** Plot showing the percentage of nests predated by humans in relation to the cost-distance values in each lake.

## DISCUSSION

One of the few publications where black caiman hatching success in the wild is reported suggested that flooding of nests is the main cause of egg mortality, while predation is negligible in two localities of the Ecuadorian Amazon (Villamarin-Jurado and Suarez 2007). For other crocodilians, predation has been identified as the main cause of egg mortality, but in many studies (Cintra 1988; Hussain 1999) the proportion of eggs taken was not higher than the number of eggs that hatched. In this study we found that a very high proportion (~70%) of nests was depredated. The presence of researchers in nesting locations could increase the probability of nests being found by non-human predators as found in *Alligator mississippiensis* (Deitz and Hines 1980). Further analyses that take this into account may explain part of the high predation that we found.

Caiman eggs in the MSDR are collected by *ribeirinhos* in most and least costly to reach lakes. Research on black caiman poaching in the MSDR suggested that the high prices paid for caiman skins in the past acted as an economic incentive for ribeirinhos to reach interior lakes to illegally hunt caimans. Once the skin trade was banned and replaced by a less lucrative meat market (which was still illegal), ribeirinhos quit hunting caimans in interior lakes but did not stop visiting those lakes in order to fish pirarucu (*Arapaima gigas*) (Da Silveira and Thorbjarnarson 1999). This suggests that pirarucu fishing is lucrative enough and justifies the distances to travel to interior lakes.

Today, illegal caiman meat hunting no longer occurs in Jarauá and apparently, there is no egg market in the low Japura river. Consequently the main use for caiman eggs in Jarauá is subsistence human consumption which does not generate economic incentives that would justify, by itself, great distances to travel. Since pirarucu fishing coincides spatially and temporally (between September and November) with black caiman nesting, we suspect that pirarucu fishing might be related to caiman egg collection. During the three-month pirarucu fishing season, no fishing activities other than those for local consumption are allowed in the Jarauá sector. The sustainable use program of pirarucu generated about 500,000 US$ in 2007 in the MSDR (Amaral and Barbosa 2008) and this strong economic incentive explains why the *ribeirinhos* are traveling to interior lakes. Black caiman eggs are a much appreciated aggregate value during pirarucu fishing activities and are a high-energy resource relatively easy to obtain once the ribeirinho is already in the interior lakes. The cost-distance model in this study considered only egg collection by the people from São Raimundo de Jarauá community because they use most often the studied lakes. There exists the possibility that people from communities in other sectors are illegally using the same lakes (Pers. obs.). Since the Jaraua community is the closest village from these lakes, to get into them from other places would mean even higher travel costs. If people from other communities are collecting eggs in the studied lakes, it strongly supports the idea that economic incentives from fishing activities (illegal in this case) justify the travel costs.

Caiman egg consumption is probably a traditional practice in the MSDR that probably existed long before the creation of the reserve, perhaps traditionally associated with fishing activities. Nevertheless, black caiman sustainable-use programs are being implemented in the MSDR and the high levels of egg consumption found in this study may compromise potential earnings from managed caiman hunting and population dynamics. In this sense, it is important to undertake discussions with local communities to address limits for egg collection activities.

It is also important that a long-term nesting monitoring program be implemented in this and other sectors of the reserve where it is possible that the relationships between nest fate and human egg collection are different.

## AKNOWLEGEMENTS

This study was financed by the Wildlife Conservation Society. The Mamiraua Sustainable Development Institute provided logistic support; the Instituto Nacional de Pesquisas Espaciais (INPE) provided the Landsat images. Benor G. de Souza provided field assistance; the Jarauá community people were always helpful in the field. The floating stations guards (Eder and Moacir) help permanently with hidrologic data collection

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# Nesting Habits of the Black Caiman (*Melanosuchus niger*) in the Rupununi Region of Guyana

**Peter Taylor.**

**ABSTRACT:** As part of a collaborative ecological study conducted with local Macushi Amerindians we report on nesting habits of locally healthy populations of black caiman (*Melanosuchus niger*). Complete data sets of detailed information, collected over two seasons report on 29 intact nests. Continuing observations of predation events and environmental perturbations were made. Parameters described included habitat usage and nest density therein, nest dimensions and composition, proximity to water, clutch mass and egg biometrics, insolation of nests, incubation period, temperatures of nests, hatching success, and maternal presence. Where possible hatchlings were later recovered and examined (N = 159 from 11 clutches). Mean clutch size for Rupununi *Melanosuchus* nests is 32. Range is 22 to 39. Eggs are very large. Egg mass means in some clutches exceed 155 grams. More than 80 % of nests are chiefly of leave and twig composition receiving little or no insolation. Most nests are observed in oxbow, lake or pond situations but also occur along rivers. Over 30 % of nests are visited by tegu lizards (*Tupinambis teguixin*) usually resulting in complete predation events. Attending female caiman are observed near nests over 80% of the time. Evidence is strong that mother caiman assist hatching of nests. Strong aftercare of offspring is observed.

# A simple method of incubating crocodilian eggs for conservation aims

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**ABSTRACT:** Incubation of crocodilian eggs has been developed through many years and several trial and error methods, beginning with the monitoring and control of wild nests (*in situ*) and subsequently with systems that utilize seminatural and artificial incubation techniques that involve the direct manipulation of eggs. Nevertheless, the two first methods do not allow for the achievement of a controlled environment in which large scale egg incubation can be attained which is a very valuable tool in commercial captive breeding programs in addition to other programs that are focused on restocking wild populations. Very often incubation chambers are of great complexity and this limits their use in rural areas where the management of several clutches is needed in order to accomplish objectives that go together with a crocodilian conservation program.
The construction and design of an incubation chamber that is both economical and functional is very important and more so being that it can be used as an important tool in the management of endangered species of crocodilians. These incubators can be used by those communities that live side by side with these reptiles and may want to benefit from the sustainable use of these species coupled with the available wetland biodiversity in general. This system has been in use for more than ten years and applied in several conservation programs that include industrial and communitarian efforts. These techniques offer an alternative and efficient option for the management of wild populations.

**RESUMEN** El manejo de la incubación de huevos de *Crocodylia* se ha desarrollado durante muchos años mediante diferentes métodos, iniciando con el control y monitoreo de las nidadas *in situ*, y posteriormente realizando métodos de incubación seminaturales y artificiales que involucran la manipulación de los huevos. Sin embargo los dos primeros métodos no permiten la realización de incubaciones controladas a gran escala lo cual es de gran utilidad en los programas comerciales y de conservación. Normalmente las cámaras de incubación son de gran complejidad y esto dificulta su instalación en áreas rurales en donde se requeriría el manejo de múltiples nidadas para lograr los objetivos de los programas de conservación de *Crocodylia.*
El diseño y construcción de una cámara de incubación de fácil elaboración se presenta como una sencilla alternativa económicamente viable y funcional sobre la cual se pueden basar ingeniosos programas de manejo de especies de cocodrílidos que estén en peligro o que quieran ser aprovechadas de manera sostenible *in situ* con la participación de las comunidades que viven de la biodiversidad de los humedales.
El sistema ha sido validado por más de 10 años en diversos programas tanto industriales como comunitarios y actualmente es una eficiente alternativa de manejo de las poblaciones naturales.

**Sex Determination in Reptiles**

In reptiles, there are numerous species in which sex is determined by differences in incubation temperature and not by genetic fixation. That is why it is probable that in times of increasing temperatures caused by global warming, crocodilians could be one of the most affected groups of vertebrates. In hypothetical cases, increases in temperature affecting a specific region (inland, wetland, beachfront or other) and where there are residing populations of reptiles in which sex determination is based on subtle temperature differences probably could be affected drastically and in a negative way by altering the natural sex proportions encountered in healthy populations.

It has been established that in 70 species belonging to 43 families of reptiles, sex is determined by TSD, (Temperature Sex Determination). In turtles there is wide variation, in *Chelidae*, *Trionchidae*, and *Staurotypidae* sex is determined genetically GSD (Genetic Sex Detrmination). Moreover TSD is prevalent in the rest of families of turtles even though there are species with GSD. Belonging to *Chelidridae* there has been a report for the Alligator snapping turtle.

With respect to the studied reptiles the vast majority exhibit GSD while TSD has been described in *Agamidae, Eublepharidae, Gekkonidae* and *Lacertidae*, in addition to all Crocodilians and Tuatara (*Sphenodon punctatus*), while in *Anfisbaenidae,* there has not been any species found to exhibit TSD, in snakes studies have been few and results cited are not determinant so that one can prove the influence of temperature in their sexual development, even though there have been studies with members belonging to *Colubridae*, *Elapidae*, *Pythonidae* and *Viperidae*.

Sex determination caused by variations in incubation temperatures have shown to produce 100% females in the following cases:

- Production of 100% female offspring at lower incubation temperatures with a pivotal temperature in which both males and females are produced in the same proportions.
- Production of 100% females at higher incubation temperatures with a pivotal temperature in which males and females are produced in the same proportions.
- Production of 100% females at lower and higher temperatures in which two pivotal temperatures are present.

The consequences of global warming are more evident in reptiles that exhibit TSD, these variations are converted into potentially dangerous situations in which a given population could generate in different breeding seasons only males or only females with dire consequences to the survival of the species, only the species that exhibit double pivotal temperatures could have an additional alternative in cases of higher temperature exposure, in addition egg losses due to these variations could be of significant importance. It has also been noted that males belonging to the order *Crocodylia* that have been incubated at the fetal stage at temperatures close to those that give rise to females develop penises of smaller size than those whom were incubated at temperatures more towards those that give rise to males (Lance, *verbatim*). At the same time numerous authors have done tests with regards to the influence that incubation temperature has on the adaptability, growth, development, and behavior of these reptiles after hatching.
Techniques focused on the study and control on the factors present in the incubation process

of reptile eggs, especially those of crocodilians have been characterized by not being invasive permitting them to finish their due course in their development naturally. The only material present being cylindrical nets around the nests used to capture the emerging hatchlings. These techniques do not diminish losses in case of flooding, human predation or increased temperatures but exhibit certain benefits as to point to the area in which the nest is located.

In those cases in which a population of crocodilians or other species of reptiles are at high risk due to the loss of nests by way of human intervention like that of floods (Bad use of hydroelectric power), global warming, increased presence of predators due to the inadvertent creation of suitable conditions such as Monitor lizards or Tegus (*Varanidae, Teidae*) in an area, it would be convenient to evaluate the advantages and risks of manipulating wild nests with the purpose of diminishing egg loss and at the same time controlling the sex proportions that are necessary in order to keep a population at an adequate sex ratio.

If studies of this nature determine these factors then it would be possible to develop an incubation technique that is economical, efficient, and precise and that can be applied to the regions where these species are distributed.

**The Incubator**

The incubator is a chamber or closed area constructed for the purpose of maintaining live organisms (mainly eggs) in suitable environmental conditions for their adequate growth and development. Efficient incubators should maintain a temperature and humidity gradient that is constant. (Medrano,2001).

Artificial incubation is a hydrothermic system utilized in order to control and stimulate the development of eggs by artificial methods. In conservation programs that involve the manipulation and monitoring of eggs as well as in production farms at an industrial level, incubation is a necessary link in the chain of knowledge and production. Success in incubation of these eggs permits the hatching and rearing of eggs obtained from captive breeding and egg ranching operations in natural nesting areas.(*op.ct*).

**History**

Incubation of crocodilian eggs is a common practice in those countries that can benefit from the use of this resource. These species are subject to commercial use and man has found a valuable use for these reptiles in the food and fashion industries.

In Colombia the need to establish an efficient technique in order to attain good results in the incubation of caiman eggs (*Caiman crocodilus fuscus*), favored the development of numerous systems and chambers with varying degrees of complexity and costs in which the priority was to achieve procedures that are simple and that allow the incubation of large quantities of eggs at the same time. This objective permitted the design of a vast number of systems and trials, some based on the scientific method and others not so much.

Very few of these systems functioned in an autonomic fashion during the long incubation process and success was attained more through discipline, observation and human effort than to the efficiency of the incubator itself.(Medrano 2001).

The use of previous methods such as water jackets[2] such as those developed in Australia were useless in the sense that these systems were developed for holding a few hundred eggs and not thousands as those encountered in Colombian breeding operations that soared above (20,000) per farm. This was a puzzle that had to be solved being that the humidity and temperature gradients were very hard to stabilize in the large chambers utilized to accommodate the large number of eggs produced year after year. The main concern was that the humidifiers generated permanent flaws in the conventional electrical wiring complicating the incubation process. (*op cit*).

The system would get even more complicated due to the mixing of eggs of different ages with no data as to what female they originated from or when the hatching date was expected. Later on the nests were placed in boxes of different materials and sizes with the objective of separating clutches. Eggs were placed on leaf, mulch and wood shavings.

Water was provided by way of spray bottles or hoses and the temperature through hot stove metal or warmed water reservoirs placed at the bottom of the chamber. This did not allow an even distribution of the humidity and temperature causing high egg mortality.

**Updated System**

Afterward new techniques were developed in order to manage individual clutches with the use of racks, trays and were supplied with water by way of light humidifiers (spray systems and nebulizers). Trays were placed over racks and suspended over water containers that were 3cm deep, air distribution was achieved through a complex system of hoses and tray covers made out of plastic with no adequate control of the trays internal humidity and the bubbling of the water generated by the air hoses caused a permanent loss of clutches. The system was simplified in the year 1994 and later in two years the dry incubator (Medrano 2001) was developed. This incubator utilized the neutral mineral (ph7.2) and reusable known as vermiculite[3]. This material has been used traditionally in several zoos and herptariums and is used as a substrate for the incubation of a wide variety of reptile eggs sometimes *Sphagnum* moss. Vermiculite can also be replaced by zeolite (aluminosilicate) and perlite (amorphous volcanic glass) those of which function efficiently as substrates.

The key to this system lies in the *water/vermiculite* ratio (volume/weight) of 10/1 or 15/1 ratio which is used in the majority of incubators that hatch caiman eggs in Colombia. The ratio most often used is the first and the other minor variations are applied with no dramatic alterations that affect the hatchability of the eggs. With this ratio and with a standard sized tray that is used in our incubation systems the ratio consisting of 1500gr of vermiculite and 150cm$^3$ water was established. This same amount of water added at the beginning is available throughout the incubation process which takes approximately 75 days. Managing this proportion and making sure that the vermiculite is dry prior to adding the water is imperative for success in hatching.

Smaller trays must be evaluated due to the volumes of air that are stored in them once closed and to which the oxygen that will be available to the eggs throughout the incubation process will be supplied.

[2]Isolated chambers in which the walls are subject to a permanent waterfall which runs down the wall in a continuous manner and is accomplished by way of a complex network of pipes, pumps, and heaters in which the desired temperature is achieved through the warming of the water itself.

[3] Expansive medium comprising of the mineral mica with a great capacity for holding water, low cost and easy to obtain.

The use of this material (vermiculite) coupled with the monitoring of other variables and careful collection of data have permitted that this same technique be applied to other species such as *Crocodylus acutus, Iguana iguana, Trachemys scripta callirostris, Kinosternon scorpiodes, Chelonoides carbonaria* and was successfully used in two clutches of Colubrids of the genus *Liophis sp*. and *Oxibelys sp*.

Due to the small volumes of water that are utilized in this system and to the fact that it is added at the beginning of the incubation process there have been reasons to name this incubator a dry incubator which differs markedly in contrast to the Australian Water Jackets. This system is used successfully in 85% of the commercial breeding programs in Colombia.

The dry incubator is of simple design and low cost permitting it to become an important tool in efforts for establishing effective conservation programs focused on many species of reptiles.

From experience it is known that one of the major limiting factors affecting reptile conservation efforts focused on the possibility of giving sustainable use to many species of reptiles such as turtles and crocodilians lies in the adequate management of nests and transfer of egg clutches for protection and control.

**Justification**

Next noted are some considerations which are gathered from Medrano (2001) for the use of artificial incubation systems.

Beforehand there must be clarity as to specify that this system of artificial incubation is an invasive system and must be managed with caution. From every point of view it is clear that natural processes are very important and must be prioritized before any type of intervention is taken. On the other hand certain aspects have to be kept in mind in order to obtain a more detailed analysis of what would be defined at this day and age as a natural process being that this concept can be evaluated in different ways:

- We should assume that global climate change with its rapid warming pattern is a natural process? Or in contrary this global warming is caused and has accelerated because of the natural activities of man? In either case the evaluation focused on whether or not artificial incubation should be developed for conservation purposes should be applied for use with the species that are most affected by these changes[4].

**Social Component**

In another sense, conservation programs must include a strong social component in the sense that native communities must benefit from this resource and must participate actively in the process. For this reason it is important to determine if the conservation program views the possibility of artificial incubation of egg clutches. Such a study must be done in an area where the community participates directly in this process and that it will only work if it is involved. If absent from the process the desired results will probably not be obtained.

[4] Increase in temperature from 1 to 3.5 degrees centigrade in the course of this century

Once there is an active role in the community, there will be a possibility to develop a conservation program that views artificial incubation as a main objective. In an intrinsic manner the development of this objective will have the virtue of being involved with the different participants belonging to these communities in which there will be recognition for women and children that otherwise would not be in contact in an educational and conservation oriented manner with the specimens that are utilized for cultural and commercial reasons.

A system of artificial incubation that is organized and technified is the fundamental principle in an environmental educational program that is focused on a successful long term commitment by way of getting to know in a practical sense two fundamental periods in the life of these organisms; incubation coupled with the delicate handling of eggs and the hatching of the new beings. With intelligent management of this material it would be very difficult for an educational program to fail.

**Development of the System**

In order to implement effectively the dry incubator in a communal environment there has to be a baseline study of the area in order to determine where exactly the system will operate. For this reason there must be a carefully selected area where the incubator will be constructed keeping in mind the following aspects:

- If possible, the area that is chosen should not be more than 15 or 20 minutes by foot from the area where the egg clutches will be collected.[5]
- There must be good shade above the incubator in order to prevent the direct rays of the sun from hitting the incubator directly.
- If this possibility does not exist an area of shade must be constructed above, it could be below the tree line or a cover may be constructed consisting on palm leaves or darkened shade cloth (75% protection) in which the incubator shall be constructed below. A good alternative could be to install and adapt the incubation chamber in a room belonging to a house of some community member that takes part in the project Medrano (2001).
- The chamber must have a volume sufficient enough for the air to be stable with respect to temperature as long as possible. Volumes below $12m^3$ are not recommended.
- Whichever the final design, an incubation chamber located in a specific place or in a makeshift room must include a thermal barrier that is effective so that the temperature of the incubation chamber never exceeds 29 degrees centigrade in the hottest day of the year. In addition to using and effective insulating material with the purpose of keeping the temperature stable inside the egg chamber. Both concepts are different, overheating can be prevented but if there is no insulating material present the temperature will rise and fluctuate rapidly generating unnecessary strain on temperature control devices (thermostats).
- If the area chosen lies with no protection against the elements the incubator must be isolated thermally by way of the previously described methods and in fact be constructed of hollow brick, wood or other insulating material that will prevent the air in the chamber to escape.
- In order to achieve thermal isolation and stability there will be a room built and surrounded by a perimetral corridor limiting with a second wall leaving one

[5] Given the case that these conditions are not encountered the area must be accessible through a motorized vehicle (terrestrial or aquatic) or of animal labor that permits the transport of clutches in a quick and safe manner

meter distance between the first and the second, a chamber within a chamber. Figure 1 and 2.

- In the interior of the chamber a system of racks made out of wood in order to place and organize the egg trays, this rack system must be designed consisting of various levels that are between 5cm above ground to 1.8 cm tall.
- The interior electrical system must contain sufficient outlets and electrical switches.
- Heat will be generated through various methods depending on the size of the chamber. Small chambers will have in their interior a spiral oven heater connected in sequence to a medium speed fan that will push the air within the chamber. This system will turn on and off depending on the thermostat (type sauter) electromecanic, set for this function or other digital device. For this reason the more thermically isolated the chamber the heating device will most likely turn on between long intervals and be in heating mode for short periods of time. If there is no electrical power in the area, the system should be energized by a small power engine. In chambers comprising of larger incubation chambers the hot air will be conducted through 6" PVC pipe that is perforated with small holes and installed.

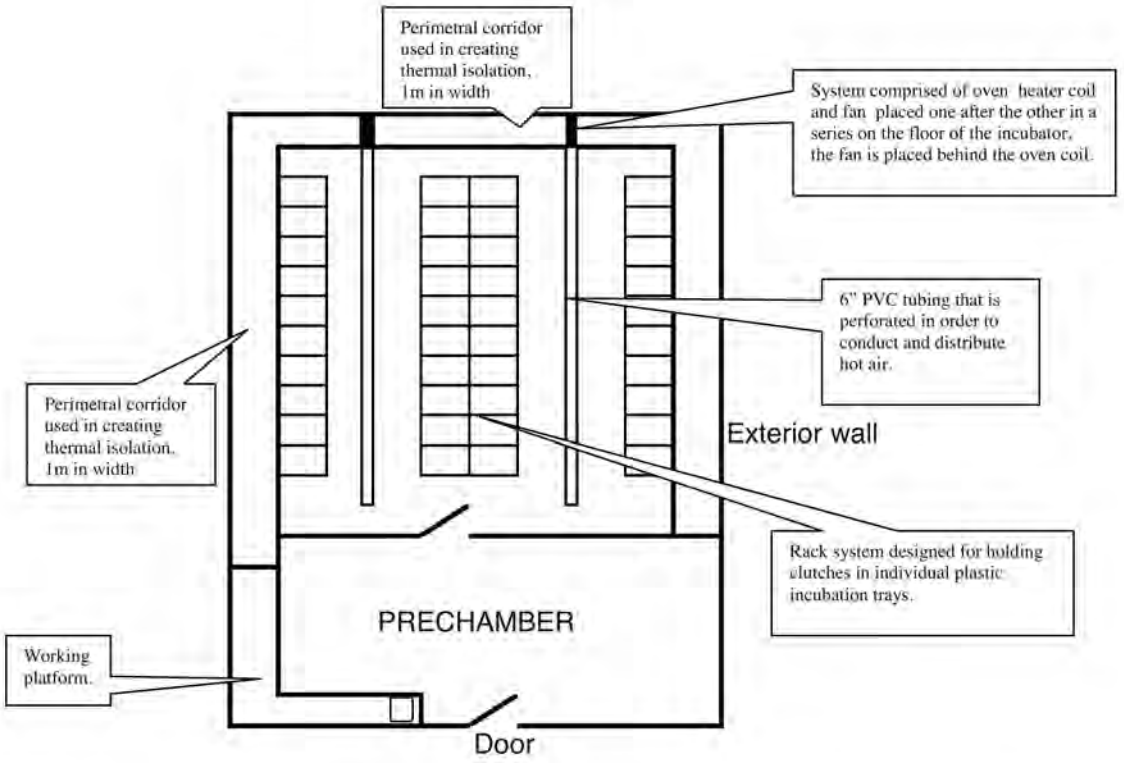



**Figure 1**. Illustration showing incubation chamber. The air chamber created between the two walls in the perimetral corridor is fundamental in keeping the incubators temperature at low Temps that should remain between 26-29 Centigrade in the hottest days.

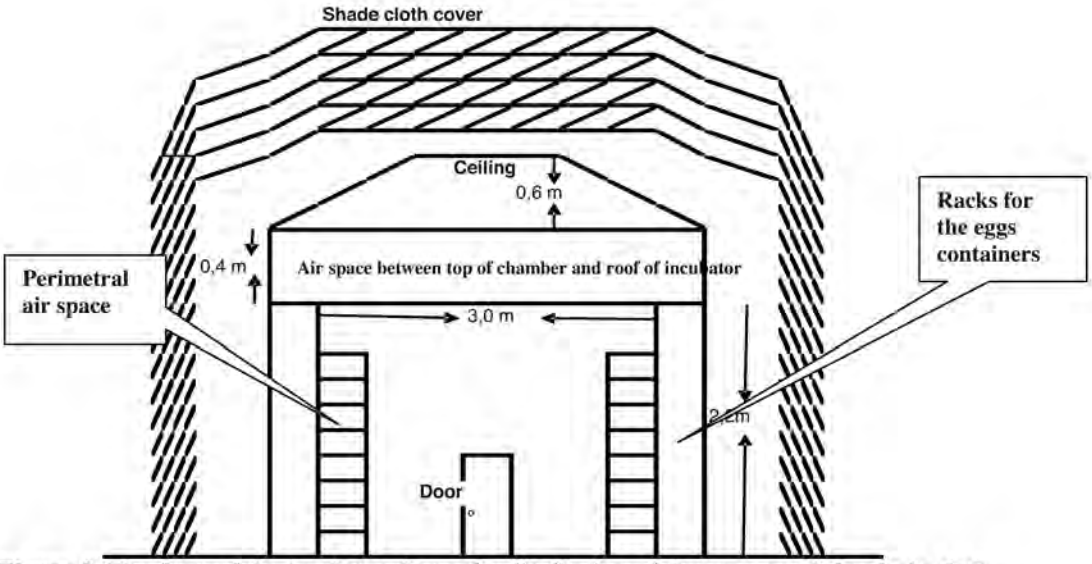



**Figure 2**. The front of the incubator shows the distribution of the spaces and the shade cloth exhibiting total coverage of the incubator, keeping the temperature in the incubation chamber within a range of 26 and 29 Degrees Centigrade in the hottest days without a need for coolers or air conditioners.

## Chamber Function

Posterior to the construction of the system and of the incubation chamber the next step would be to manage in a routinely basis the revisions and collection of data in which the specific information pertaining to egg clutches will be gathered. One system will operate in a permanent and efficient manner in accordance to the following principles.

a) Individual egg tray clutch management.
b) Collection of individual data for each of the nests in which the information pertaining to the date of recollection, place and time of nest retrieval, number of eggs, sterile eggs, level of incubation[6], monitoring of mortality in a periodic manner in periodic time lapses (days), date of hatching, total hatched and finally incubation length (Table 1).
c) Follow up on temperatures in the different levels of incubation and collection and filing of data in a format specially designed for this purpose.

**INCUBATION YEAR 200__**

**Species**_____
**Place**_____
**Person in charge**_____

| Clutch date | Nest No. | N° of eggs | Sterile eggs | Incubation Placement level | MORTALITY CHECK | | | | | | | | Date Hatched | Total hatchlings | Incubation Length. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 5 | 15 | 25 | 40 | 55 | 70 | H* | N** | | | |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |

**Table 1.** Register of data collected from clutches incubated artificially in an incubator under controlled conditions, H* refers to fetuses that are completely formed but fail to hatch and N** registers the neonates born but dead in the incubator. The numbers under the column "mortality check" refer to days that pass until another revision is due.

[6] The placement of the clutch in the incubator can determine the sex of the hatchlings keeping in mind that incubators without permanent internal ventilation tend to exhibit thermal stratification which give rise to differences of even 3 degrees centigrade between the lower and upper areas. the lower could give readings of 29 degrees centigrade and the higher of 32 degrees with an approximate distance of 2.2m between the two.

In the majority of farms embryonic death occurred due to poor recollection techniques that gave rise to a significant percentage occupying an important place in diagnostic studies. Keeping this in mind the following factors are presented that must be controlled and that have an effect in the successes or failures of the systems of artificial incubation. In addition to this it is important to note that embryonic death occurs principally within two stages of embryonic development, at the beginning of incubation and at the end. This is due to the fact that early in development embryos die because of nutritional deficiencies exhibited in the breeding females, damage that occurs during recollection and transport or alterations in their genetic makeup. At the same time embryonic death at the end of incubation is due mostly to the lack of energy stores in the egg reserves of genetically deficient eggs.

Considering the previously stated facts there should be an extremely careful and diligent application of these techniques in order to minimize embryonic death due to nest and egg manipulation. Next we will present some of the factors that must be observed, analyzed and if possible controlled due to the fact that they influence the success or failure of artificial incubation systems.

- Habitat characteristics, availability of nesting sites and material for nest building.
- Detection routines and recollection techniques.
- Clutch placement in plastic trays
- Careful management of ratios between vermiculite and water in each tray.
- Daily supervision of the incubation system
- Daily monitoring of temperature inside the egg chamber

If these steps are followed it is possible to attain a75% or above hatch rate.

**Final Considerations**

For the specific case of crocodilian conservation it is emphasized that natural management must prevail over just good intentions that are usually accompanied by unfounded manipulation of the term conservation. Nevertheless in cases where populations that are studied present a certain degree of decline and detriment in their structure any effort in conservation no matter at what level (eggs, hatchlings, juveniles or adults) a well founded conservation program is a good and viable alternative. With respect to artificial incubation, this technique that has already been applied with success in various species of reptiles can be replicated with sure results and is an important tool for acquiring information that otherwise would be difficult to attain. Being that we are dealing with conservation programs in wild habitats, the surrounding communities must be well informed and active and must participate fully of all expectations an results of such programs.

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# Variation of incubation temperature in nests of *Crocodylus moreletii*

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**ABSTRACT:** To date, very few studies on the evaluation of the variations of the incubation temperature in nests of crocodiles in natural conditions have been developed. The main goal of this study was to analyze and the variation of incubation temperature in nests of *Crocodylus moreletii*, in relation with the environmental conditions. During the harvesting season of 2007 of *C. moreletii* (June-August), eight nests located near Campeche City, Mexico were selected: six nests were located in the Centro de Estudios Tecnológicos del Mar N° 2, and the other two were located at a local breeding farm. In each nest, three digital thermometers were placed at the bottom, in the middle and at the upper parts of the egg cavity. Data loggers were programmed to record incubation temperature every 30 minutes, until the eggs hatched. In general, incubation temperature showed significant differences in the different levels inside the egg cavity, recording the highest temperature in the middle part. On the other hand, the incubation temperature is higher during the night than during the day in all layers. Environmental temperature and precipitation were the variables with more influence in the variation of temperature in the egg cavity.

**RESUMEN:** Hasta la fecha son pocos los estudios enfocados a evaluar las variaciones de la temperatura de incubación en nidos de cocodrilos en condiciones naturales; debido a ello, el objetivo de este estudio fue analizar y comparar las variaciones de la temperatura de incubación en nidos de *Crocodylus moreletii,* así como sus variaciones dentro del nido y la relación de la temperatura de incubación con las condiciones ambientales. En la temporada de reproducción 2007 (junio-agosto), fueron seleccionados ocho nidos ubicados cerca de la ciudad de Campeche, México: seis nidos localizados en el Centro de Estudios Tecnológicos del Mar N°2 y otros dos en un criadero local. En cada nido se colocaron tres termómetros digitales en diferentes estratos de la cámara de incubación (superior, media, inferior). Los termómetros fueron programados para monitorear cada 30 min la temperatura, desde el momento que fue localizado el nido hasta su eclosión. En general, la temperatura de incubación presentó diferencias significativas en los diferentes estratos de la cámara de incubación siendo mayor en el estrato medio. Por otro lado, la temperatura de incubación es mayor durante la noche que en el día en todos los estratos, y en cuanto a la relación con el clima, la temperatura ambiental y la precipitación son las variables que más influyen en su variación.

# The invasion and subsequent radiation of Caimaninae in the Neotropics, and the relationships of the extant species, with special reference to the *Caiman* complex

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**ABSTRACT:** Fossil records indicate a Laurasian origin of Caimaninae, with the split from Alligatorinae about 69 mya. Unfortunately, caiman evolutionary history is perhaps the least complete of all crocodilians. The earliest fossil caiman from the Neotropics (Argentina) is dated at about 50 mya. Fossil evidence for the extant genera is virtually non-existent and their evolution is unknown. Geologic studies during the last two decades have produced new information on potential faunal corridors through Central America, and a new vision of the genesis of the great river basins of South America. Molecular data from this study is used to demonstrate the relationships of the extant Caimaninae taxa. Divergence of *Caiman yacare* and *C. crocodilus* is estimated at about 7.5 mya. Results indicate that *C. yacare* is a distinct Evolutionary Significant Unit that merits full species status. Analyses of *C. c. fuscus* from Honduras are presented and it also is distinct, significantly different from both *C. c. crocodilus* and *C. yacare* in all tests. These data, combined with recent geologic findings, are used to hypothesize the potential evolution within the *Caiman* complex, including a recent re-invasion of Central America.

## INTRODUCTION

Crocodylian origins are estimated to have arisen over 200 million years ago (mya) (Brochu 2001). A remarkable radiation of species developed from their Archosaurian ancestors and this rich evolutionary trajectory continues today. Modern forms are traditionally divided into three lineages: Crocodiloidea ('true' crocodiles and relatives), Gavialoidea (gharials), and Alligatoroidea (alligators and caimans), all with fossil records dating from the Late Cretaceous (Brochu 2003).

The extant species of Alligatoroidae have developed relatively recently and very little fossil evidence exists for any of the caiman species. Their morphological characters lend scant inference to phylogeny for the group. Molecular analyses were employed to define the topology and to add information on their possible evolution. Details on laboratory procedures have been omitted for simplicity. Molecular clock calculations, along with calibration from fossil evidence, were used to make divergence date estimations. Recent geological findings give new insights into the emergence of the Central American isthmus, closure of the Panama Seaway, and the development of the major river basins in South America. The development of the Neotropical caiman species resulted as a consequence of these processes. I consider below how historical biogeography might have occurred.

The Alligatoroidea (*Alligator mississippiensis* and all crocodylians closer to it than *Crocodylus niloticus* or *Gavialis gangeticus*) is composed of two *Alligator* species and a monophyletic

clade of 6 caiman species, the Caimaninae. This subgroup is composed of *Caiman crocodilus* and all crocodylians closer to it than to *Alligator mississippiensis*. This includes *Caiman yacare, C. latirostris, Melanosuchus niger* and the more primitive *Paleosuchus palpebrosus* and *P. trigonatus*.

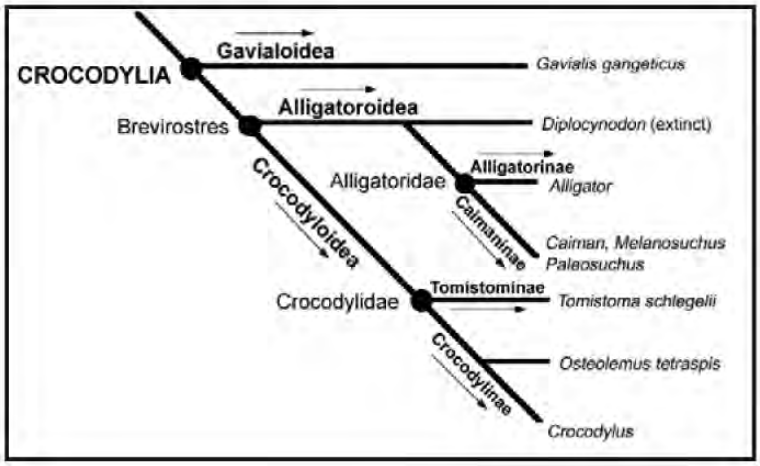



**Figure 1.** Diagram of phylogenetic nomenclature for extant crocodylians. Arrows indicate stem-based group names (groups including a species and any other more closely related to it than to another species), and black circles denote node-based group names (last common ancestor of two or more species and all of its descendents) (simplified from Brochu 2003).

The early fossil records place alligatorid development in Laurasia due to fossils found in Europe and North America from the late Cretaceous (Taplin and Grigg 1989). *Alligator* appears to have originated in North America with fossils from the late Eocene, about 35 mya (Brochu 1999) and a subsequent radiation to Asia led to *A. sinensis.*

Few fossils exist for Caimaninae and Brochu (2003) describes the knowledge of the caiman evolutionary history as the least complete of all crocodilians. Brochu (2004a,b) estimates the alligator-caiman divergence occurred by the lower Paleocene, about 65 mya. Early caiman fossils from the Eocene have been located from North America. An ancestral lineage is believed to have dispersed to South America where subsequent radiation resulted in the living species. The Caimaninae appears to have an exclusive South American radiation with a recent invasion of Central America by *Caiman crocodilus.*

*Eocaiman cavernous* remains have been recovered in southern Argentina from Eocene strata dated at about 50 mya. *Eocaiman* is believed to have given rise to the modern genera (Langston 1965; Brochu 2003), but the fossil record provides no clear information about their relationships. *Melanosuchus* has the best fossil record for the group with specimens dating to the Pliocene (Medina 1976). Fossil remains attributed to a precursor of *Melanosuchus* and *Caiman latirostris* date from the Miocene (Langston 1965). No fossils have been reported for *Paleosuchus*. The two *Paleosuchus* species have features distinct from the rest of the Caimaninae and are generally considered more primitive. Biochemical and morphological analyses place *Paleosuchus* basal to the rest of the group (White and Densmore 2001; Brochu 2003).

## MATERIALS AND METHODS

All of the samples prepared for this study consisted of fresh tissue, usually blood, and some published sequences were also taken from GenBank. Blood samples were taken from the cervical sinus using sterile 2 cc syringes and needles (Olson *et al*. 1975). A sample of about 1 ml was drawn and introduced into a vial containing blood lysis buffer (protocol modified from White and Densmore (1992). All DNA isolations were performed with a phenol-chloroform isoamyl alcohol (PCl) isolation protocol modified from Hillis *et al*. (1996) with 95% ethanol precipitation.

All samples were obtained with proper documentation. The Bolivian Wildlife authorities issued CITES permit No. 00470 for export of specimens. Entry into the US was under CITES import permit No. 816827. Argentine samples were exported by Proyecto Yacare, S.A. with CITES permit No. 023752 and imported under US CITES permit No. 03US714329/9. Honduran tissue samples were obtained by the FLMNH during a CITES funded population survey mission. Venezuelan material was obtained by FLMNH from the caiman survey team of ProFauna, the Venezuelan Government Wildlife Service. Alligator samples were provided by the Florida Fish and Wildlife Conservation Commission. *Paleosuchus trigonatus* samples from the St Augustine Alligator Farm, St. Augustine, Florida were kindly provided by Dr. Kent Vliet.

### Molecular Investigations

Once DNA isolations of the samples were completed, molecular analysis began. The following samples resulted in unique mitochondrial haplotypes (in brackets) *Caiman c. crocodilus* (n=12[4]), *Caiman c. fuscus* (n=20[1]), *Caiman yacare* (n=213[25]), *Caiman latirostris* (n=5[1]), *Melanosuchus niger* (n=5[2]), *Paleosuchus palpebrosus* (n=3[3]), and *Paleosuchus trigonatus* (n=2[2]).

The entire mitochondrial (mt) genome for *Caiman crocodilus* (Janke *et al*. 2001) had been published and served as the nucleotide base reference sequence. The *Caiman* mtDNA genome is 17,900 bp long and all further position references described below are relative to the *Caiman* mitochondrial genome of Janke *et al*. (2001) unless otherwise noted. Published sequences for *Alligator mississippiensis* (Janke and Arnason 1997), and *A. sinensis* (Wu *et al*. 2003), were incorporated in analyses. Additional sequences for *Caiman crocodilus* and *Melanosuchus niger* from Farias *et al*. 2004 were also used.

Birds have Archosaurian ancestry and are considered the closest living relatives to crocodylians (Sereno 1999; Brochu 2001). Sequences for the domestic chicken, *Gallus gallus* (Desjardins and Morais 1990) were used for comparison as an outgroup in some analyses. Recent molecular studies have determined that Testudines are likely a sister clade to crocodylians (Kumazawa and Nishida 1999; Iwabe *et al*. 2005). The mtDNA genome for the green sea turtle was published (GenBank accession number NC_000886.1) and sequences were also used as an outgroup in some analyses.

The highly conserved 16S rRNA gene was chosen for phylogenetic analyses. The *Caiman* 16S rRNA gene is 1593 base pairs (bp) long. I was able to successfully amplify a fragment over 1100 bp long for all taxa in this study. The highly variable cytochrome (cyt) *b* gene

was chosen due to great value for phylogenetic research and widespread use in intraspecific relationship analyses (Avise 2000, 2004). The *Caiman* cyt *b* gene is 1150 bp long, from position 14,461 to 15,610. I was able to amplify a sequence about 1200 bp long for all the taxa. The reconstructed fragment, corresponding from positions 14,461 to 15,660, represents the entire cyt *b* gene. Most PCR amplifications above were 25 µl volumes and the product were processed on ABI 3700 automatic sequencers.

### Data Analysis

Sequences for 16S and cytochrome *b* were separately evaluated, and aligned with Sequencher 4.2. The resulting 16S contigs were then trimmed of missing data resulting in consensus sequences of about 1077 bases depending on insertion or deletions. The same process was performed for the cytochrome b data, resulting in sequences of about 1145 bases depending on indels. For one data set, the two sequences were joined into one string of approximately 2200 bases per species. Both *Gallus gallus* and *Chelonia mydas* served as out groups for initial analyses of Alligatoroidea as mentioned above. Files were then imported into PAUP 4.0b (Swofford 1998) for phylogenetic analyses and tree generation. The data set was subjected to the hierarchical likelihood ratio test (LRT, Huelsenbeck and Rannala 1997), by means of Modletest v.3.06 (Posada and Crandall 1998) to assign the most appropriate of 56 evolutionary models. A general time reversal plus rate heterogeneity among sites (GTR+G+?) was selected. Maximum likelihood (ML), minimum evolution (ME) distance and maximum parsimony (MP) trees were constructed by means of branch and bound or tree-bisection-reconnection algorithms. Analyses were performed using 16S and cytochrome *b* separately and combined. Additional statistical support was provided by Bayesian inference using MrBayes v. 3.1.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003).

## RESULTS AND DISCUSSION

The constructed data set of 16S rRNA and cytochrome *b* sequences was employed for a preliminary analysis of Alligatoroidea. One sequence for each of the following species was used for the analyses: *Alligator sinensis* (As), *A, mississippiensis* (Am), *Caiman latirostris* (Cl), *C. yacare* (Cy), *C. crocodilus* (Cc), *Paleosuchus palpebrosus* (Pp), *P. trigonatus* (Pt), *Melanosuchus niger* (Mn), as well as *Gallus gallus* (Gg) and *Chelonia mydas* (Cm) as outgroups.

### Alligatoroidea

Figure 2 shows the relationships between the species of Alligatoroidea under criteria for maximum parsimony. The general topology follows that given in recent publications using DNA (White and Densmore 2001), and a combination of DNA and morphological characters (Brochu 2003). There were a total of 2328 unordered characters of equal weight, 1167 constant characters, 517 parsimony-uninformative variable characters and 644 parsimony-informative variable characters. Gaps were treated as missing data. Starting trees were obtained via simple stepwise addition using *C. crocodilus* as the reference taxon. Tree-bisection-reconnection (TBR) branch swapping algorithm was employed. Bootstrapping was performed with branch-and-bound search and a 50% majority-rule consensus tree was enforced.

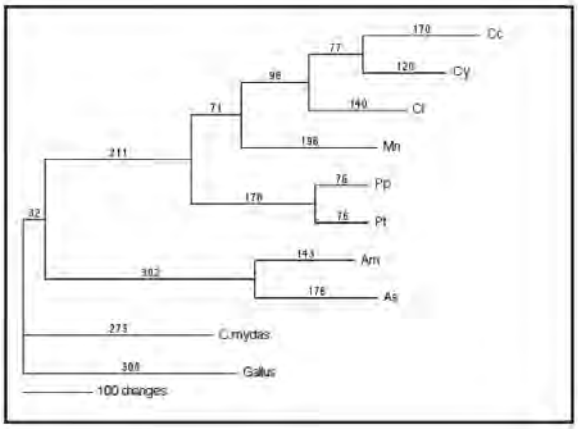



**Figure 2.** Maximum parsimony phylogram representing base changes for 2200 bp concatenated partial sequences of mitochondrial 16S and cytochrome b genes. Tree length is 2057, CI=0.761, RI= 0.565, RC=0.430 and HI=0.239. Species abbreviations are: Cc (*Caiman crocodilus*), Cy (*C. yacare*), Cl (*C. latirostris*), Mn (*Melanosuchus niger*), Pp (*Paleosuchus palpebrosus*), Pt (*P. trigonatus*) Am (*Alligator mississippiensis*), As (*A. sinensis*) C. mydas (*Chelonia mydas*), Gallus (*Gallus gallus*).

A maximum likelihood model of evolution (GTR+G) was established by Modeltest v3.06 using the Akaike Information Criterion (AIC). The assumed nucleotide frequencies (A=0.31910, C=0.30880, G=0.14440, T=0.22770) were calculated from the data set. A substitution matrix was constructed from the data set by Modeltest and six substitution types were used. Starting branch lengths were obtained using the Rogers-Swofford approximation method and no molecular clock was enforced during the test. All but one branch had uniformly high bootstrap agreement (see Figure 3).

This analysis supports the historic assumption of monophyly within Alligatoroidea and also within Caimaninae. Traditional systematic diagnoses for the clade have relied heavily on morphological cranial characters with certain assumptions on derived states.. When *Alligator* serves as the most distant group, the bootstrap percentage values for unrooted trees following the same criteria above equal 100 for MP (10,000 replicates), and 100 for ML (1000 replicates) for the *Paleosuchus* branch in question.

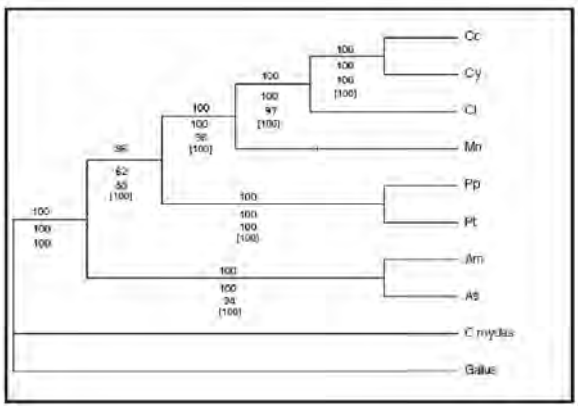



**Figure 3.** Bootstrap values for support of Alligatoroidea phylogeny. Bootstraps values are maximum likelihood (ML-top), minimum evolution distance (ME-middle) and maximum parsimony (MP-bottom). Bootstrap values are for 1000 replicates. Bayesian inference in brackets ($5x10^6$ generations, 50,000 trees, 25% burnin). Species abbreviations as in Figure 2.

**Caimaninae**

With the basic Caimaninae phylogeny firmly established for this data set, the next step was to determine the relationships within *Caiman*. A new data set was constructed in which *Gallus* and *Chelonia* were eliminated. A sequence was constructed for *Caiman c. fuscus* from Honduras, near the northern extreme for the species, and added to this data set. The subspecies diagnosis is not clear as there is great overlap in characters with *C. c. crocodilus*, and the detailed distribution has not been determined. Questions remain whether the taxon represents a distinct evolutionary lineage or simply clinal variation within the Caiman crocodilus distribution. Results of analyses were inconclusive regarding the relationship of *C. c. fuscus* with *C. c. crocodilus* and *C. yacare* even with a reduced data set which eliminated distant *Paleosuchus*.

A new data set was constructed using only cytochrome b sequences. The 16S sequence was removed to eliminate possible confounding effects on these closely related lineages from this slowly evolving gene (see Figure 4). A 1092 base sequence was used for each taxa.

Tree generation under various criterion resulted bootstrap associations of *C. fuscus* with *C. yacare* over 50% of the time as seen by the bootstrap values in ML, ME and MP hierarchies, but sometimes was weakly associated with *C. crocodilus* (ML=29, ME=11, MP=20). Alternatively, *C. yacare* was associated with *C. crocodilus* (ML=11, ME=35, MP=20) (see Fig. 4).

A new data set was constructed for Alligatoroidea from cytochrome b sequences, with *Gallus* as the outgroup. Estimates of evolutionary events were subsequently made using rooting time estimates found in the literature. The early origin of birds has been calculated at about 240 mya (Brochu and Norell 2000) and the split between alligator and caiman was estimated in literature from 65 mya (Brochu 2003) to 75 mya (Wu *et al*. 2003). With my data set, the alligator–caiman split was estimated at 69 mya, consistent with the above calculations.

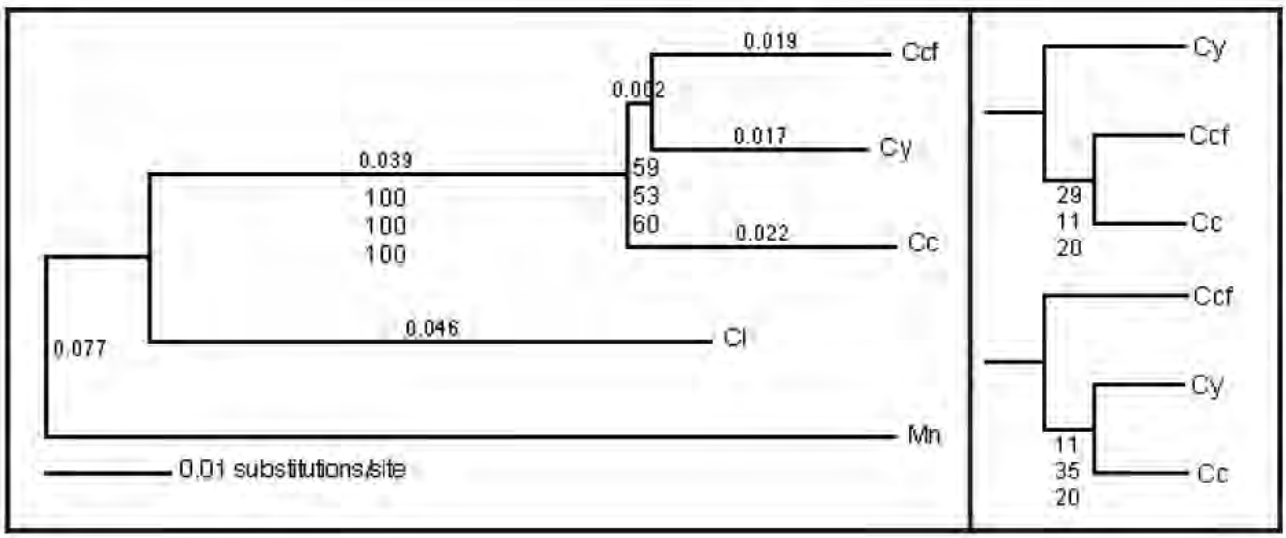



**Figure 4.** Minimum evolution distance phylogram (uncorrected 'p') for cytochrome *b* sequence comparison rooted with *Melanosuchus* (score 0.22141, branch length above; 1000 bootstrap replications with values below branch: top-ML, middle-ME, bottom-MP). Alternate trees and bootstrap values on the right. Species abbreviations are: Cc (*Caiman c. crocodilus*), Ccf (*Caiman c. fuscus*), Cy (*C. yacare*), Cl (*C. latirostris*), Mn (*Melanosuchus niger*).

Based on ML distance values generated with enforcement of a molecular clock using the cytochrome b only and combined with 16S data sets, the Cc-Cy/Ccf split occurred about 7-8 mya, with the emergence of *Caiman yacare* and *C. c. fuscus* at ~7 mya. This predates the rise of the Isthmus of Panama and closure of the Panama Seaway between the Atlantic and Pacific basins. Migration scenarios to account for this possible occurrence are discussed below.

Further investigation was undertaken to clarify the *Caiman crocodilus* complex and a new data set was constructed with cytochrome b and partial Control Region sequences of 1193 bases. Since many authors differ on the subspecies of the common caiman, analyses were constructed to represent one species with various populations. Sequences for two haplotypes from each of the major basins occupied by *C. c. crocodilus* (Orinoco and Amazon) and *C. yacare* (Amazon and Paraná) were selected for comparison with the *C. fuscus* haplotype. *C. yacare* and *C. crocodilus* were consistently separated (see Figure 5). With this data set *C. fuscus* is usually associated with *C. yacare* but with weak support and little additional resolution was established. Alternate trees associated *C. fuscus* with different arrangements of *C. yacare* haplotypes (ML=43, ME=35, MP=46) more often than with *C. crocodilus* (ML=32, ME=23, MP=31).

A new data set was constructed with cytochrome *b* sequences for analysis to determine not only the relative position of *Caiman c. crocodilus* with *C. c. fuscus*, but also to include comparisons with *C. yacare*, often regarded an additional subspecies. A total of 274 caiman representing 40 haplotypes were used for analysis.

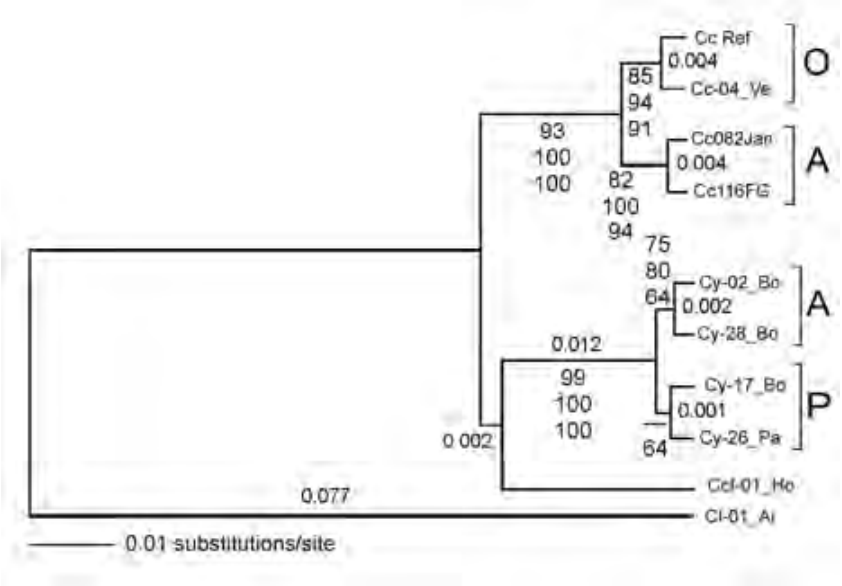



**Figure 5.** Minimum evolution distance tree for cytochrome *b* data set using GTR+Γ evolutionary model; score=0.15676. Bootstrap branch support for Ccf not strong and the relationship remains unclear. Bootstrap values (1000 replications): top-ML, middle-ME, bottom-MP. Species abbreviations are: Cc (*Caiman c. crocodilus*), Ccf ((*Caiman c. fuscus*), Cy (*C. yacare*), Cl (*C. latirostris*). **O** = río Orinoco, **A** = río Amazon, **P** = río Paraná.

For the purpose of analysis, the six discreet populations were associated geographically into 4 groups referring to the sample localites: Central America (Honduras), Orinoco (Venezuela), central Amazon (French Guiana, Brazil) and southern Amazon (Bolivia, Paraguay). The French Guiana (FG) sample is grouped with the Brazilian population due to the most

parsimonious geographic connection for resulting gene flow. This logic is also applied to associating the *C. yacare* populations even though the Paraguay haplotype technically lies outside the Amazon basin boundaries, as does the FG sample. An AMOVA pairwise difference test revealed 83.55% variation among groups, 12.63% variation among populations within groups, and 3.82% percent variation within populations, all significant at the p=0.05 level. Significant differences also were found for the population pairwise $F_{st}$ test results at an alpha level of 0.05 (see Tables 1, 2)

**Table 1.** Pairwise $F_{st}$ test results, all significant at an *a* level of 0.05.

| | Honduras | Venezuela | Fr Guiana | Brazil | Bolivia | Paraguay |
|---|---|---|---|---|---|---|
| 1) Honduras | | | | | | |
| 2) Venezuela | 0.9853 | 0 | | | | |
| 3) French Guiana | 0.9972 | 0.9000 | 0 | | | |
| 4) Brazil | 0.9915 | 0.9073 | 0.6002 | 0 | | |
| 5) Bolivia | 0.9559 | 0.9543 | 0.9548 | 0.9563 | 0 | |
| 6) Paraguay | 0.9713 | 0.9639 | 0.9664 | 0.9682 | 0.7581 | 0 |

**Table 2.** Population average pairwise distances. Above diagonal: Average number of pairwise differences between populations $(\pi_{XY})$. Diagonal elements: Average number of pairwise differences within population $(\pi_X)$; Below diagonal: Corrected average pairwise difference $(\pi xy-(\pi x+\pi y)/2)$, all significant at an $\alpha$ level of 0.05.

| | Honduras | Venezuela | Fr Guiana | Brazil | Bolivia | Paraguay |
|---|---|---|---|---|---|---|
| 1) Honduras | 0.0000 | 46.4286 | 47.2500 | 46.3750 | 38.4615 | 39.8462 |
| 2) Venezuela | 45.5934 | 1.6703 | 12.4643 | 11.5893 | 42.2028 | 42.2187 |
| 3) French Guiana | 47.0000 | 11.3791 | 0.5000 | 1.6250 | 41.2780 | 41.1885 |
| 4) Brazil | 46.0145 | 10.3936 | 1.0145 | 0.7210 | 40.4309 | 40.3135 |
| 5) Bolivia | 37.4821 | 40.3882 | 40.0486 | 39.0910 | 1.9589 | 7.4128 |
| 6) Paraguay | 39.0995 | 40.6369 | 40.1918 | 39.2063 | 5.6868 | 1.4933 |

As seen in column 1 of the two distance tables above, the closest group association to Central America (Ho) is southern Amazon (Bo, also row 1 in Table 2). As seen in by the $F_{st}$ values (Table 1), there is no gene flow among *C. fuscus* and the other populations. The lowest value is still very high (0.6002) between Brazil and French Guiana.

The results from this data set indicate that the *C. fuscus* samples used here represent a distinct evolutionary lineage. Further investigation will be required to determine if there is a clinal variation from Honduras to the distribution extremes of southern Ecuador and western Venezuela. The caiman of the central Venezuelan coast have not been clearly identified, and the relationship to *C. crocodilus* in rest of the country is unclear. The *C. fuscus* populations west of the Andes have been isolated from *C. crocodilus* populations for about 5 million years by vicariance events as outlined below.

In the hypothetical reconstruction of conditions during the time frame leading up to and

including the proposed migration events, three interrelated processes must be taken into consideration:

1) The geologic evolution of northern South America including the influence of Andean tectonics and development of the Amazon and Orinoco basins
2) The dynamic conditions of changing paleocurrents affecting the Panama Seaway and environs
3) The progression of emerging land mass and eventual closure of the Seaway by the isthmus.

**Northern South America and caiman migrations**

South American geography has been strongly influenced by the Guyana and Brazilian shields. These pre-Cambrian massifs dominated drainage and erosion patterns in the pre-Andean landscape. Early continental drainages flowed off the shield in a generally western direction to the Pacific (White *et al.* 1995). The influence of these highland regions continues today and they are the source for many affluents of the Orinoco, Amazon and Paraná drainages.

The South American Plate has been impacted by several peripheral plates. Most notable has been the convergence with the oceanic Nasca Plate along most of the western margin, leading to the rise of the Andes. This mountain building process began at least 90 mya and has not been uniform over the 7000 km length. The Andes north of Ecuador rose most recently and have divided into 3 principal branches (cordilleras): Western, Central and Eastern. The rising Andes fundamentally changed the drainage and weather patterns over the continent and the world.

As the mountains rose, a parallel axial trough was formed by crustal downwarping in the eastern foreland basin. As the previous drainage to the west blocked, new drainages off the forming Andes then flowed east into the trough. Fluctuating sea levels produced periodic intrusions that penetrated the continent along these trough basins. From the Caribbean, penetrations reached into the western Amazon and central Bolivia and from the south Atlantic, intrusions into the proto-Paraná area reached southern Paraguay (Haq *et al.* 1987; Hoorn et al. 1995; Lundberg *et al*. 1998).

By the late Cretaceous, the central Amazon appears to have had two important drainages: a minor one flowing east toward the present delta, and the other draining north toward the alternately brackish or marine foreland basin. The early Paleocene hydrology was characterized by reduced marine intrusions and increased fresh water flow northward from a huge watershed. The caiman lineage is believed to have invaded South America from Central America at about this time. Although no evidence exists for a stable land bridge between continents, there was a large volcanic arch that may have aided dispersal (see Figure 6).

This proto-Amazonian drainage system dominated much of South America from ~67 to ~8 mya (Lundberg *et al*. 1998). A fresh water plume would have extended outward from the coastal margin and created brackish conditions for a great distance. Successful migration may also have depended on the oceanic currents to extend this brackish environment along the volcanic Ave arc. Fluctuating sea levels could have, at times, reduced inter-island distances and provided a ‘stepping stone’ archipelago enhancing faunal connections. There are numerous records of continental interchanges in both directions between the late

Cretaceous and early Paleocene, including freshwater fish, birds, reptiles and mammals (Hallam 1994).

The pattern in northern South America continued for the next 50 million years. As the Andes grew in height and width, it imposed strong weather modifications. A western Amazon inland sea, "Lago Pebas', has been proposed due to pooling of fresh water and periodic marine intrusions (Hoorn 1993, 1994). The cyclic marine intrusions continued but, partly due to erosional infilling of the forebasin, they did not reach the previous interior extensions.

Flow increased northward into the proto-Orinoco basin from a system that extended from Bolivia. Outflow existed in a large area near present day Lake Maracaibo and Falcón further east (Hoorn *et al.* 1995). Collision with the Caribbean Plate to the north and Cocos Plate to the northwest produced profound changes. There were significant drainage disruptions as the eastern Cordillera began to rise (ca. 12 mya) in Colombia and Venezuela. The Magdalena river valley was isolated and its large input to the Maracaibo basin ceased. From ~10 to ~8 mya there was an increased drainage via the Maracaibo and Falcón basins. Deep marine incursions ceased and to the south, the proto-Paraná drainage extended from southern Bolivia (Lundberg *et al.* 1998).

In the intervening time period to the present, active tectonism in the northern Andes resulted in closing the Maracaibo basin and forcing the río Orinoco eastward. The course of the Amazon also changed to the current Atlantic outflow. Major rearrangements occurred resulting in multiple interbasin exchanges of species (Albert *et al.* 2005). This accounts for the apparent incongruent distributions of many aquatic species and may be reflected to some degree in caiman distribution. A dynamic mosaic of marine, brackish and freshwater environments were evolving over a huge tropical area. Conditions were excellent at this time for caiman species development in northern South America.

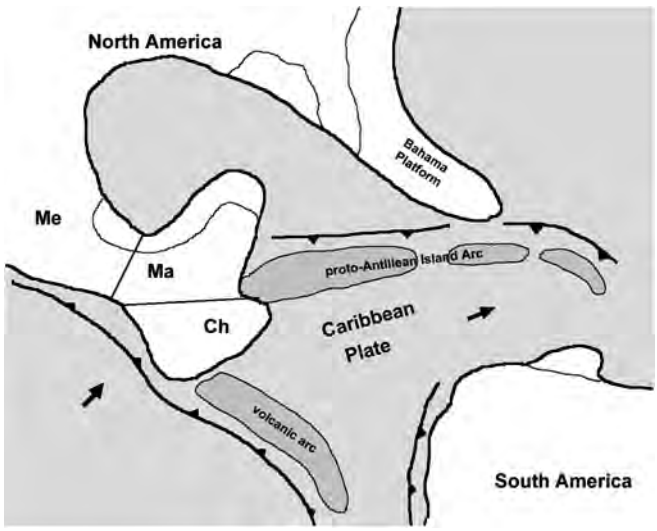



**Figure 6**. Possible configuration at the end of the Cretaceous (ca. 65 mya) when the caiman lineage may have migrated to South America. Major Central American blocks have sutured allowing faunal movement (Me=Mexican, Ma=Mayan, Ch=Chortis). Arc ultimately moved east, forming the Lesser Antilles. Arrows indicate plate movement (after Donnelly 1988).

Measurements of the outflow of the río Amazon today indicate the magnitude of potential effects of an ancient river draining into the Maracaibo basin and adjacent Falcón basin. The

present Amazon annual average discharge is about 175,000 $m^3$/second (Sioli 1984) and the 250 km wide sediment fan reaches 680 km into the Atlantic (Putzer 1984). Depending on the season, the fresh water plume is transported northwest 600-800 km to French Guiana (Goulding *et al*. 2003). The ancestral plume of fresh water flowing north into the Panama seaway would have affected the salinity of near shore seawater for many hundreds of square kilometers.

Alligatoroidea do not possess lingual salt glands or display urine modification as seen in *Crocodylus* (Leslie and Taplin 2001). The lack of resistance to marine conditions has been attributed as a primary reason for geographic restriction of the group. But despite apparent physiological restrictions, caiman (and alligators) are not restricted solely to fresh water. Caiman populations resulting from natural migration events are found on islands off Colombia, Venezuela, Nicaragua and Panama, as well as Trinidad and Tobago. Occupied habitats within caiman distributions include brackish lagoons, estuaries and mangrove swamps (Medem 1981, 1983; Gorzula and Seijas 1989; Grigg *et al*. 1998). These occurrences demonstrate partial salt tolerance and the possibility for coastal marine migrations. In addition to effects from a river discharge plume and partial salt tolerance, proposed north and western oceanic currents at the time period would favor transport of both caiman and brackish water mass toward the emerging peninsula.

**Evolution of the Isthmus**

In the Middle Miocene (15.1-12.9 ma), the shallow seaway was open allowing warm Atlantic water to enter the Pacific and continental vertebrate faunas were generally isolated. By the late Middle Miocene (11.8-7.0 ma), there was partial emergence of the Panama Isthmus as sea levels varied, causing a disruptions of warm, westerly Atlantic currents. The cool California current flowed south along the west margin of Central America. Terrestrial faunal interchanges were first recorded at ~9.3-~8.0 ma (Marshall et al. 1979, 1982; Webb 1985; Duque-Caro 1990).

As time progressed, sea levels fell and the isthmus emerged as continuous (or nearly) dry land. The Great American Biotic Interchange (GABI) commenced in force about 3 ma (Stehli and Webb 1985; Webb 1991). Sea level changes may have produced temporary seaway connections in the late Pliocene in southern Panama. (Cronin and Dowsett 1996). Even today, areas of the Darien in Panama have a maximum height of only tens of meters above sea level.

Addition physiological factors may have contributed to successful colonizations of Central America by caiman before a continuous terrestrial landscape. Multiple paternity has been shown for *Alligator mississippiensis* (Davis *et al*. 2001b). There is also evidence that caiman (*Paleosuchus*) may employ sperm storage in reproduction (Davenport 1995). If these factors prove true also for *Caiman,* a single migration event of a gravid female could have resulted in the establishment of a new population with a robust genetic compliment.

There is compelling evidence that the conditions outlined above were advantageous for caiman expansion northward. Land mammals such as ground sloths migrated north from South America during the Late Miocene. Procyonids from North America traversed the route south about the same time. In addition to vagile mammalian species, several freshwater fish

taxa made successful migrations to Central America before a continuous land connection (Lundberg 1992; Bermingham and Martin 1998; Martin and Bermingham 2000; Lovejoy and Collette 2001).

The Central American freshwater icthyofauna is largely of South American origin (Bussing 1985). In Bermingham and Martin's (1998) analysis, they described freshwater fish species the Central American Atlantic coast slope drainages as more isolated than the Pacific slope drainages. They found the extant fish fauna to be the result of multiple colonizations across the isthmus beginning about ~7 mya near the close of the Miocene. Phlygeographic signal due to sea level rise and the re-establishment of the seaway (Haq *et al*. 1987) is observed in several studies (Bermingham and Martin 1998; Knowlton and Weigt 1998; Zeh *et al*. 2003).

The biogeographic history of *Caiman c. fuscus* may have followed a pattern similar to these histories. Andean tectonism, starting in northern Colombia and western Venezuela ~10 mya (Hoorn *et al.*1995), gave rise to the Eastern Cordillera. This produced drainage division and vicariant separation of the existing caiman populations. The area biogeography outlined above, produced conditions for caiman colonization of Central America. The current evolutionary lineage, *Caiman c. fuscus,* is considered a subspecies of *Caiman crocodilus* but today is reproductively isolated west of the Andes. The Honduras population, the most distant Atlantic drainage *C. c. fuscus* from the source *C. c. crocodilus* population, has a ~7 ma history and has significant sequence divergence.

**Conclusions**

The following conclusions are given with the caveat that this data set is limited to molecular analyses of mitchondrial DNA sequences from 16S and/or cytochrome *b* genes only. Given the immense distribution of the Caimaninae, point locality sampling produces large intervening gaps in the data and transitional intermediate haplotypes certainly exist undetected.

1) Alligatoroidea forms a monophyletic clade derived from a common Archosaurian ancestor as recognized historically by conventional, morphlogical systematics.
2) The alligator-caiman split occurred about ~69 mya, similar to estimates by Brochu (2004) of 64 mya, and Wu (2003) of 75 mya.
3) Caimaninae forms a monophyletic clade with Paleosuchus in the basal position and *Caiman yacare* as the most recently derived species.
4) The lineage leading to *Paleosuchus* split from the other caiman ~53 mya (54 mya; Brochu 2004). *Caiman crocodilus* dates from about 7.5 mya.
5) The lineage leading to *Melanosuchus* split from the group at about 30 mya, with a more recent splitting of the *C. latirostris* line at about 24 mya.
6) *Caiman crocodilus* from the Orinoco and Amazon basins are currently geographically (reproductively) isolated and have formed distinct genetic populations. Divergence is estimated at ~2.75 mya and within basin radiation occurring in the last million years.
7) *Caiman yacare* and *Caiman c. fuscus* (Honduras population) are unique and separate evolutionary lineages, which arose independently from *Caiman crocodilus* ~6 mya in a combination of vicariance and dispersal events. They are also geographically, and hence reproductively, isolated from populations of *C. crocodilus* of the Orinoco and Amazon basins.

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# The Enigmatic Cuban Crocodile

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**ABSTRACT:** The Cuban crocodile is the most morphologically, ecologically and behaviorally distinctive member of the genus *Crocodylus*. We argue that this is a result of species having evolved largely to fill the niche of a terrestrial predator in Cuba and adjacent Caribbean islands during the Pleistocene. During this period Cuban was home to megafauna of several species of ground sloths and no species of large mammalian predators. Contemporary fossil remains of Cuban crocodiles indicate the species grew significantly larger than it does today and. In this presentation we discuss evidence to support the hypothesis of Cuban crocodile was preying to a large extent on terrestrial mammals and much of its unique character are derived from its evolution as a terrestrial or semi-terrestrial predator. We also discuss the consequences of the extinction of the mammalian megafauna on Cuba some 6,000 years ago, and interpretations of how the Cuban crocodile has adapted. This information is vital for planning a conservation future for the species.

# Use of archival tags to track large estuarine crocodiles in 3-dimensions

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**ABSTRACT:** Satellite and radio-telemetry has been successfully used to remotely monitor the movements of crocodilians in their natural habitats. The advantages of using this type of telemetry include being able to monitor the position and movement of animals over large spatial and temporal scales. However, monitoring the fine scale movements, behaviours and activities of crocodiles through telemetry is problematic because of the high rates of data collection and transmission required. It is here, that the use of archival tags (data-loggers) comes to the fore as high sampling frequencies (e.g. >4 Hz) and large data storage capacity (>1 Gb) can now be easily achieved. There are however problems with the use of dataloggers, one being that the logger needs to be jettisoned from the animal and retrieved. In a recent study we attached data loggers to estuarine crocodiles that could determine their movements in 3-dimensions, including accelerations and compass bearings. I describe a novel method to allow for a timed release of the data-logger from the crocodile and present some preliminary data analysis.

# Den construction and use by the American crocodile (*Crocodylus acutus* Cuvier) at the Monte Cabaniguan Wildlife Refuge, Cuba

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**ABSTRACT:** The American crocodile (*Crocodylus acutus*) is a proficient den digger; crocodiles of different ages use dens for thermoregulation, and as shelters to protect themselves or their offspring from predators, to prevent being swept along by too fast streams, and to store food.

In the five-year period comprised between 2003 and 2007, we documented several aspects of den construction and their use by American crocodiles of different sex and age classes, at the Monte Cabaniguan Wildlife Refuge, South-west Cuba. Our main subjects of research were den site selection, den dimensions, structure, and the structure-related role of red and black mangrove root systems; differences in the use of dens by hatchlings, juveniles, adult males and females; the relationship between den temperature and external temperatures of air and water, and their role in thermoregulation. We also describe den digging techniques, and the role of dens in the transformation of the swamp landscape by the crocodiles.

# Genotoxicity of the herbicide formulation Roundup® (glyphosate) in *Caiman latirostris* evidenced by the Comet assay and the Micronucleus test.

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**ABSTRACT:** The genotoxicity of pesticides is an issue of worldwide concern. Any damage induced in essential portions of the DNA can become particularly pronounced when the lesions are produced during embryogenesis, when rapid organogenesis is taking place. The present study was undertaken to evaluate the genotoxic potential of a widely used herbicide formulation, Roundup® (glyphosate), in erythrocytes of broad-snoated caiman after *in ovo* exposure. Caiman embryos were exposed at early embryonic stage to different sub-lethal concentrations of Roundup® (50, 100, 200, 300, 400, 500, 750, 1000, 1250 and 1750 µg/egg). At time of hatching, blood samples were obtained from each animal and two short-term tests, the Comet assay (CA) and the Micronucleus (MN) test, were performed on erythrocytes to assess DNA damage. A significant increase in DNA damage was observed, at concentration of 200 µg/egg or higher, compared to untreated control eggs ($p < 0.05$). Results from the CA revealed a dose-dependent effect. This study demonstrated adverse effects of Roundup® on DNA of *C. latirostris* and confirmed that the comet assay and the MN test applied on caiman erythrocytes are useful tools in determining potential genotoxicity of chemical agents.

# Identification of structures and reproductive stage in *Caiman crocodilus fuscus* females through ultrasound methodology

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**ABSTRACT:** In December 2006, we examined and identified the reproductive structures in 723 females of *Caiman crocodilus fuscus* using ultrasound methodology. The females averaged 63.2 cm snout-vent-length) and 7.1 kg body mass. These individuals are part of the breeding stock in Salazar crocfarm close, to Barranquilla, Colombia. December is the month when courtship begins, therefore none of the females would have ovulated or nested. A total of 521 females without visible follicles (immature or non-reproductive) were diagnosed. Only 180 presented vitellogenic follicles between 3 and 30 mm in the ovary. Ninety of these females showed vitellogenic follicles in both ovaries; 51 females showed vitellogenic follicles in one ovary only; 39 females with follicles in ovary and/or oviduct on the right side. 22 females had calcified eggs from 32 mm to 69 mm in the process of reabsorbtion in different parts of the abdominal cavity. One female had 5 eggs in different states of resorption within a sac located in abdominal cavity and a ruptured oviduct around an egg. These abdominal eggs came from ruptured oviducts, failure to enter the oviduct after ovulation, or reverse oviductal peristalsis. Also we determined that the reabsorbtion process could occur in different parts of the abdominal cavity. This was observed after sacrificing 6 individuals to corroborate diagnoses.

# Presence of red fire ants (*Solenopsis invicta*) in broad-snouted caiman (*Caiman latirostris*) nests.

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**ABSTRACT:** Sustainability of crocodilian ranching programs is based on the high losses of both eggs and hatchlings. *Solenopsis invicta* (Red Fire Ant - RFA) infestations on Caiman nests were related to a possible cause of the death of hatchlings before they can leave the nest, but this aspect was not studied in the original distribution of this ant. Our goal in this work was to analyze the proportion of *Caiman latirostris* nests infested with RFA during the last four nesting seasons. In order to know the amount of infested nests, we search for RFA in wild caiman's nests during the entire nesting season. We observed that the proportion of infested nests increased as the season advanced, and by the last month, of incubation the infection was as high as 50%.

# Rainfall effect on the reproduction of *Caiman latirostris* (Crocodylia: Alligatoridae)

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**ABSTRACT:** Weather affects crocodilians' biology, such as reproduction. Studies that could provide future information about nesting are extremely important for management programs based on nests harvesting. In this work, we analyzed the relationship between number of caiman nests collected and rainfall in Santa Fe province (Argentina) on six reproductive seasons with the aim of predicting future number of nests. First, we worked within a local scale; analysing number of nests in a certain area and rainfall for a nearby place. We then searched for a relationship in a regional scale. We found no relationship when looking at local scale, however, when we examined the regional scale, number of nests and clutch size were related to precipitation. Precipitation during September had a positive effect on clutch size. In addition, number of nests was affected by rainfalls during reproductive seasons 2002-2008.

# Crocodiles Of the Regional Park Of W of Niger: Status and Perspectives of the Conservation

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**ABSTRACT:** Niger is a Sahélian country which contains significant faunal potentialities. In spite of these natural resources, the country has only one national park: The Regional Park of W of the Niger River. Thanks to its statute of integral and absolute protection, this park conceals more than 80% of the biological diversity of Niger. That is made up of approximately 150 species of reptiles and Amphibians. Among these reptiles one notes a very noticed presence of the crocodile of the Nile (*Crocodylus niloticus*) in the three (3) principal rivers (River Niger, Rivière Mékrou, and Tapoa River) which are on the way today to become, its sanctuary. One also finds the crocodile of the Nile in the multiple interior water points in particular on the level of certain ponds, permanent (Pérélégou, Bata, and Anana), semi permanent (Adamou pond, pond of Moussiémou) and some temporary (pond of Tchirobi Gangani). However, no situation nor given quantified complete on the manpower of the population of the crocodiles of the Park W is available before the partial census of November 2007 at the time of the 1er African Western Congress of the group of the specialists in the crocodiles. This study proposes to fill this vacuum through the realization of a counting of the crocodiles in the water levels of the Park W. With this intention; three (3) methods of counting were used: the direct counting of the individuals; indirect counting by Traces and Deposit; and the counting of the Burrows. With regard to direct counting, it was carried out on the level of the permanent water points of the rivers Mékrou and Tapoa, the river part of the park as well as the pond of Pérélégou. Counting of the traces and lees especially related to the drained point of water i.e., semi permanent and temporary. The counting of the burrows was carried out on all the water levels.

**Key words:** Niger, Regional Park W, Crocodiles, Counting, Pond, river, Burrows, Deposit,

# Crocoland SRL: First experience on farming and ranching of *Caiman yacare* in Bolivia

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**ABSTRACT:** Crocoland S.R.L. is the first *Caiman yacare* farm installed in Bolivia. Under a management proposal that combines ranching and captive breeding systems. Crocoland began its activities in September 2006. The farm initiated with a parental stock of 2,000 yacares, 1,600 female and 400 males, extracted of wild populations from the Bolivian Pantanal, relocated at 29 kilometers of Santa Cruz de la Sierra city in 8 lagoons of 60 meters in length by 18 meters in width, with a density of 250 yacares in each lagoon. During the first year (2007) 853 nests were harvested from wildlife, producing 25,590 eggs, with a hatchling percentage of 73%. From the closed cycle, 312 nests were collected (9,360 eggs), with a hatchling percentage 75.01%. The total hatchling obtained (less 40 cm of total length) was of 26,291. In the first semester the mortality was of 11.75%, reducing the stock to 23,863 yacares. The ranching in wildlife in 2008 was 1,300 wild nests like that 35,932 eggs. For the closed cycle the nests production increased in 37%, obtaining 430 nests (like 12,480 eggs).

**RESUMEN:** Crocolond S.R.L.es el primer zoocriadero de *Caiman yacare* instalado en Bolivia. Bajo una propuesta de manejo que combina sistemas de *ranching* y *farming*, Crocoland inicia sus actividades en septiembre de año 2006. El zoocriadero cuenta con un plantel parental de 2.000 ejemplares, 1.600 hembra y 400 machos, extraídos de poblaciones silvestres del pantanal boliviano, reubicados a 29 kilómetros de Santa Cruz de la Sierra en 8 pozas artificiales de 60 metros de largo por 18 metros de ancho, con una densidad de 250 animales por poza. Durante el primer año de rancheo (2007) se cosecharon 853 nidos, de los que se obtuvieron 25.590 huevos, con un porcentaje de eclosión del 73%. Ese mismo año, en el ciclo cerrado, se produjeron 312 nidos, 9.360 huevos, con un porcentaje de eclosión del 75.01 %. El total de neonatos menores a 40 centímetros logrados fue de 26.291. En la etapa de desarrollo de los neonatos (6 a 7 meses), la mortalidad fue de 11,75%, produciéndose un plantel juvenil total de 23.863 animales. En el rancheo de año 2008 se colectaron 1.300 nidos silvestres, 35.932 huevos. Para el ciclo cerrado la producción de nidos se incrementó un 37%, obteniéndose 430 nidadas de las que se recogieron 12.480 huevos.

**(Invited Paper)**[7]

# The status of the Saltwater crocodile (*Crocodylus porosus*) inhabiting the Nilwala River, Matara District, Sri Lanka, and its impact on the community

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## INTRODUCTION

Two species of crocodiles: the Mugger or Marsh crocodile (*Crocodylus paluster* Lesson 1831) and the Saltwater or Estuarine crocodile (*Crocodylus porosus* Schneider 1801) are known from Sri Lanka (Deraniyagala 1939; Whitaker & Whitaker, 1979; Das & de Silva, 2005; de Silva 2007). References to crocodiles are found in the early literature of the country such as Buddhist Jataka stories (written around 5-8th century AC) e.g. Vanara *Sumsumara Jataka, kumbila*. The ancient chronicle *Culavamsa* (1: 70.4) also records that during the reign of king Gajabahu 1132-1153 AC, King Parakkramabahu's men could not pass the deep waters at Yatthikanda and Dumbara due to man-eating crocodiles (Geiger, 1929).

P. E. P. Deraniyagala was one of the first to conduct studies of both species of crocodiles of Sri Lanka (1930, 1939). However, the first status and census survey of crocodiles of the island was carried out by Rom Whitaker & Zai Whitaker in 1977 (1979). The present study could be mentioned as the first detailed investigation of the saltwater crocodile inhabiting one specific locality: the Nilwala River (south Sri Lanka). The present study also investigated several other related aspects of the saltwater crocodile, such as approximate numbers, status of their habitats, including nesting, human conflict and the knowledge and attitude of the people living in crocodile habitats along the Nilwala River. Awareness programs too were conducted to vulnerable people of the precautions that they need to take to avoid attacks by crocodiles and the importance of these reptiles in the river ecosystems.

*Crocodylus porosus* is highly a threatended reptile in Sri Lanka. It has only a few favoured natural habitats left, and presently most of these habitats are cleared, altered and under pressure by human activites. The study gave an overall perspective on where crocodile concentrations still occur (especially outside the Wildlife Protected Areas) and identify places where crocodiles are likely to continue to survive given some assistance with awareness and protection to the community in these areas.

## STUDY AREA AND METHODOLOGY

Nilwala River, Matara, from Modara (Lands End) (North 5º 56’ 42.7” and E 80º 32’ 26.0”) to Paraduwa (North 06º 04’ 09.3” and East 080º 30’ 55.0”), land water interface area, riverine mangroves and vulnerable people at Fort and Piladuwa, Matara (See Figures 1).

[7] The importance of this paper relates to the fact that this appears to be the first report from Sri Lanka since 1979. Since then we have had very few reports on the status of Saltwater crocodiles in this region.

## METHODOLOGY USED

A structured questionnaire was administered to 53 householders in Fort and Piladuwa, Matara by the chief investigator and a survey team of six people from Sri Lanka Red Cross (DP) team.

Conducted boat cruises in the Nilwala River in the study area from 0800 to 1300 hours on three days for physical verification of the presence of crocodiles, assess the status of their habitats and discuss possible plans of action with people residing along the riverbank within crocodile locations.

The same river route was cruised at night (2000 to 2400 hours) to conduct the night "eye-shine" sampling technique using powerful spotlights. The survey team was dressed in dark clothes so they were not conspicuous to the crocodiles.

Interviewed fishermen to estimate the number of crocodiles accidentally trapped and killed in their nets.

Interviewed family members of victims who were killed by crocodile attacks as well as people who sustained minor injuries due to attacks.

Collected samples of mangrove plants and mangrove associate plants along the riverbank. These were immediately placed into separate plastic bags, and secured with rubber bands. These plants were subsequently taken to the National Herbarium, Botanical Gardens, in Peradeniya for identification.

## RESULTS (SUMMARY):

- Observed fourteen *C. porosus* yearlings (under 600 cm) and 13 adults including a large (approximately 4.5 m) specimen.
- Encountered 31 and 39 pairs of eye-shines respectively during the night 'eye-shine' technique, which was conducted on two nights.
- Observed about 25 species of riverine mangrove plants and mangrove associate plants
- Investigated eight people who were attacked and killed by crocodiles.
- Investigated ten people who sustained minor injuries from crocodile attacks.
- Investigated 70 abandoned and in use 'crocodile exclusion pens' and 3 crocodile 'fences' that have been installed along Nilwala River.

## THREATS

### Direct threats to crocodiles due to human activities

Direct threats to crocodiles by human activities are: killing of supposed 'man-eaters', destroying eggs to control the growth of the species, killing of yearlings and sub adults which get trapped in fishing nets, (some drown in the fishing nets). In fact, one fisherman informed the author of killing five sub-adult crocodiles that had got trapped in his fishing nets in the past. Evidence of similar instances of fishery related mortality was revealed while interviewing several other fishermen. Furthermore, there is an increase in the fishing industry in Matara. Occasional attacks on humans give crocodiles a bad reputation. Just a single confirmed man-eater inevitably leads to many of the crocodilians in the vicinity being killed.

### Direct threats to crocodile habitats due to human activities

Vast extents of the riverine mangroves and mangrove associated plants along the Nilwala River have been cut down for firewood, cleared for agricultural purposes as well as other development projects over the years.

### Predators

The water monitor (*Varanus salvator*) attacks and feeds on large number of animals, including other reptiles, such as venomous and non-venomous snakes and crocodile yearlings and crocodile eggs. Deraniyagala (1939) also reports water monitors feeding on crocodile eggs. In addition, eagles, hawks and mongoose have all been observed to prey on yearlings. Large water monitors were common in the Nilwala River.

## CONCLUSIONS

The KAP (Knowledge, Attitude and Practice) survey indicated that vulnerable people were unaware of most of the general facts about crocodiles. However, many important and interesting observations can be learned about the activities of crocodiles from these people as they observe them daily.

Regarding the human-crocodile conflict and attacks, investigations revealed that in all cases studied the fault was on the part of human beings. Yet many use insecure crocodile exclusion pens for bathing and washing. Thus, an intensive awareness program coupled with protective measures like installing ‘crocodile exclusion pens’, ‘crocodile exclusion fences’ and installing warning sign boards in risk areas were suggested. As far as crocodile attacks on pet and farm animals is concerned, most of these attacks have taken place in the river-land interface, pointing to the negligence of the respective owners of these animals. During our survey of residents living near the river we observed that some tie their dogs in the backyard of their homes adjoining the river, which prompts the dog to bark at the slightest disturbance. This unfortunately helps to attract crocodiles. During the survey, we encountered five such cases.

Regarding the direct threat to crocodiles by humans, such as killing of supposed ‘man-eaters’, destroying eggs and yearlings and sub-adults that get trapped in fishing nets could be minimized by educating the relevant people. In addition, killing crocs for its flesh is an additional threat. In areas where crocodile flesh is eaten, we surveyed people who related that the flesh tastes good and is of high medicinal value.

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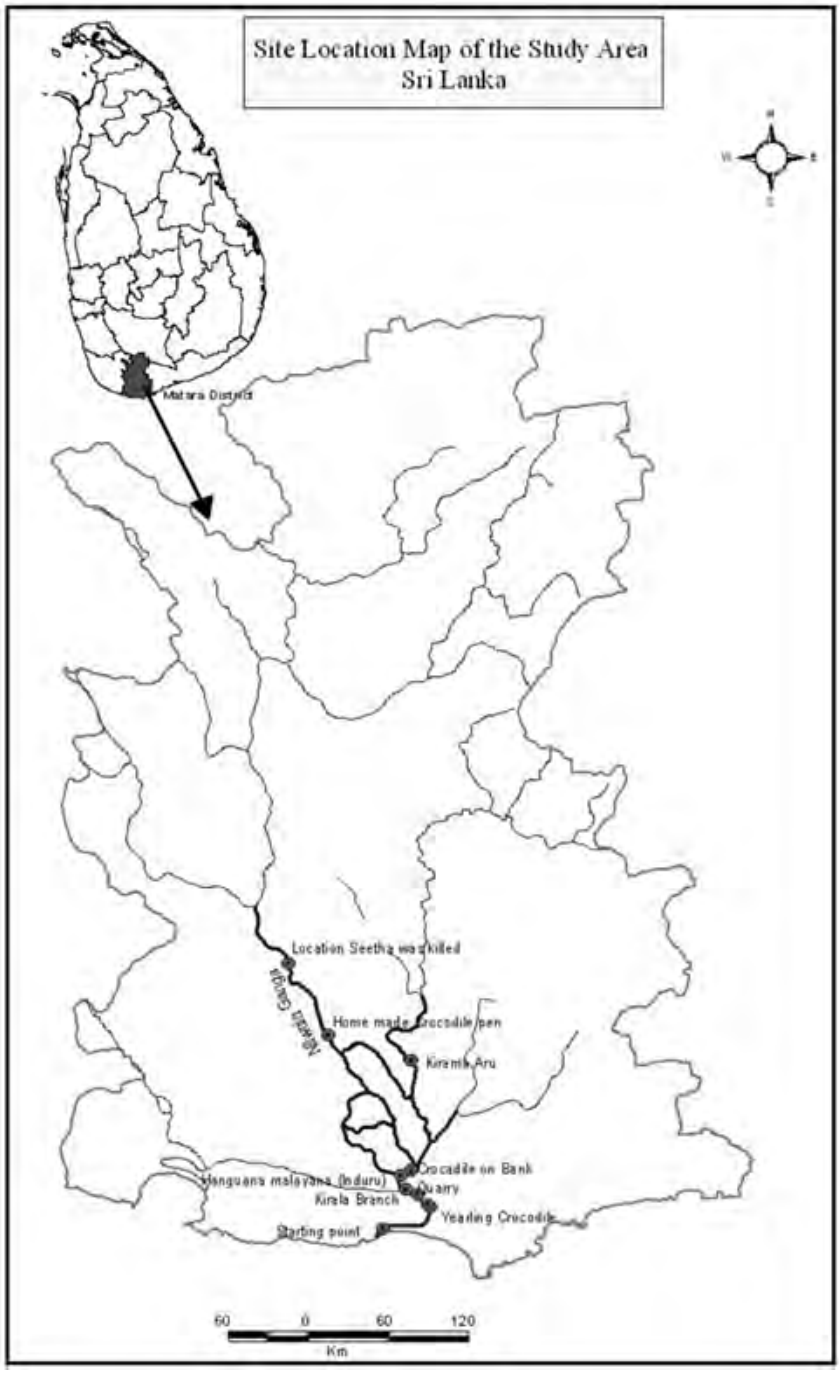
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# A nearing to caiman ethology (*Caiman crocodilus fuscus*) in conditions of captivity, Crocodile Museum, Zoomat, Chiapas, México

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**ABSTRACT:** The ethology of Mexican crocodilians is poorly studied, reason why made a first approach with *Caiman crocodilus fuscus* searching to create basis lines for future studies. This work was performed on the areas of the Crocodile Museum, in the Regional Zoo Miguel Álvarez Del Toro, located in Tuxtla Gutierrez, Chiapas, Mexico. We worked with 6 reproductives specimens; 2 males and 4 females between 10 and 17 years old, from the Coast of Chiapas. Made between August and September of 2007, with a total of 21 days of observations; it was used a chronogram with stratified sequences of observations to have the entire spectrum light during day hours. The used technique was the focal animal, based on an ethogram to *Crocodylus moreletii*. We observed 16 different behaviors where the most frequent were those of visible eyes, visible nose and sunning d. statistically significant differences where not found in the behavior of males and females; however, it is possible to infer that one of the males would be acting dominant. The reproductive behavior was not observed, this was because of the stress that individuals suffered by their recent change of site in 2006.

**RESUMEN:** La etología de los cocodrilianos mexicanos ha sido poco estudiada, por lo que se hizo un primer acercamiento con *Caiman crocodilus fuscus* buscando crear líneas bases para futuros estudios. Esto se realizó en las áreas del Museo Cocodrilo, del Zoológico Regional Miguel Álvarez del Toro, ubicado en Tuxtla Gutiérrez, Chiapas, México. Se trabajo con 6 ejemplares reproductores; 2 machos y 4 hembras; de entre 10 y 17 años de edad, originarios de la Costa de Chiapas. Realizándose entre agosto y septiembre 2007, con un total de 21 días de observaciones; se utilizó un cronograma con secuencias de observaciones estratificadas para tener todo el espectro durante las horas luz. La técnica utilizada fue la de animal focal, basado en un etograma para *Crocodylus moreletii*. Se lograron identificar 16 conductas distintas, donde las más frecuentes fueron los ojos visibles, nariz visible y asoleo d. Estadísticamente no se encontraron diferencias significativas en los comportamientos de machos y hembras; sin embargo, se puede infererir que uno de los machos estaría actuando como dominante. No se observaron los comportamientos reproductivos, esto se atribuyó al estrés que sufrieron los individuos por su reciente cambio de sitio en el 2006.

## INTRODUCTION

The crocodilians have a series of adaptations that have allowed them to survive by many million of years like group: the habitat in wich they are, the structural characteristics for feeding, the way they capture their prey, the complex social behavior that they exhibit,

and their reproduction are some of the things that make it attractive for study (Casa-Andreu, 1969).

The state of Chiapas has the three species of Mexican crocodilians: *Crocodylus acutus* species predominantly of the Mexican Pacific, in the Atlantic from the coast of Florida and in the islands of the Caribbean to Venezuela, *Crocodylus moreletii* which is in the Gulf of Mexico, in Belize and Guatemala; and *Cayman crocodilus fuscus* wich distribution is from the south of Oaxaca to the Paraguay River in South America. In Mexico and Central America is restricted to the coast, but in South America it occupies the coast of the Atlantic and penetrates to the interior of the continent (Álvarez del Toro & Sigler. 2001).

The state of Chiapas has been divided in several physiographical regions, of which, four have populations of crocodilians, the similarity in them is the tropical climate, the abundance of water and the altitude of 600 meters on the level of the sea. In general, the custom and behavior of the mexican crocodilians are poorly studied (Sigler, 1996); reason why made a first approach with *Caiman crocodilus fuscus* (*C. c. fuscus*) searching to create basis lines for future studies is important.

## MATERIALS & METHODS

**Workplace:** Crocodile Museum (MUCRO), Regional Zoo Miguel Alvarez del Toro (ZOOMAT), located in Tuxtla Gutierrez, Chiapas, Mexico. This work was performed in the area well-known like outside exhibition (Fig. 1), in where are reproductive specimens of *C. c. fuscus.* The total area of the pool is of 158.74 $m^2$ of which 89.77 $m^2$ correspond to earth and 68.97 $m^2$ correspond to water. The total amount of water is 34.49 $m^3$ (Fig. 1). The work was made in the months of August to September of the 2007 year.



**Figure 1**. pool outside exhibition 3 of reproductive specimens of *C. c. fuscus.*

**History of the individuals:** The observed individuals have been in conditions of captivity approximately for 10 years, they come from Puerto Arista and were donated to ZOOMAT in October of the 2006. The data of the individuals is shown in table 1.

**Table 1:** Data of the observed individuals.

| Origin | Date | Sex | Name | Staple | Year of birth | Age | weight (gr.) | length (mm) |
|---|---|---|---|---|---|---|---|---|
| Do. Pto Arista. | 28/10/06 | Male | Hocicudo | 293 | 1990 | 17 | 34000 | 1730 |
| Do. Pto Arista. | 28/10/06 | Male | Colamocha | 300 | 1990 | 17 | 25000 | 1680 |
| Do. Pto Arista. | 28/10/06 | Female | Manota | 296 | 1990 | 17 | 20000 | 1420 |
| Do. Pto Arista. | 28/10/06 | Female | Chiquis | 298 | 1990 | 17 | 14500 | 1335 |
| Do. Pto Arista. | 28/10/06 | Female | Morena | 294 | 1990 | 17 | 25000 | 1530 |
| Do. Pto Arista. | 28/10/06 | Female | Lady | 295 | 1990 | 17 | 23000 | 1560 |
| Villa Flores | 2001 | Male | Taiwan | 298 | 1996 | 11 | 19500 | 1501 |
| Chiapas de Corso | 2003 | Female | Lupita | ZMT606 | 1997 | 10 | 9000 | 1145 |

**Observations:** The observations were done using the technique of focal animal, in which the specimen was identified for each characteristic of the individuals. We used an ethogram (16 different behaviors) made for *C. moreletii* (Orozco, 2006). An extra behavior (semi submerged) was added. Semi submerged: head, and back outside the water, the rest of the body is submerged. The individual is static in that position or it can move (Fig. 2). In addition for this work semi submerged sunning was considered to the behavior taken from Orozco as a type of sunning to which we denominate sunning e.



**Figure 2.** specimen semi submerged.

A chronogram of observations was made, using a sequence of observation stratified with variation day to day to have the entire spectrum light during day hours. This chronogram followed during 21 days, doing a total of 81 hrs. of observations. They studied to 6 individuals

that coexist in the pool, in day 19 of work was introduced a pair of *C. c. fuscus* that they were in the area of handling of the MUCRO, located in bathtubs, being a total of 8 specimen that correspond to 5 females and 3 males (table 1). Daily, the temperature of the atmosphere, the wind, the cloudiness and rain, were written to determine the possible zones of ovoposition.

**Analysis:** We used Microsoft Excel software to make the tables and the graphics, and Info Stat to make the statistical analysis (ANOVA).

## RESULTS AND CONCLUSION

As follow, an histogram with the behaviors frequencies (Fig. 3) showing that the most frequent behaviors are visible eyes with a total of 1364 observations, sunning d with 1020 observations and visible nose with 912 observations, making the 74.47% of total, whereas the remaining (26.53%) is distributed in the other 14 behaviors.

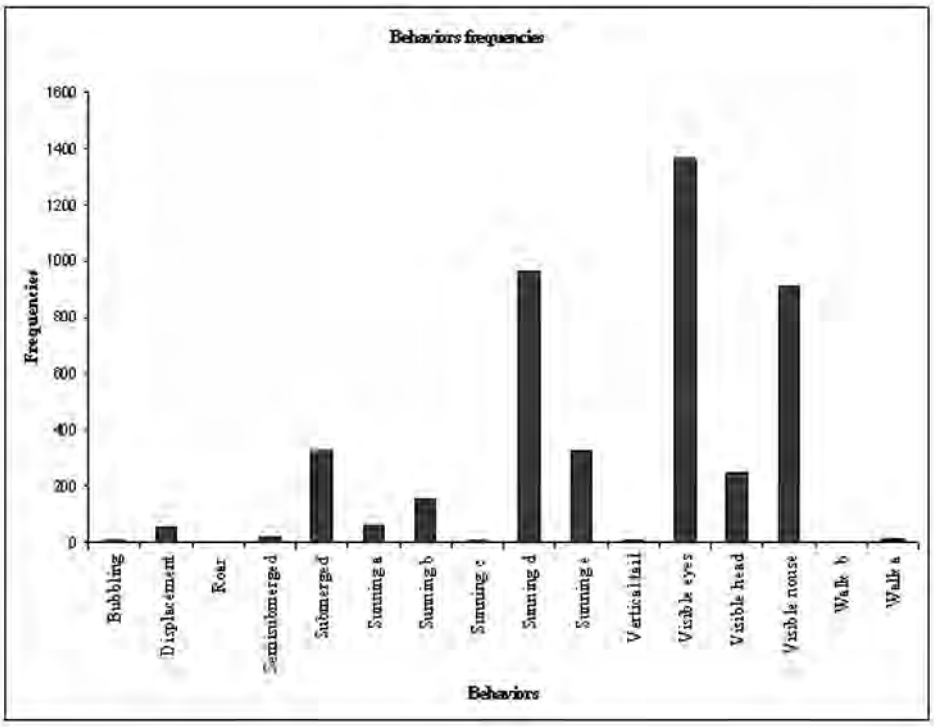



**Figure 3.** Frequencies of the observed behaviors total.

We made an ANOVA to observe if these differences were significant obtained: walking b, roaring, sunning c, vertical tail, walking a, bubbling, submerged, displacement and semi submerged are significant different with sunning b, sunning a, visible head, visible sunning, nose, eyes and sunning d.

The comportamentales frequencies for males and females are show in Fig. 4 & 5.

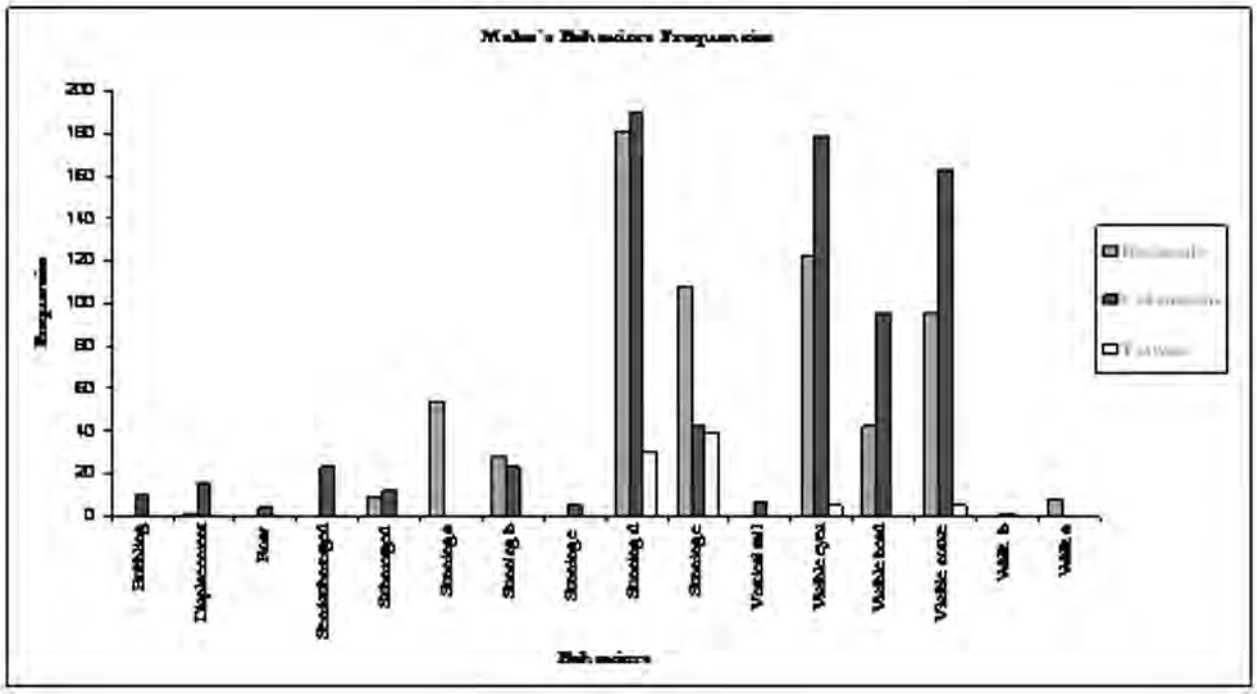


**Figure 4.** Behaviors frequencies of the males.

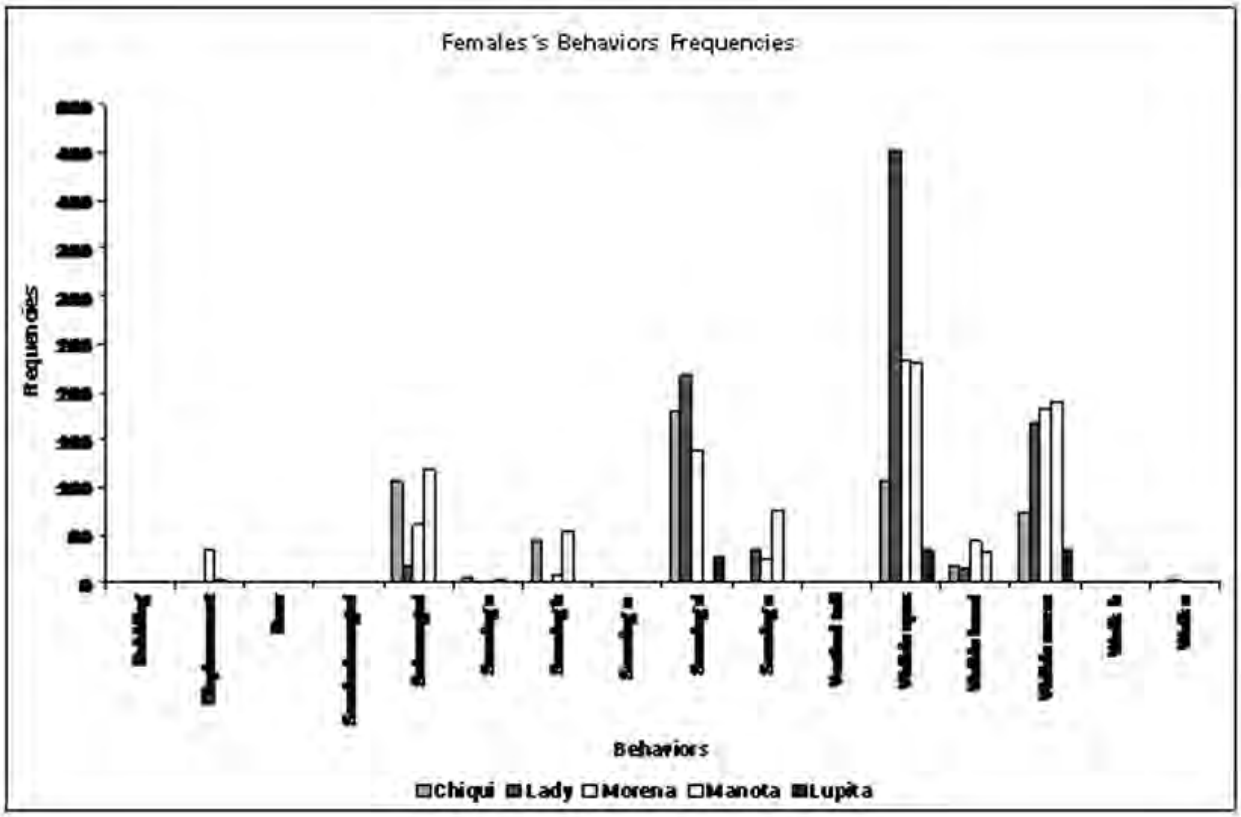



**Figure 5.** Behaviors frequencies of the females.

For the three males observed the most frecuent behaviors were shows in Fig. 6, 7 & 8.

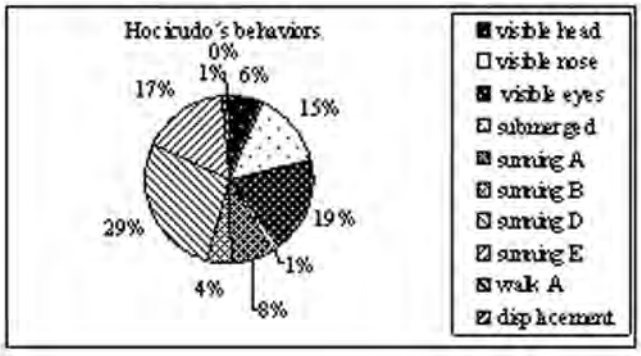



**Figure 6**. Hocicudo´s behaviors.

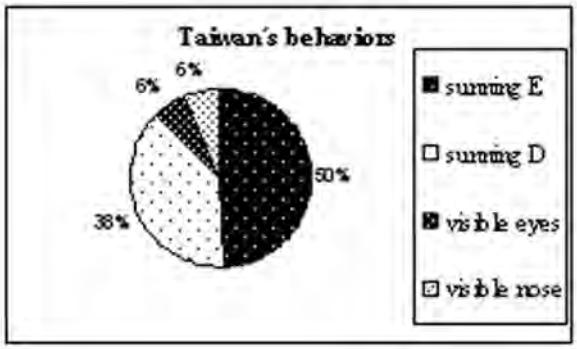



**Figure 7.** Taiwan´s behaviors.

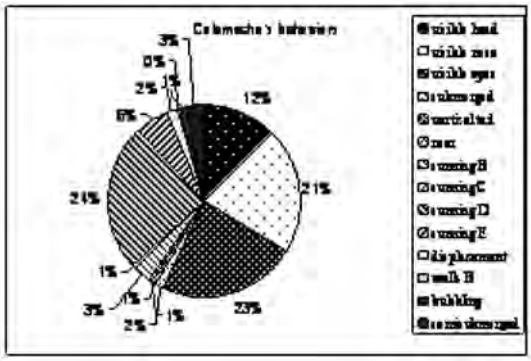



**Figure 8**. Colamocha´s behaviors.

The onlyone wich have territorialidad and courtship behaviors was Colamocha; three of these behaviors were observed, that are the bubbling, the vertical tail and the roar that constitute 2.59% of the total. This percentage is low but if it is compared with the other individuals, could be indicating a dominancia of Colamocha on Hocicudo.

Taiwan, male who was introduced in day 19 of the study, like for Hocicudo had behaviors of sunnings and visible nose and eyes, representing the sunning e 49.37% of the behaviors and sunning d 37.98%. It is possible to mention that these two behaviors were outside the water mirror. In addition we observed that in this introduction, Colamocha increase the frequencies in the territorialidad behaviors as the bubbling and semisubmerged, too increase the frequencies of displacement, which would be sustaining the dominancia of Colamocha mentioned previously. According to the results exposed in Fig. 6 the behavior most frequents for females are visible eyes, sunning d and visible nose.

Figures 9, 10, 11, 12 & 13 show the percent for each female.

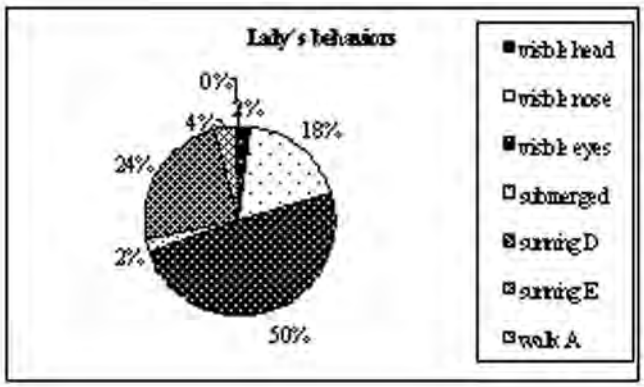



**Figure 9.** Lady´s behaviors.

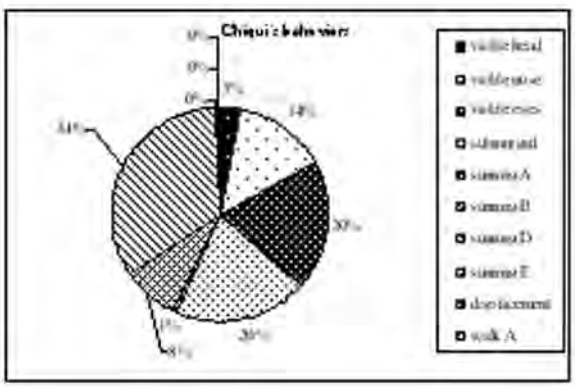



**Figure 10.** Chiqui´s behaviors.

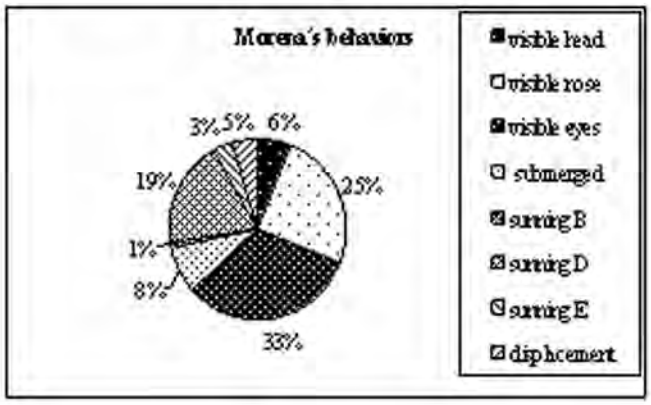



**Figure 11**. Morena´s behaviors.

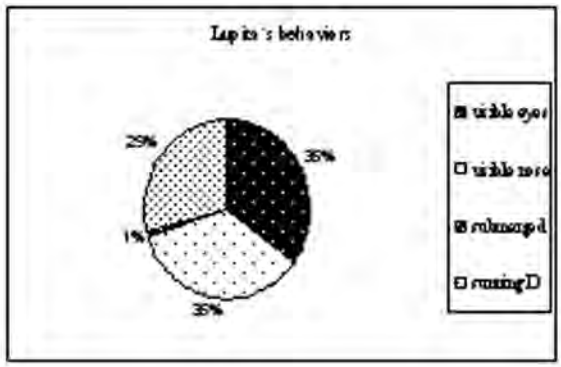



**Figure 12.** Lupita´s behaviors.

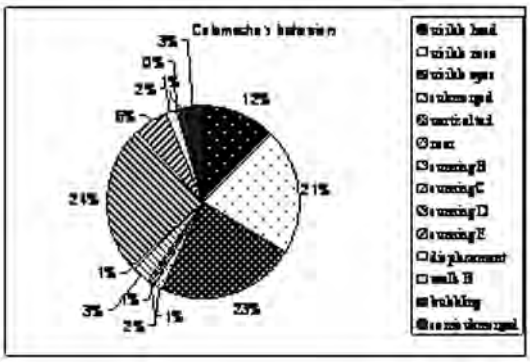



**Figure 13.** Manota´s behaviors.

For Lupita, the female introduced with Taiwan, observed that the frequent behaviors were visible eyes and nose with 35.5% constituting both 71% of the total. This would indicate that the female did not have any type of disadvantage in its introduction to the pool, its was not attacked for any of the individuals that were in.

From the exposed results previously we can say that for the females and males, the most frequent behaviors are those of location (visible nose and visible eyes) and sunning d.Then an ANOVA were made to analyze if there are differences betwen the individuals obteining that there are no significant differences between the individuals that coexist in the pool. Although according to the observed (Fig. 4) only Colamocha had territorial behaviors. In the 3 last days of observation this individual showed a modified behavior of tipe the alert, semisubmerged in water, whereas Taiwan stayed outside the pool occupying a very small space and always near the border. Nevertheless Taiwan was not used for the statistical analyses because it was only observed during 3 days, for that reason believe that the result had been different if the observations had continued. For the other hand females behave of equal way, as it was not to be expected becouse the time in which the study was made was the time of reproduction and nesting which did not take place.The reproductive behaviors was not observed possibly due to the stress of the individuals, becouse these relocated to another place in where latitude and altitude were different, as well as the average temperature. For the same reason the possible zones of nesting could not verification (Fig. 14).



**Figure 14.** Possible zones of nesting for reproducers of *C. c. fuscus.*

Then, in figure 15 observed the relationship between behaviors and temperature. Displacement, to walk b and to walk a; occurred when the temperature is about $27^0$C. Respected to sunning the most frequent is d (head high and closed snout) which has two tips ($27^0$C & $24^0$C) this type of sunning does not depend of the temperature. The other types of sunnings stay constant in the different temperatures, except for the sunning type b (head elevated with open snout) which is minimum in low temperatures ($23^0$C to $26^0$C) and maximun at high temperatures ($26.5^0$C to $27.5^0$C); this would indicate that *C. c. fuscus* opens the snout to expel heat and it does not for take it (Fig. 15).

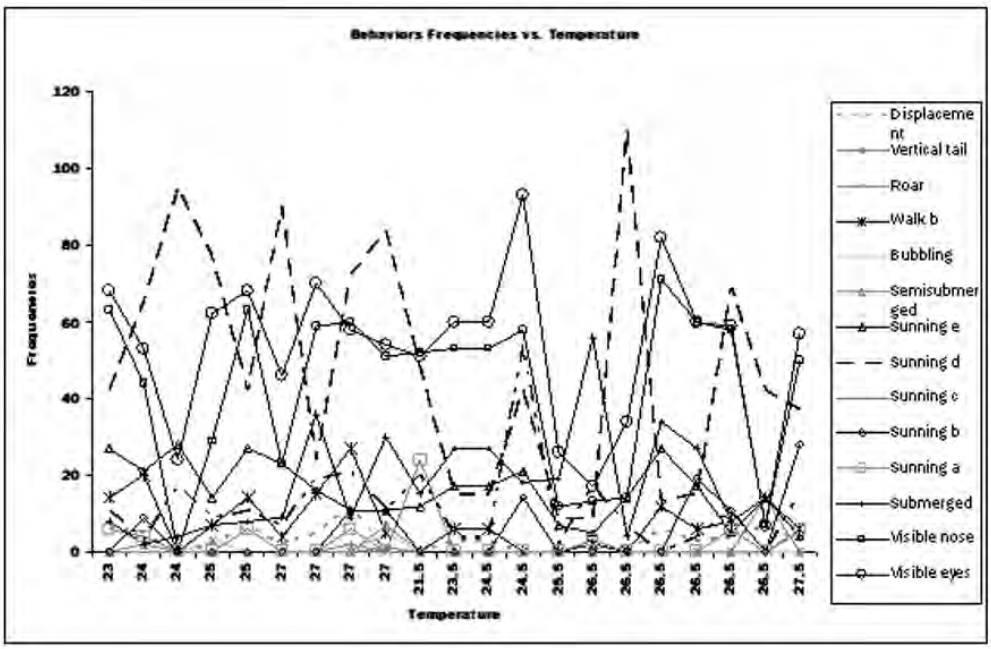



**Figure 15.** Behaviors frequencies in relationship with temperature.

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Orozco, Deyli de León, 2006 Conducta prereproductiva y reproductiva del cocodrilo de pantano *Crocodylus moreletii* bajo condiciones de cautiverio.

# Abundance and Population Structure of *Melanosuchus niger* and *Caiman crocodilus* (Crocodylia, Alligatoridae) in Araguaia National Park, Tocantins, Brazil.

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**ABSTRACT:** Information about abundance and structure are essentials to determine the current conditions of a population. Several studies about crocodilians biology and ecology were conducted in Brazil, however it knows not much about the species present in the Tocantins state. This work aims to extend the knowledge about population ecology of *Melanosuchus niger* and *Caiman crocodilus* around the Araguaia National Park, Tocantins. The study was conducted between August 2004 and November 2006, with spotlight surveys using an aluminum boat in 50 km of shoreline in the Rio Javaés. We counted 4887 caimans, of which, 774 belonged the specie *M. niger* (0,1 to 2,5 animals per Km of shoreline) and 1849 of *C. crocodilus* (0,1 to 8,6 animals per km of shoreline). Individuals of all size class were found, indicating a possible stability in the development of these populations. We observed that species occupied different microhabitats, where *M. niger* remained in the main bed of the river and in the parts deepest of the reentrance while *C. crocodilus* occurred most frequently in shallow or grassy areas. The data obtained in this study help in assessing the current status of conservation of these species in a little studied region of the Brazilian Amazon.

# Abundance and population structure of Spectacled Caiman (*Caiman yacare*) in Isla del Encanto and El Combate communities (Yapacani and Grande rivers), Santa Cruz - Bolivia.

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**ABSTRACT:** Surveys of Spectacled Caiman (*Caiman yacare*) were conducted during December 2005 and January 2006 (corresponding to the wet season) and in September 2006 (dry season). This population assessment was carried out in Grande and Yapacani rivers, and floodplain lagoons (Obispo Santistevan province, department of Santa Cruz) under the National Harvesting Program of Spectacled Caiman (*Caiman yacare*) We selected 9 water bodies in the area of Grande and Yapacani rivers, and we conducted night surveys with 3 replicates on different days in two seasons to establish the abundance and population structure of spectacled caiman, and to find differences between the results found in wet and dry season.
*C. yacare* is widely distributed throughout all the study area but not in a uniform way, we found a population median of 6.83 ind / km of shore, while the population structure is dominated by Class II juveniles, and with low percentage of Class IV individuals (13.10%); this could suggest a population which has been subjected to extraction of adult males.

**RESUMEN:** En el marco del programa Nacional de aprovechamiento del *Caiman yacare,* se realizaron evaluaciones poblacionales de este recurso en la provincia Obispo Santistevan del departamento de Santa Cruz en los ríos Grande y río Yapacani y lagunas fluviales durante el mes de diciembre del año 2005 y Enero del 2006 correspondiente a la época húmeda y en Septiembre del 2006 correspondiente a la época seca.
Se establecieron 9 cuerpos de agua en la zona en los ríos Grande y Yapacani realizando conteos nocturnos con 3 repeticiones en diferentes días en dos épocas del año para establecer la abundancia y estructura poblacional del lagarto *Caiman yacare* y determinar diferencias entre los resultados obtenidos en época húmeda y época seca.
*C. yacare* está ampliamente distribuido en toda la zona de estudio pero no de una manera homogénea, se encontró una mediana poblacional de 6.83 ind/km de orilla recorrida, mientras que la estructura poblacional se encuentra dominada por individuos juveniles clase II (50 a 120 cm de longitud total) con un bajo porcentaje de individuos clase IV (13.10 %), lo cual podría sugerir que se trata de una población que ha sido sometida a extracción de machos adultos.

## INTRODUCTION

America concentrates the greatest diversity of crocodiles that can be found in relation to other areas of the world, throughout history, this region has been the largest source of commercial exploitation of crocodiles on the planet. This fact, boosted also by an increasing decline in suitable habitat, caused a quantitative decline of wild populations; led in some cases, extinctions (Messel, 1991, quoted in Prado, 2003).

Crocodiles are an important ecological component of tropical fauna and represent a natural resource of considerable value, primarily by the quality of their leather, as well as their meat by-product, has great potential especially for export. The main causes that produce the decline in natural populations of these reptiles are illegal hunting and habitat destruction (Aparicio, 1999; Bost, 1983; Pacheco and Aparicio, 1996; Thorbjarnarsson, 1991). In Bolivia, hunting of spectacled caiman (*Caiman yacare*) acquired greater importance since the 70's, when the populations of black caiman (*Melanosuchus niger*) and the broad snouted caiman (*C. latirostris*) almost disappear as a result of the strong pressure of commercial harvest (Aparicio, 1997; Llobet and Goitia, 1997).

The great potential which represents the species *C. yacare* like an usable resource as well as its important role in the dynamics of the ecosystems they inhabit, makes an urgent implementation of programs to ensure the conservation of this species and make possible sustainable development (Llobet and Goitia, 1997) since the use of diverse wildlife species in the neotropics constitutes an alternative element as economic and food for local people, indigenous and peasant (Bodmer *et. al.*, 1996).

## OBJECTIVES

Determine the relative abundance and population structure of the spectacled caiman (*Caiman yacare*) in the cattle ranche Isla del Encanto and community El Combate, in the fluvial lagoons of the Grande river and Yapacani river, in two different seasons of the year.

Compare the population abundance and the population structure in the two seasons of the year (humid and dry time).

## METHODOLOGY

This study was conducted in the municipality of Mineros province Obispo Santistevan in the Northwest of the department of Santa Cruz-Bolivia to 243 km. to the city of Santa Cruz de la Sierra in the towns of Isla del Encanto and El Combate community. The town Isla del Encanto is surrounded by rivers Yapacani (old and new channel) Piraycito, and the Grande river. The community "El Combate" is based on the Grande river north of the Isla del Encanto.

Counts were conducted during the night into the wet and dry season. Corresponding to the wet season the campaigns of December 2005 and January 2006. During the dry season only a raid was conducted fieldwork in September 2006 for lack of funds could not be entering a second time this season. 9 aquatic ecosystems were sampled in two communities settled in the course of the rivers Yapacani and Grande.

In each of these 9 water bodies were conducted with 3 counts repetitions carried out on different days trying to start night counts at the same time and under similar climatic conditions such as moon phase, rainy days.

Individuals were located at night using 12-volt spotlights, 500000 candles power. Maglite flashlights of 3 batteries and 4.5 volts 22000 candles power.

The abundance of spectacled caiman population was calculated including individuals observed from the population for classes II, III and IV and is expressed in individuals per kilometre from shore (ind / km). (Aparicio 1997, Godshalk 1994, King and Videz Roca, 1997; Godshalk 1997, Llobet and Aparicio 1999, Llobet and Goitia 1997, Pacheco 1993), individuals found in Class I were not taken into account when calculating the abundance due to the high mortality rate that has during the first year of life.

To determine the population structure were conducted Histograms size, following criteria established by Velasco and Ayarzagueña (1995). According to these, the animals were divided into four classes according to size the following characteristics:

- Class 1: animals with a total length (snout-tail) of 50 cm. corresponding to 25 cm. length ventral (snout-sewer). Includes most of the juvenile in the first year of life. This kind usually not taken into account in determining the characteristics of the population, because in this stage of life is estimated that the rate of survival this coming more than 20% of individuals who are born, which makes this class suffers strong monthly changes.
- Class 2: animals with a length of 50 to 119 cm. (snout tail). This class is composed of young males and females.
- Class 3: adult animals with a total length of 120 to 179 cm. In this class include the full range of reproductive females and a portion of adult males reaching about 20% of the Class.
- Class 4: adult animals with a greater length of 180 cm. The class consists almost entirely by adult males reproductive and is being used for the program of commercial exploitation of spectacled caiman in Bolivia.
- Eyes Only: when it was not possible to estimate the size of an individual identified spectacled caiman, is assigned to the category Eyes Only (OS).

The population structure was carried out three counts of repetitions in each of the water bodies in the two seasons of sampling. Applying the method of minimum and maximum Messel (1991), this method is to take the maximum number of individuals observed obtained from the three repetitions in each size class.

We compared the results statistically abundance and population structure of spectacled caiman between the two seasons of sampling. The analysis of the data was carried out with the statistical programs Infostat and Statsoft (Statistica 6.0) where the results obtained during the seasons samplings were compared statistically, this way to be able to determine if significant differences exist among bodies of water or among sampling time. In the case of the population abundance using a nonparametric test (Kruskal - Wallis test) and for the structure population contingency tables with Chi squared.

**RESULTS**

It succeeded in registering a total of 672 individuals between the two field campaigns, covering a total of 189.76 kilometers from the shore of the 9 places sampling during the 2 sampling seasons (wet and dry). We show a square with the individuals found by sampling seasons and the total kilometers of shore sampling among the two seasons by water bodies

**Table 1.** Number of individuals found by sampling season and km of shores sampled.

| Place | Rainy season | Dry season | Km. of shore |
|---|---|---|---|
| Río Yapacani | 19 | 14 | 18.00 |
| Río Grande | 83 | 83 | 48.00 |
| Laguna Buey sarazo | 52 | 44 | 24.00 |
| Laguna Media luna | 21 | 12 | 10.80 |
| Río Yapacani2 | 20 | 43 | 48.00 |
| Laguna El Placer | 5 | 92 | 8.40 |
| Laguna Las Petas | 7 | 21 | 10.80 |
| Laguna Yapacani río seco | 20 | 103 | 18.96 |
| Laguna El Bufeo | 7 | 26 | 2.80 |
| **Total** | **234** | **438** | **189.76** |

Based on the night counts in the water bodies in the study area we found that the highest index of abundance is found in the lagoon El Placer during the dry season with 23.57 ind / km followed by the lagoon El Bufeo with 18.57 ind / km from shore during the dry season. While the lowest values found in the Yapacani river 2.50 ind / km in the rainy season, followed by the lagoon El Placer with 3.57 ind / km.

**Table 2.** Relative abundance of spectacled caiman (Ind/km) in the different water bodies.

| Place | Water bodies | Abundance Rainy season (ind/km) | Abundancia Dry season (ind/km) |
|---|---|---|---|
| Isla del encanto | Río Yapacani | 5.33 | 4.67 |
| | Río Grande | 9.50 | 7.00 |
| | Laguna Buey Sarazo | 11.75 | 11.00 |
| | Laguna Media luna | 10.56 | 6.67 |
| | Río Yapacaní 2 | 2.50 | 5.38 |
| El Combate | Laguna El Placer | 3.57 | 23.57 |
| | Laguna Las Petas | 3.89 | 11.67 |
| | Laguna Yapacaní | 6.01 | 12.34 |
| | Laguna El Bufeo | 5.00 | 18.57 |
| Median | | 6.01 | 7.00 |
| Standard Deviation | | 3.28 | 6.75 |

We contain the abundances for sampling time to see if they present significant differences for the season, not finding significant differences for the relative abundances in the sampling season to 95% and 99% (OR = 25.5 p = 0.18).

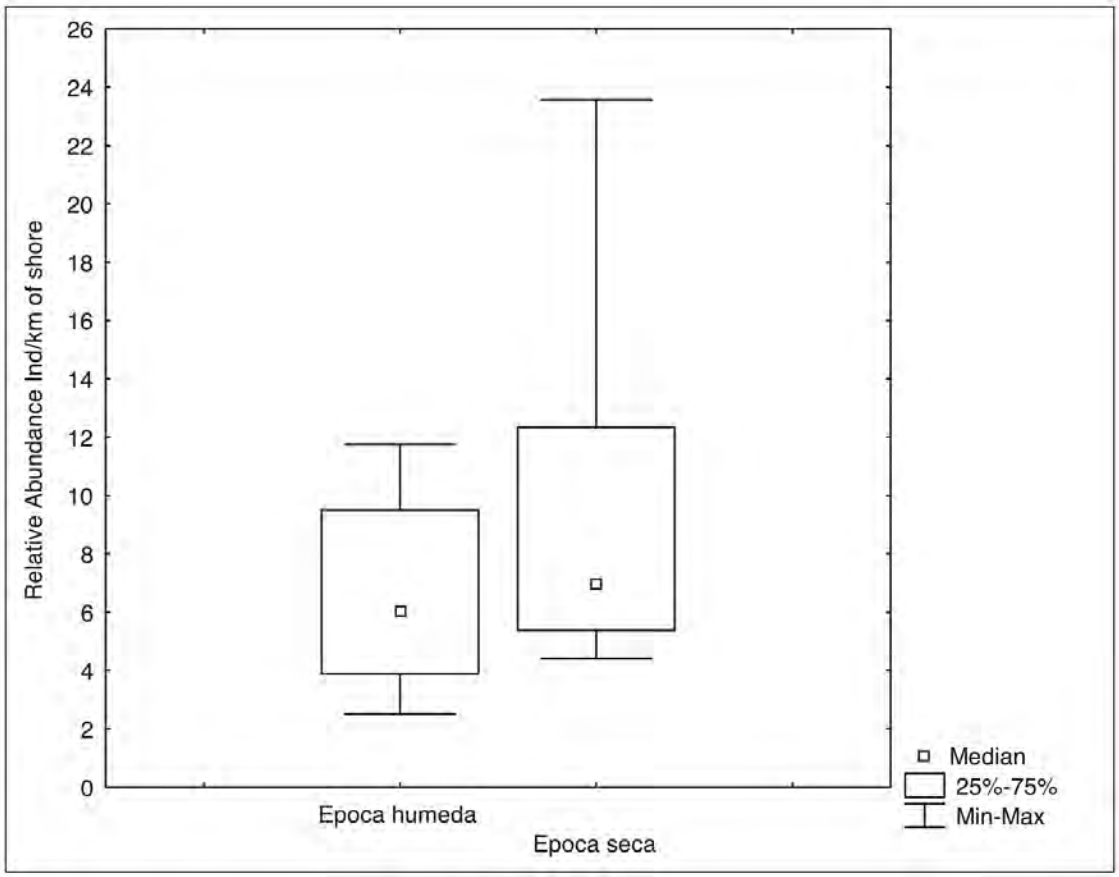



**Figure 1.** Relative abudance by sampling season.

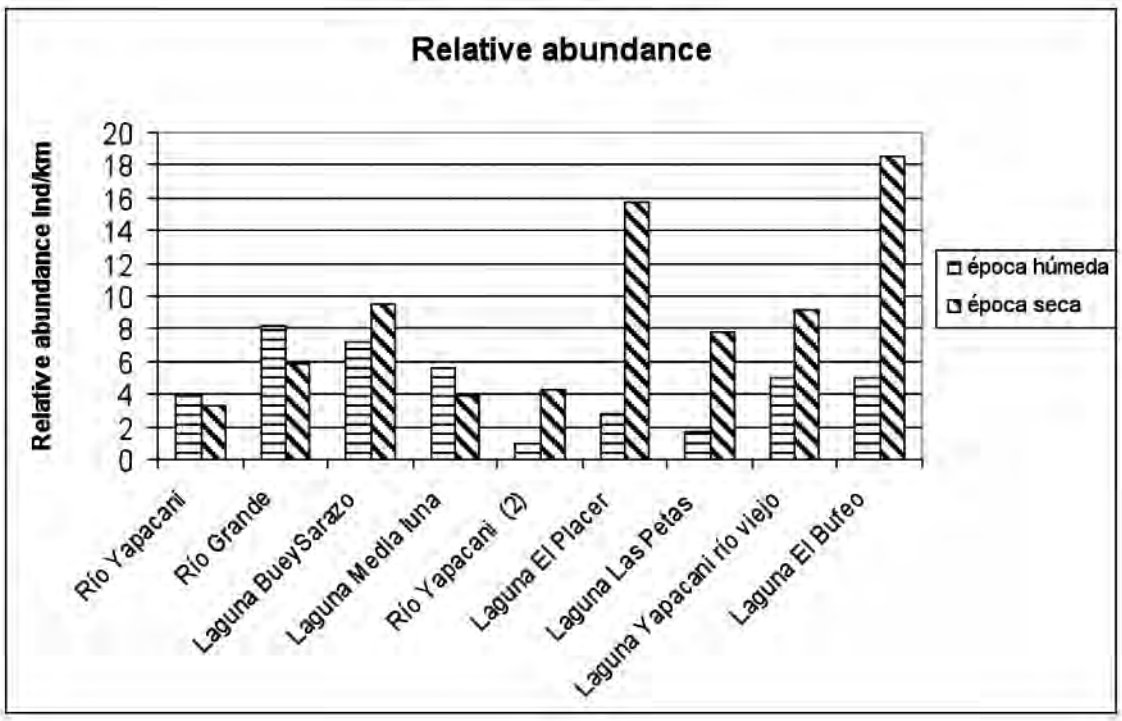



**Figure 2.** Relative abundance of spectacled caiman were found in water bodies in the two seasons of sampling.

An analysis containing the values of abundance by type of water bodies using two categories: running water (rivers) meanders (lagoons of fluvial origin). Among the two categories they registered 189.76 kilometers for shore sampled in the two seasons of sampling, which correspond 114kilómetros near running water (rivers), and 75.76 miles are along meanders (lagoon origin river). The analysis of the abundances by type of water bodies shows that there are no significant differences (U = 6.0, p = 0.44).

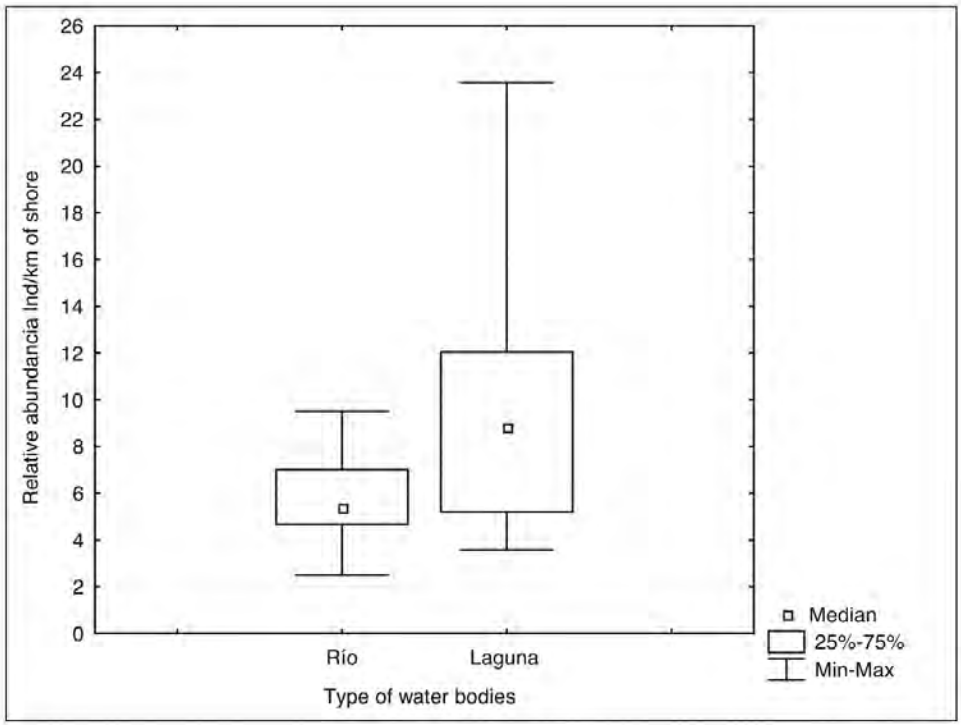



**Figure 3.** Relative abundance by type of water bodies.

As for the population structure we can see that is dominated by youngs animals Class II up to 120 cm. total length, followed by adult class III, then individuals infants Class I and finally the adult class IV.

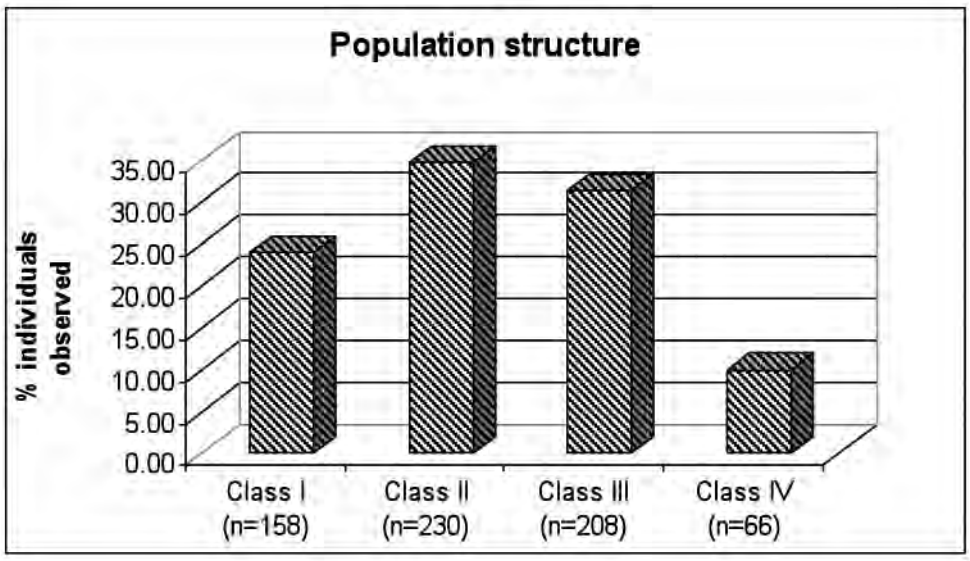



**Figure 4.** Population structure of spectacled caiman expressed as a percentage of individuals found to the area sampling.

Regarding the population structure for sampling seasons find that the rainy season is dominated by youngs individuals class II, continued by individuals adults class III, later individuals class IV (figures 5).

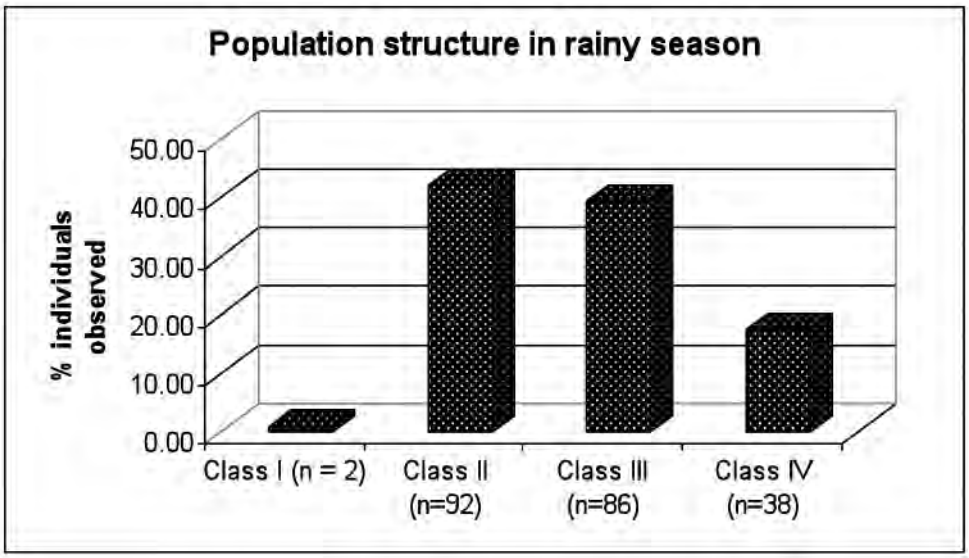



**Figure 5.** Population structure in rainy season.

In the dry season we found that the population structure is dominated by individuals infants Class I, followed by youngs individuals Class II, adult Class III and finally adult class IV (Figure 6).

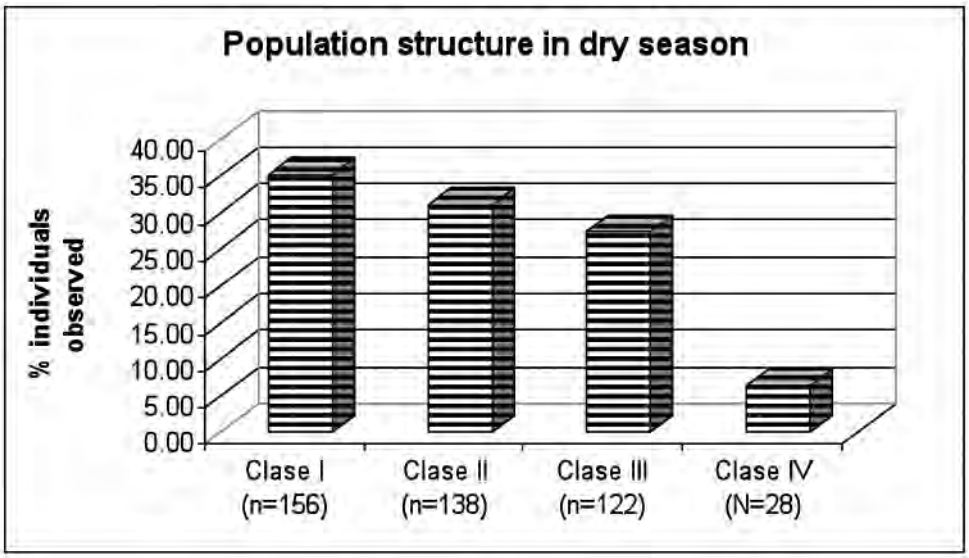



**Figure 6.** Population structure in dry season.

When comparing the structures for sampling times applying a Chi squared test ($X^2$) it shows us that highly significant differences exist in the population structure ($X^2$ = 101.75; p = 0.0001), these significant differences could be due to the presence and absence of individuals infants in the population structure in dry season and rainy season.
Similarly comparisons were made in the population structure by type of water body (rivers and lagoons) regarding the season (rainy season and dry season). That shows that there are highly significant differences for the two seasons: rainy season ($X^2$ = 14.32, p= 0.0025); dry season ($X^2$ = 37.75, p <0.0001).

## CONCLUSSIONS

The species *Caiman yacare* is distributed throughout the study area, covering all water bodies in the region, including lagoons of tectonic origin, fluvials lagoons and rivers, but is not an evenly distributed. The species *C. yacare* was the only crocodilian species recorded in the region during the nights counts. However, We note the presence of black caiman *Melanosuchus niger* by the presence of the head skull of a specimen recently killed by a community member of El Combate, and testimony from community members who claim the presence of black caiman *M. niger*, which did not comment records for the area.

The spectacled caiman *C. yacare* presents a median annual population abundance of 6.83 individuals per kilometers, showing a higher value to different records of this species to other areas. In rainy season presented an average of 6.01 ind / km in dry season and a value of 7.00 ind / km.

The records of Class IV for the populations of *Caiman yacare* in the study area show a fairly low percentage by far of classes of individuals' youth, suggesting that these stocks, on the one hand have a high-pressure hunting. This became evident with the report of the prefecture of extracting more than a thousand skins of spectacled caiman, from across the Grande River Basin. In addition to information for community residents El Combate ensure that intermediaries come from the Beni department to market hides extracted by community members in exchange for commodities of basic necessities.

As to factor human intervention, even though it was not conducted an analysis of the index of shyness could demonstrate that there is a high level of intervention because of the existence of communities on the river Grande performing harnessing spectacled caiman.

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# Alligators and Crocodiles as Indicators for Restoration of Everglades Ecosystems

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**ABSTRACT:** Ecological monitoring is a key part of adaptive management and successful restoration. Not everything within an ecosystem can be monitored so it is important to select indicators that are representative of the system, show clear responses to system change, can be effectively and efficiently monitored, and are easily communicated. Crocodilians (alligators and crocodiles) are one of the indicators that meet these criteria within the Everglades ecosystem. The alligator indicator uses relative density, body condition, nesting effort and success, and occupancy rates of alligator holes, while crocodile indicators use growth and survival, and trends in their populations related to hydrology. Correlations between biological responses and environmental conditions contribute to an understanding of species' status and trends over time. Positive or negative trends of crocodilian populations relative to hydrologic changes permit assessment of positive or negative trends in restoration. The crocodilian indicator is currently stable; with alligator trends negative in seven management areas and stable in two, and crocodile trends in Everglades National Park and Biscayne Bay Complex showing a stable trend. Restoration success or failure can be evaluated by comparing recent and future trends and status of crocodilian populations with historical or reference population data and model predictions.

# An enrichment experience for hatchling researchers

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**ABSTRACT:** As the interest for crocodilian conservation programs and the inherent demands for new researchers grow, the Instituto Mamirauá and the Wildlife Conservation Society promoted the "II Workshop for Training in Alligators Research", wich ocurred during December 1st to December 15th in the Reserves for Sustainable Development Mamirauá and Amanã, both situated next to Tefé, Amazonas, Brazil.

The course offered an unique opportunity to young researchers (which included recently graduated, Master students and Phd students) from South America to interact and share their experience while learning with experienced researches. Nine students were present and came from Argentina, Bolivia, Brazil, Colombia, Equator and Peru.

There were lectures and discussions about subjects concerning crocodile's biology and conservation. Standardized methodology such as night counts, nest search, capture of individuals, collection of blood samples and analysis of diet and wounds were also part of the training course. Three documents were produced using the data collected during the field training.

The workshop achieved its goals by stimulating the formation of a new network of South American alligator researches and standardizing the methodology for scientific surveys, contributing to a greater exchange of information. We hope this experience could be eventually repeated in a III Workshop.

# Broad-Snouted caiman growth (*Caiman latirostris*) under different periods of UV radiation (UVA-UVB) exposition

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**ABSTRACT:** UV radiation is necessary in many vital processes; all organisms depend directly or indirectly on UV radiation. In natural conditions, quantity and quality of radiation received by organisms depends on numerous factors. Under artificial conditions, it is possible to regulate UV exposure in order to estimate how long broad-snouted caiman would have to be exposed to affect specific biological processes. This study will allow us to evaluate *Caiman latirostris* growth, as well as serum calcium and phosphorus homeostasis, under different periods of artificial UV radiation, as well as make an estimation of the effects and implications of a possible greater environmental radiation exposure. Growth, and serum phosphorous and calcium concentrations, were measured in 72 broad-snouted caiman (6 month old) maintained with diurnal cycles of 8 or 16 hours of UVR (UVA-UVB) exposure, and another control group maintained in complete darkness during 90 days with controlled temperature.

# *Caiman latirostris* embryos exposed to pesticides in a field experiment simulating agrochemical application practices

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**ABSTRACT:** Roughly 85–90% of pesticides applied agriculturally never reach target organisms directly, but they disperse through the air, soil and water. In Argentina, some areas of the broad-snouted caiman habitat are situated near agricultural lands where high amounts of agrochemicals are applied.

The practice of creating pesticide cocktails/mixtures can produce a potentiation of the individual properties of chemicals, such as genotoxic effects, which may be negligible when individual chemicals are considered.

The aim of this study was to determine the effects of pesticides on *C. latirostris* exposed *in ovo*. We carried out a field experiment in which common agricultural pesticide applications were used to simulate the maximum environmental exposure that a caiman nest can receive in neighbouring croplands habitats. Three groups of artificial caiman nests were constructed and eggs placed inside. One group remained untreated, the second received an application of a glyphosate herbicide formulation and the third was spread with a pesticide mixture consisting on this herbicide and two insecticides: endosulfan and cypermethryn based products.

Neonate caiman were examined using morphological endpoints (mass and length) and genotoxic effects were probed via the Micronucleus test and the Commet assay in order to compare data from caiman exposed to different pesticide applications with untreated animals.

# Caiman's Abundance in Sustainable Development Reserves Mamirauá and Amanã, Central Amazonia, Brazil

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**ABSTRACT:** We studied the relative abundance of the crocodilian's species presents in the Sustainable Development Reserves Mamirauá y Amanã through night counts in metallic canoes with motor, traveling distances up to 15 kilometers in rivers, floodplain and lakes, including areas of várzea and igapó. The relative abundance was obtained by means of the rate of encounter that consists of the number of individuals detected divided by the whole range.

The results showed a greater amount of caimans in várzea, this would be because várzea has a greater amount of organic matter, resulting in a greater amount of food available for these animals. Considering the microhabitat where the caimans were seen, it was observed that the greater amount of caimans were in macrophytes. The greater rate of encounter took place in the sector of Paranã de Jarauá, finding 11.1 caimans per kilometer of border; the lower rates of encounter occurred in the Sustainable Development Reserves Amanã, with a rate of encounter of 0.07 caimans per kilometer of border.

# Center educational and demonstrative of the program “Yacarés de Entre Ríos”

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**ABSTRACT:** One presents the experience that arises in the Program "Yacarés de Entre Ríos" from the of integration approach that is done on having spoken about Cayman latirostris as natural inhabitant of the sweet water. The analysis of the problematics of the natural resources and of the environment, it is done relating his presence to the good availability of water and ideal offer of food, taking the method as a practical example of ranching in the commercial exploitation of the species when we speak of " develop sustentable ". By means of holding a permit visits, photos, chats, projections, walks, there is visualized the narrow interrelationship that exists between the different animal, vegetable, species and his environment, and the way that the evil affects in the quality of life of the communities use of pollutant products or inadequate managing of the soil, water or mount. The aim is to generate an environmental culture from conducts that allow a suitable use of the natural resources and the environment, revaluing the resource sweet waters down on having defended the crocodile.

# Characterisation of Endogenous Retroviruses in Crocodilians

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**ABSTRACT:** Endogenous retroviruses (ERVs) are copies or remnants of exogenous retroviruses that integrated into a host genome at some stage in the past. ERVs are vertically transmitted from the host to the progeny. Most ERVs are defective, having accumulated random inactivating mutations and are not normally pathogenic. However, functional ERVs are potential agents of disease. Previous studies have identified the presence of a distinct clade of ERV from six species of crocodilians. Here we analyse the functionality, distribution and phylogenetic relationships of ERVs in twenty extant species of crocodilian. The ERV *pol gene* (~1 kb) was amplified, cloned and sequenced. Preliminary analyses show that two types of ERV sequences, possessing stop codons and deleterious mutations are distributed in most species of crocodilian. Thus, crocodilian sequences are generally, if not universally, defective as has been observed in many other ERVs. Phylogenetic analyses show that crocodilian ERVs cluster in two clades within the Retroviridae family. One clade in particular appears to be specific to most species of crocodilians, suggesting that this type of ERV may have infected the crocodilian lineage before the modern lineages diverged from the common ancestor about 250 million years ago. Further analyses to assess the evolutionary relationships and look for evidence for functional ERVs within crocodilians are underway.

# Cliteropenis'morphology of hatclings *Caiman latirostris*: Sex separations based on simple measures

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**ABSTRACT:** We study the cliteropenis (CTP's) morphology of *Caiman latirostris*, a species with temperature sex determination. We used 19 hatchlings which eggs were incubated at 30 ºC and 33 ºC, with the aim of ensure that males and females will be availables. In order to determine positively the sex of the animals, we inspectioned by direct observation the gonads after the sacrifice. CTP's measurements were: Total Length, Lateral width and Head width. This structure are single medial organs and the body develops on the ventral wall of the cloacae, both penis and clitoris protrude from the cloacae in early embryonic stages but disappear into it, sometime before the hatch moment. We found significant differences ($\alpha$=0,05) between sexes in lateral width of CTP ($p < 0,01$) and head width of CTP ($p < 0,01$). From this results and other analysis, we developed a preliminary protocol that's allow the discrimination of sexes based on the differences of the CTP measurements of *C. latirostris* at piping moment. The conclusion of this research is that based on simple morphometric measures of hatchlings, it is possible to sex them with a confidence of 89.4 %.

# Comparison of Analytic Assays to Detect Sexual Hormones in *Caiman latirostris*

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**ABSTRACT:** Sexual hormones detection is a key factor for *in situ* and *ex situ* wildlife handling. Knowledge of these hormones values will help us to understand even more about the species biology, make estimations regarding sex proportion in newly born animals by nest or in juveniles, as well as monitor potentially contaminated environments, among others. Sensitivity and specificity of detection methods are always subjects of study and continuous advances. The aim of this study is to compare three methods to detect Estradiol and Testosterone in *Caiman latirostris* juveniles' serum. Serum concentrations of these hormones were analyzed by the following methods: RIA (radioimmunoassays), MEIA (Microparticle Enzyme Immunoassay) and QL (Quimiolumniscense). Identifying more sensitive methods than those traditionally used will let us detect smaller concentrations in small sample volumes and evaluate the concentration of these hormones in different fluids.

# Contribution to the Knowledge of the Population State of the Yacaré Overo, *Caiman latirostris* (Reptilia, Alligatoridae), in Entre Ríos Province, Argentina

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**ABSTRACT:** In this work are presented the obtained results of the campaigns carried out during the year 2006 and 2007 in the frame of the "Yacarés of Entre Ríos Program" that has been developing since the year 2000 in the north-central region of the Entre Ríos province, Argentina. Day and night censuses were carried out on foot and oar boat using information obtained from the local settlers. For the night censuses halogen lamps of 1.500.000 B. and hand lanterns were used. The places of nest building were registered by a GPS Garmin 72. The observed caimans were classified under categories of classes of sizes (I= 0-40 cm; the II = 41-1,30 cm; the III= 1,31-1,70 cm; the IV= more than 1,70 of entire length) for the evaluation of the population structure. Surveys of 43 bodies of water and 34 km of coast were done. A whole of 247 individuals were detected registering a predominance of individuals of type the II and III, and 50 nests were located. The age structure of *Caiman latirostris* in the north-central region of the province would indicate that the specie is in state of recovery after decades of intense hunting pressure because of the demand of its leather.

# Croc FISH – Fluorescence in Situ Hybridisation of *Crocodylus porosus*

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**ABSTRACT:** Fluorescence *in situ* hybridisation (FISH) is a molecular cytogenetic technique which is commonly used in the creation of physical genome maps. Here we present the initial stages in the physical map of the saltwater crocodile (*Crocodylus porosus*). In conjunction with construction of the linkage map, a bacterial artificial chromosome (BAC) library was established with 2.8x coverage of the *Crocodylus porosus* genome. Several BAC clones containing microsatellite markers were fluorescently labeled and used to anchor informative linkage markers onto chromosomes. We also present further refinement of the standard G banded *Crocodylus porosus* karyotype, including production of a chromosome ideogram with band allocation providing a standardized reference.

# Crocodiles as a Bushmeat Resource in the Lac Tele Community Reserve, Republic of Congo

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**ABSTRACT:** The Lac Tele Community Reserve (LTCR) is a protected area of 4,400 km$^2$ located in the vast Likouala swamp forests of northern Republic of Congo. As Congo's only Ramsar site, the LTCR protects important populations of resident and migratory bird populations, but is also home to high densities of lowland gorillas, forest elephants, chimpanzees, buffalo and all three species of African crocodiles. The goal of the Reserve, with a human population of ≥16,000 inhabitants living in 26 villages, is to protect the natural resources and ecosystems services provided by the wetlands while sustaining the livelihoods of its human inhabitants. Although fish constitute the largest source of protein for Reserve inhabitants, some 43 species of non-fish vertebrates are harvested for food or local sale. Of those non-fish species, the African dwarf crocodile (*Osteolaemus osborni*) contributes the greatest volume to the harvest. Beginning in August, 2006, I developed and implemented the first program for long-term harvest monitoring of fish and wildlife resources in the LTCR. Training national Reserve staff and village assistants, we have collected monthly harvest data in five villages spanning the major habitat types found in the region. I present preliminary data on seasonal shifts in the resource base, habitat-specific harvest composition and the role of crocodiles in the harvest and village economies.

# Cross-Species Amplification of Microsatellites in Crocodilians

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**ABSTRACT:** Microsatellite DNA loci have emerged as the dominant genetic tool for addressing questions associated with genetic diversity in many wildlife species, including crocodilians. Despite their usefulness, isolation and development of microsatellite loci can be costly and labour intensive, thus limiting their wider use in many crocodilian species. In this study, we investigate the cross-species amplification success of 82 existing microsatellites previously isolated for the saltwater crocodile (*Crocodylus porosus*) in 18 other crocodilian species; *Alligator sinensis, Caiman crocodylus, Caiman latirostris, Caiman yacare, Melanosuchus niger, Paleosuchus palpebrosus, Mecistops cataphractus, Crocodylus acutus, Crocodylus intermedius, Crocodylus johnstoni, Crocodylus mindorensis, Crocodylus moreletii, Crocodylus niloticus, Crocodylus novaeguineae, Crocodylus palustis, Crocodylus rhombifer, Crocodylus siamensis,* and *Osteolaemus tetraspi*. Our results show a high level of cross-amplification with an average success rate of 90% in Crocodilidae species and marginal success in the Alligatoridae species. These results make available many polymorphic markers for a range of crocodilian species previously lacking informative genetic markers.

# Culture of chorioallantoic fluid and neonate mouths

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**ABSTRACT:** Frequently, organisms live in contact with a great number of diverse microbes, especially bacteria. Most of them are commensal and do not under normally cause illnesses. Flora normal composition can be different. Some microorganism in the normal flora can vary to pathogen if physiological or environmental conditions are modified or when these microorganisms enter sterile places. In order to verify the presence of bacteria or fluids or cavities that should be sterile we have taken samples from neonate mouths and its chorioallantoic fluid (CAF). CAF was taken by sterile needle and syringe by punction of the chorioallantoic membrane. Samples were taken by means of hyssops being the neonate still inside the egg. They were cultured in base agar and Mac Conckey Agar in aerobiosis during 24 hours and then identified the genus as possible.

# Development of Crocodile Blood Collection Process on Animal Life Maintains for Innovation of Crocodile Blood Product

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**ABSTRACT:** Crocodile blood collection process on animal life maintains of captive Siamese crocodile (*Crocodylus siamensis*) was developed. By collecting the anterior dorsal sinus blood in a 100 milliliters volume from 9 crocodiles, there were divided into 3 groups according to the duration time in each crocodile blood collection as following. The crocodiles that their bloods were collected every 4, 8 and 12 weeks. And the control group (n= 3) were taken 10 milliliters every 4 weeks. The results were evaluated by crocodile blood donor behavior i.e. food feeding and social behaviors, determination of hematological and biochemical values, and the concentration of cortisol hormone. These data revealed no significant differences between control group and treated groups ($p$>0.05). Therefore, the crocodile blood collection might be taken at least 100 milliliters in volume and the duration time for each collection should not less than 12 weeks. However, the optimal blood volume has remained to be elucidated.

## INTRODUCTION

Crocodile blood and other products have been widely consumed not only for its nutritious composition, but also for its claimed medicinal value. The practice of consuming crocodile blood for improving human health is found in the traditions of many Asian cultures. The anti-microbial activity of crocodile blood has been scientifically investigated (Chaeychomsri *et al* 2003 ; Siruntawineti *et al* 2003, 2004, 2005 ; Merchant *et al*. 2003, 2004, 2005). Crocodile blood is a waste material in slaughtering industry. Recently, it was added value as a supplemented food product (Chaeychomsri *et al*. 2006 ; Siruntawineti *et al*. 2006). The process of freeze-dried crocodile blood in capsule was developed (Thai patent application No. 0601001179, 16 March 2006) for maintaining nutrients such as protein (83.1%), iron (164 mg/100gm) and calcium (90 mg/100 gm) in a blood. The crocodile blood in a capsule can be preserved more than one year (Toraktakul *et al*. 2007). For production of the crocodile blood capsules, the bloods are collected from captive crocodiles before slaughter. In the future, it may not adequate for blood supplying if the demand of crocodile blood material increase. Therefore, the blood collection process on animal life maintain of captive Siamese crocodile should be an interesting option for sustainable use.

The purpose of this study was to determine the duration time of blood collection from normal crocodile for life maintain without health hazard. It was intended that these data would provide guidelines on repeated blood collection from captive crocodile. Moreover, they would promote a sustainable use and increase the production of crocodile blood as an alternative way for consumers who do not want animals to be killed for their blood.

## MATERIALS AND METHODS

*Animals:* Twelve captive Siamese crocodiles (*Crocodylus siamensis*) used in this study were juvenile crocodile (4 males, 8 females) about 5 years old. Total length and body weight were about 177.42 cm and 21.28 kg, respectively. All crocodiles were hand captured and snared with catapult. They were divided into a control group and 3 treated groups according to duration of time for blood collections: - 1) Collected blood every 4 weeks, 2) Collected blood every 8 weeks and 3) Collected blood every 12 weeks. The identification number was tagged on the crocodile before releasing back into the pen. The control and group 1- crocodiles were retained in the same pen, and the other groups were maintained in another pen.

*Blood collections:* Blood sample was collected with an 18 gauge needle from anterior dorsal sinus. A total volume of 100 ml of blood was collected from treated groups (group 1, group 2 and group 3) according to duration of planning time for blood collecting and 10 ml of blood were collected from control group in every 6 weeks. To evaluate the health status of these crocodiles, the blood was withdrawn ten milliliters from each crocodile at the week-1, -6 and -12 of experiment for hematological, biochemical and cortisol measurements. Two milliliters of blood was placed immediately into tube containing EDTA - anticoagulant, well mixed and stored at $4^{o}$C until measurement of hematological values. Another was placed into a clot activated tubes. Upon returning to laboratory (3-4 hours) blood samples were centrifuged at 5,000 rpm for 5 minutes. Serum were separated and stored at $-20^{o}$C until analysis of biochemical values and cortisol levels.

*Temperature and relative humidity measurement:* As temperature and humidity could influence the cortisol levels, hematological values, the temperature and relative humidity of captive pens were record three times a day.

*Behavior observation:* After blood collection, crocodile behaviors including feeding, basking, diving and social behaviors were observed. The food intakes of crocodile were determined and recorded.

*Hematological measurements:* The hematological tests included hemoglobin, hematocrit, red blood cell count, white blood cell count (WBC), differential WBC - count, and red cell indicies (MCV, MCH and MCHC) were performed. Hemoglobin was measured by using Cyanmethemoglobin method. Hematocrit was determined using microhematocrit centrifugation. Red blood cell count and white blood cell count were measured by using hemocytometer. Thinwedge smears of blood were made and Wright-Giemsa's strain was used for microscopic differential counts.

*Biochemical and cortisol measurements:* Biochemical values including blood urea nitrogen (BUN), creatinine, cholesterol, triglyceride, total protein, albumin, globulin, aspartate aminotransferase (AST) and alanine transaminase (ALT) were analysed by using automatic biochemical analyser (Hitachi 912, Japan). For quantitative analysis of cortisol, the serum was analysed by radioimmunoassay (RIA).

*Statistical analysis:* The results were reported as mean ± SE, analysed by using one way ANOVA and followed by Duncan's multiple-range test. A value of $p<0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

Body weight and length of all groups had no significant difference from initial values. These values were not difference between the groups ($p$>0.05). As this study was performed during winter, low temperature might be the cause of stress and might affect immune system (Huchzermeyer 2003). The maximum and minimum temperatures around the pen were 29.60$^{O}$C in the 7$^{th}$ week and 25.38$^{O}$C in the 12$^{nd}$ week, respectively. In addition, the maximum and minimum relative humidity was 60.2% in the 9$^{th}$ week and 38.98% in the 5$^{th}$ week, respectively.

After blood collection, all crocodiles in this study showed the normal in basking and diving behaviours. Feeding behavior reduced 2 weeks post blood collection, and then increased gradually. The crocodiles of both pens represented similar feeding behavior, although blood collection was not taken from group 2 and group 3 that stayed in another pen. It might be involved by disturbance, temperature and distress call of crocodiles. Although, the food intake of the crocodiles in this study after two week of the blood collection was increase, but it still lower than other nearby crocodiles that not involved in this experiment. Hence, the blood collection in group- 1 (designed for blood collecting every 4 weeks) and group- 2 (designed for blood collecting every 8 weeks) were not performed.

About crocodile health status determination, almost crocodile was no significant difference in hematological values and biochemical values between treated groups and control group or with in group ($p$>0.05). However, there was significant difference in cholesterol levels. This may due to food available in the pen, stress, sex, age and environment. The significant difference in AST and ALT within group 3 were be demonstrated. Anyway, these measured values were within reference range when compared with saltwater crocodiles (*Crocodylus porosus*).

The total blood volume of crocodile is approximately 4-5% of body mass (Huchzermeyer, 2003). In this study, collection of 100 ml of blood is about 10% of blood volume. Malikides *et al.* (2001) found that, the optimal blood volume for horse blood donor was about 25% of total blood volume. This blood volume causes some changes in the tested values. However, they recovered rapidly within 24-48 hours to their normal rang values. Moreover, heart rate and respiratory rate increased during blood collection then declined within 1-2 hours post collection and reach their normal values within 31 days post collection.

## CONCLUSION

This study of crocodile blood collection process on animal life maintains demonstrate that crocodile could be donated the blood 100 ml in volume very 12 weeks with normal behaviours and no significant difference in hematological values, hormonal levels and biochemical values between treated crocodiles and control group. The repeated 100 ml of blood collection is not hazardous to crocodile health and welfare. However, the optimal blood volume has remained to be elucidated.

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**Keywords:** Crocodile blood, Crocodile blood collection, Crocodile blood product

# Diet of the Broad Snout Caiman (*Caiman latirostris*, DAUDIN, 1802) in urban environment located in two municipal natural parks, Rio de Janeiro, Brazil

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**ABSTRACT:** *Caiman latirostris* in Rio de Janeiro lives in urban coastal lagoons (Tachas and Marapendi Lagoons situated in Municipal Natural Park Chico Mendes and Marapendi, respectively). Our goal was to analyze how the animals live in the urban environment and if they present a specific diet. Previous reports on the diet of the broad-snouted caiman include several invertebrates and vertebrates. In this work we analyzed stomach contents from 74 animals, 36 in Tachas Lagoon, 26 in Marapendi Lagoon and 12 in Tachas channel. The Lagoons are two different water bodies that are interconnected by Tachas channel. We observed that Caimans from Tachas channel at Tachas Lagoon presented a poorer diet, composed of hexapods associated with polluted environments, than those from Marapendi's pond, including vertebrate and invertebrate organisms, including fishes and crustaceans but mainly small invertebrates as prey items. The diet of adult caiman´s was different to that of juveniles at both sites. Despite the large variety of prey available in Marapendi Lagoon the adults there consume mainly small invertebrate prey. In this study, C. latirostris did not show preferences for any specific prey, feeding on all possible prey available in the environment. Our results suggest that prey items found in stomach contents of *C. latirostris* could be a reflection of the prey's diversity in the environment, which indicates a great capacity for adaptation in this species.

# Effects of Alligator Leukocytic Peptides on Antibiotic-Resistant Human Bacterial Pathogens

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**ABSTRACT:** Treatment of clinical isolates of human pathogenic bacteria, which were known to be resistant to multiple antibiotics, with refined leukocyte extracts from the American alligator (*Alligator mississippiensis*) resulted in a concentration-dependent reduction in growth. The alligator leukocyte extract exhibited the strongest antibacterial effect on *Klebsiella pneumoniae* and *Acinobacter baumannii,* followed by *Enterococcus faecium*, and then *Pseudomonas aeruginosa*. The antibacterial activities were heat stable at 70$^{O}$C for up to 30 minutes, stable in the presence of 50 mM ethylenediaminetetraacetate, and sensitive to protease treatment (10 units/mL protease for 30 minutes). Collectively, these data strongly suggest that the molecule(s) responsible for the observed antibacterial activities are small, cationic peptides. These peptides may prove to be useful as a new class of antibiotics for human use.

# Effects of Alligator Serum on Antibiotic-Resistant Human Bacterial Pathogens

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**ABSTRACT:** Treatment of clinical isolates of human pathogenic bacterial strains, which were known to be resistant to multiple antibiotics, with serum from the American alligator (*Alligator mississippiensis*) resulted in a concentration-dependent reduction in growth. The bacteria were grown in the absence, or in the presence of 10%, 25%, 50%, 75%, and 100% alligator serum. The alligator serum had the strongest growth inhibitory effect on *Klebsiella pneumoniae*, followed by *Acinobacter baumannii, Pseudomonas aeruginosa*, and *Enterococcus faecium*. The antibacterial properties were completely inhibited by heat treatment of the serum at 56$^{O}$C for 30 minutes, addition of 10 units/mL protease, or by the addition of 50 mM ethylenediaminetetraacetate, indicating that the activity was due to serum complement protein activity

# Effects of El Niño on growth in captivity of *Crocodylus acutus* in Tumbes, Peru

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**ABSTRACT:** Temperature affects the growth rate of crocodiles. However, there is no information about the effects of temperature variation caused by El Niño-South Oscillation (ENSO) on crocodiles. Therefore, our goal is to determine the effect of ENSO on growth rates of *Crocodylus acutus* in captivity during 1997 to 2001. Total length and body weight of 40 captivated crocodiles were recorded in the Centro de Acuicultura La Tuna Carranza, located in Puerto Pizarro, Tumbes, Peru. We did not observe statistical differences in total length rate among years, but we did found differences in body weight rates among years. We observed that warm episodes of ENSO represented higher average in total length rate and cold episodes represented a higher average of body weight. The sea surface temperature was significantly related with total length rate; however, the relationship between sea surface temperature and the growth rate of body weight was not significative. As a conclusion, we suggest that ENSO events are an important factor that affects growth rates of crocodiles, thus it should be considered in management policies of captive populations.

**RESUMEN:** La temperatura afecta el crecimiento de los cocodrilos. Sin embargo, hasta la fecha no existe información acerca del efecto de la variación de la temperatura durante los eventos de El Niño sobre los cocodrilos. Por ello el objetivo del estudio fue determinar el efecto de El Niño sobre el crecimiento de *Crocodylus acutus* en cautiverio en el período 1997-2001. Se trabajó con un grupo de 40 cocodrilos cautivos en el Centro de Acuicultura La Tuna Carranza, localizado en Puerto Pizarro, Tumbes, Perú. Se midió la longitud total y la masa corporal de los cocodrilos. En general, se observaron diferencias en el incremento de peso por año, pero no se observó una diferencia en la longitud. Por otro lado, observamos que durante los episodios cálidos de El Niño la tasa de crecimiento en longitud es mayor mientras que durante los episodios fríos fue mayor el aumento en peso. Observamos también una relación significativa de la temperatura superficial del mar con la tasa de crecimiento en longitud, pero no con el peso. En conclusión, consideramos que los eventos de El Niño son un factor importante que afecta el crecimiento de los cocodrilos por lo cual deben ser considerados dentro del manejo de las poblaciones.

# Evaluation of genotoxic effects induced by UV radiation in *Caiman latirostris*: Progress report

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**ABSTRACT:** Ultraviolet (UV) radiation is a component of the solar energy emission spectrum. The ecological importance of UV light has been extensively studied during many years, on account of stratospheric ozone depletion in certain areas of the planet, which has led to an excessive UV radiation over ecosystems. Overexposure of organisms to UV radiation may produce different biological effects as a result of photochemical absortion by significant molecules such as proteins and nucleic acids. Among them, DNA damage induced by UV-A/UV-B exposure, which included mainly thymine dimers and chromosomal fragmentation, are considered of prime importance. The aim of this study is to evaluate genotoxic effects of UV-A and UV-B radiation in juvenile *Caiman latirostris*, using the Micronucleus (MN) test as a biomarker.

Seventy two juvenile caimans were reared during $3^{1}/_{2}$ months under one of three conditions: 24 hours darkness and exposure of 8 or 16 daily hours of artificial UV-A/B light, respectively. Blood samples were collected at the beginning and at the end of the experiment to determine the incidence of UV/A-B radiation on erythrocyte MN frequences and evaluate differences between treatments. Up to the moment, 75 % of the samples have been analysed.

# Evaluation of the Population of *Caiman yacare* at Estancia San Miguelito, Santa Cruz, Bolivia

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**ABSTRACT:** The designation of protected areas is one of the mechanisms created to promote the conservation of wildlife. However any protected area was established specifically for the conservation of these animals. San Miguelito, is a property with approximately 40,000 hectares, an area designated as Private Reserve in the San Julian River Basin, conditions ecological, in fact it is an alternative to preserve the biological richness of this lowland region.
This study summarizes preliminary information of the current status of alligators populations present in the San Miguelito area. In 2005 count at night were performed data of abundance (Indv/Km) were obtained in the Rio San Julian (37.31), Rio Zapoco (33.34) and Rio Tuná (67.44). In relation to population structure, the three rivers showed a high level of the individuals on the Class III on the Class IV. Its proportion was more than 60%.

**RESUMEN:** La designación de áreas protegidas es uno de los mecanismos con que se cuenta para favorecer la conservación de cocodrilianos. No obstante ningún área protegida fue establecida específicamente para la conservación de estos animales. La estancia San Miguelito, es una propiedad ganadera de aproximadamente 40.000 ha, y incluye una superficie categorizada como Reserva Privada en la cuenca del Río San Julián, considerando sus condiciones físicas y biológicas, en la actualidad es una alternativa para conservar la riqueza biológica de este sector de las Tierras Bajas de Santa Cruz.
El presente estudio resume información preliminar del estado actual de las poblaciones de lagartos presentes en la estancia En el año 2005 se realizaron conteos nocturnos en los cuales se obtuvieron datos de abundancia (Indv/Km) en el Río San Julián (37.31), Río Zapoco (33.34) y Río Tuná (67.44). En cuanto a la estructura poblacional, los tres ríos presentaron un alto dominio de los individuos clase III, sobre los de clase IV, su proporción fue más del 60 %.

# Experimental treatment in *Caiman latirostris* (Reptilia, Alligatoridae) on the "Yacarés of Entre Ríos program", Entre Ríos, Argentina.

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**ABSTRACT:** In the year 2006, on fields of the "Yacarés of Entre Rios program", were 206 yacares (*caiman latirostris*) in captivity, which were born in March of that year. Those animals, whit 5 month old, started to present the followings symptoms: Opistotono, extensives pupils, swimming inability, difficult breathing, distencion abdominal, commuting of the head. After individual blood test, fecal matter, food, etc. we determinate that those animals had high- glycemia, which was made by routines tests of glucose with values over 600mg/dl. Until that moment, the animals were feeding with a mixing of meat, chicken and fish in the same percentage. When the high- glycemia was determinate, the diet was replaced by a mixing of meat without fat 30% and food balanced for diabetic cat 70%. Supported this diet for 10 weeks, the levels of glycemia began to diminish and since November of 2006 we obtained values flanked by 64mg/dl and 120mg/dl, depending on the time of fasting of the animals. This allows us to conclude that the replaced of the diet was favourable for the decrease of the glycemia and for the normal development of the animals, since the weight and size to the year of birth were ideal for the age.

# First experience in *ex-situ* incubation of wild clutches of the American Crocodile (*Crocodylus acutus*) in Machiques de Perijá, Zulia State, Venezuela.

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**ABSTRACT:** As part of a conservation proposal for the American crocodile (or *Kanta* in Bari indigenous dialect) which will involve colonist and local and indigenous people, the first experience in searching, collecting and then artificial incubation of American crocodiles eggs took place in January 26 and 27, 2007. Two nest were found, one in river Negro (24 eggs) and the other in river Santa Rosa (38 eggs). Sixty of the collected eggs were incubated in three plastic boxes, using sand from the original nests. These boxes were kept in a house of the Barí community in Senkay. From April 25 to 28, forty three eggs hatched successfully. The smallest and largest hatchlings were 240 and 300 mm in total length, respectively.

**RESUMEN:** Como parte de una propuesta de conservación de *Crocodylus acutus* o *Kanta* en dialecto indígena Bari que involucrará actores locales e indígenas, se realizó una primera experiencia de búsqueda e incubación forzada de nidadas durante enero 2007. Los días 26 y 27 se encontraron 2 nidadas en los ríos Negro y Santa Rosa respectivamente. Las nidadas fueron de 24 y 38 huevos para los ríos mencionados. 60 huevos en total se incubaron en 3 cavas de plástico y utilizando la arena de ambos ríos. Se incubaron en una casa de la comunidad Bari de Senkay. Del 25 al 28 de abril de 2007 nacieron 43 caimancitos, los de menor y mayor tamaño midieron al nacer 240 y 300 mm respectivamente.

# Front or Rear, Top or Bottom: Measuring and Using Snout-Vent Length in Crocodilians

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**ABSTRACT:** Crocodilians have been monitored globally for decades and long-term data within and between species and regions has typically been collected using different procedures, resulting in data sets that can be difficult to standardize and compare. The most common data collected on crocodilians are morphometric measurements (e.g., head length, snout-vent length, total length, and weight). These measurements are used in a wide range of analyses, from ecological to taxonomic to evolutionary. However, these analyses are only as accurate as the data from which they are derived. When measurements are taken by different individuals using different landmarks, comparisons between species and across regions can be difficult or impossible to make. For example, snout-vent length (SVL) have been measured along both the dorsal and ventral surfaces of a crocodilian and culminated at both the anterior and posterior portion of the vent. The magnitude and importance of effects of different techniques of measuring SVL are unknown. Here, we compare effects of using different SVL measurement techniques in calculating indices of body condition for three species of crocodilian, *Crocodylus acutus, C. moreleti*, and *Alligator mississippiensis*.

# Importance of Evolutionarily Significant Units to conservation and management *Caiman crocodilus*

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**ABSTRACT**: Facing radical acceleration in the rate of extinction, habitat transformation and niche displacement, conservation biologists and decision makers play a very important role in safeguarding biodiversity. Better conservation and management plans should be made to achieve the goal of preserving natural diversity of independent evolutionary lineages, their habitats, and their ecological relationships; and also to maintain the ecosystems that they inhabit, which will enhance human well-being. The *Caiman crocodilus* complex is a widely distributed, charismatic, and economically important Latin American crocodylian species. The complexity of *Caiman crocodilus* taxonomy, systematics and ecology, in combination with high levels of exploitation, require a multidisciplinary approach to ensure its long-term conservation. The goal of this research was to define more realistic conservation units based on objective operational methodology to be used for conservation and management purposes. This methodology integrates taxonomy, systematics, population genetics, biogeography, ecology, natural history (historical and contemporary use and threats), political and social information, as well as resent trends and published predictions. Our results support conservation efforts for five independent conservation units which are genetically differentiated, reproductively isolated, and ecologically distinct, each with a unique evolutionary trajectory. This research offers the opportunity of using a coherent methodology to define conservation units that can be replicated with other taxa.

**RESUMEN:** En esta época en la que nos encontramos enfrentado una aceleración de la tasa de extinción, transformación de hábitats y desplazamiento de nichos los biólogos de la conservación y los tomadores de decisiones juegan un papel muy importante en el resguardo de la biodiversidad. Mejores planes de conservación y manejo deben desarrollarse para alcanzar la meta de preservar la diversidad natural de linajes evolutivos independientes, sus hábitats y sus relaciones ecológicas, y a la vez mantener los servicios ambientales que sostienen el desarrollo humano. *Caiman crocodilus* es un complejo de linajes independientes ampliamente distribuido por América Latina; este taxa es carismático y de gran importancia económica para América Latina. La complejidad de la taxonomía, la sistemática y la ecología de este grupo de cocodrilos, en combinación con los niveles de explotación hacen necesario un acercamiento interdisciplinario para lograr su conservación a largo plazo. La meta de esta investigación fue definir unidades de conservación más acordes con la realidad basándonos en una metodología operacional de múltiples niveles. Esta metodología integra la taxonomía, la sistemática, la genética de poblaciones, la biogeografía, la ecología, la historia natural (incluye amenazas históricas y contemporáneas), así como también se incluye información

política y social del área donde se encuentra el recurso y efectos del mercado. Al mismo tiempo se adiciona al análisis información acerca de las tendencias y predicciones de las posibles amenazas a la sobrevivencia del grupo a largo plazo. Nuestra investigación propone concentrar los esfuerzos de conservación en cinco unidades independientes de conservación que son genéticamente diferente, que están aisladas reproductiva y ecológicamente y que a su vez tienen historias evolutivas distintas; principalmente en *Caiman crocodilus chiapasius*. Estas unidades de conservación fueron diseñadas en base a una metodología coherente que se puede replicar en otros taxones.

# Influence of two different diets on size and body weight of *Crocodylus acutus* hatchlings in Manzanillo Breeding Center, Cuba

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**ABSTRACT:** An experiment was designed with 90 newborn specimens that were raised and fed with the standard diet during 50 days and with two experimental diets for 30 days , in three experimental square tanks (A, B C), during 30 days. The influence is analyzed, on size and corporal weight increment, of two types of diets against the standard diet in *Cocodrylus acutus* of the Manzanillo Zoocriadero (Province of Granma) and the cost of same. The fish diet (B) is much more effective than the standard diet (fish, and cow liver and lung) and than the one with larval flies since the growth rate was of 3,5 mm / day and grew 1,35 times more. The weight increase was of 3,71 g./day, a number superior to the values of the other two diets. The relation coefficient (Cr.) between the size and weight by tanks was calculated in order to express the prevalence of the size over weight or vice versa according to the applied diet. It is demonstrated that with diet (B), the newborn specimens grow 1.35 times more than with pattern diet (A) and larval flies diet (C). However, each newborn specimen fed with diet (B) costs 14 times more than the ones fed with the other two diets.

**RESUMEN:** Se diseñó un experimento con 90 neonatos recién nacidos que fueron criados y alimentados con la dieta patrón por 50 días y dos dietas experimentales por 30 días, en tres cuartones (A, B C). Se analiza la influencia, sobre la talla y el incremento del peso corporal, de dos tipos de dietas contra la dieta patrón en *C. acutus* (caimán americano) del Zoocriadero de Manzanillo (Provincia Granma), y así como el costo de las mismas. La dieta con pescados marinos (B) es mucho más efectiva que la dieta patrón (compuesta de pescado, hígado y pulmón de res) y la de larvas de moscas ya que la tasas de crecimiento fue de 3,5 mm. / día. Por tal razón los ejemplares con las dieta (B) y crecieron 1,35 veces más y la de con un incremento en peso de 3,71 g. / día, valores superiores a los valores de las otras dos dietas que incluyó la patrón. Se calculó el coeficiente de relación (Cr.) entre la talla y peso por cuartones para determinar el predominio de la talla sobre el peso o viceversa según la dieta suministrada. Se demuestra que con la dieta B los neonatos crecen 1.35 veces más que con la dieta patrón (A) y larvas de moscas (C), sin embargo cada neonato alimentado con la dieta B cuesta 14 veces más que con las otras dos.

# Lack of Acute Phase Response to Infection in the American Alligator *(Alligator mississippiensis)*

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**ABSTRACT:** Five juvenile alligators (6.4-8.1 kg) were injected intraperitoneally with a mixture of bacterial lippopolysaccharides (LPS) derived from *Escherecia coliform, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*. Blood was collected prior to treatment, and 1, 2, 3, 4, 5, 7, 9, and 11 days post-injection. The serum was subjected to clinical agarose gel electrophoresis to determine the changes, relative to pretreatment condition, of protein expression in the serum. Surprisingly, unlike the drastic changes in protein expression observed in mammalian and avian systems upon infection, no changes in protein expression were detected after LPS-treatment. Injection of LPS intramuscularly and in the hind foot pad revealed the same lack of acute phase response. Injection of other substances, such as phytohemagglutinin (an immune system stimulator) and heat-killed bacteria also yielded negative results. Several attempts to isolate C-reactive protein and serum amyloid A, acute phase proteins that are expressed at low levels, but increase up to 100-fold in mammals upon infection, yielded no results. These data lead us to believe that the American alligator, and perhaps other crocodilians, do not exhibit the typical acute phase response, characterized by large changes in serum protein expression, observed in more modern vertebrates.

# Microsatellite DNA markers applied to detection of multiple paternity in *Caiman latirostris* in Santa Fe, Argentina

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**ABSTRACT:** This work describes a method for using microsatellite markers to examine the mating system of broad-snouted caiman. Also, we provide the first data concerning the detection of multiple paternity on wild populations of this species. We obtained DNA samples of four nest-guarding *Caiman latirostris* females and their hatchlings from Santa Fe Province, Argentina. Eight microsatellite primers were tested and amplification products were analyzed by electrophoresis on 10% polyacrylamide gels and visualized with silver staining. Of the eight markers tested, four were found to amplify reliably and yield useful data. The methodology of using polyacrylamide gels with silver staining provided sufficient resolution to obtain the individual genotypes. To assess the presence or absence of more than two parents in each clutch we used the single locus Minimum Method, and applied Cervus 3.0 and Gerud 2.0 software in parentage analysis. Our results indicate more than one father in, at least, two families. These data could suggest multiple paternity in these families, although, a wider sampling of nests and loci is necessary to corroborate this behavior. Understanding the mating system may be important in maintaining viable populations under management programs like those of *C. latirostris.*

# Mitochondrial DNA analysis of saltwater crocodiles (*Crocodylus porosus*) from northern Australia

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**ABSTRACT:** This study presents preliminary analyses of the mitochondrial DNA control region (mtDNA CR) sequence variation and phylogenetic relationships of more than 50 wild Australian saltwater crocodiles from Australia's Top End (Northern Territory). CR sequences were aligned and compared with saltwater crocodile sequences in Genbank from Queensland in Australia, Southeast Asia and the western Pacific Ocean, as well as other crocodilian species. CR phylogenetic analyses show that saltwater crocodiles from Australia clustered in a separate clade from specimens from other countries. Surprisingly, one GenBank *C. porosus* sequence from Queensland clustered with specimens from the Asia Pacific. This unexpected clustering may suggest natural migration from this Pacific clade into Australian populations. Alternatively, smuggling of some specimens into Australia could be considered although there is currently no evidence to support this. CR pairwise genetic comparison shows that the level of divergence within the saltwater crocodile is lower than that observed within the wild Central American Morelet's crocodile, similar to that observed in wild American Alligators and higher than that observed in captive Chinese alligators and the other crocodile species included. Further studies are underway to increase our understanding of the genetic diversity within the Australian saltwater crocodile.

# Modeling of caiman abundance as a tool for the management of *Caiman yacare* in Bolivia

**Alvaro Crespo, Dennis Mendez, Fernando Cisneros, Paul A. Van Damme**

**ABSTRACT:** The Bolivian Program for the Conservation and Sustainable Use of spectacled caiman (*Caiman yacare*) foresees the annual harvesting of 45000 adult males, mostly in the Beni and Santa Cruz states. The deficient system of assignation of harvest quota is one of the main weaknesses of the Program. There is an urgent need to develop tools that can be used to improve caiman management. The objective of the present study is to develop a predictive model for caiman distribution and abundance in the Bolivian Amazon using vegetation units as spatial units. The model is based on the hypothesis that there exist a high correlation between vegetation structure and relative abundance of the species. Vegetation structure reflects abiotic factors such as temperature, precipitation, altitude, and inundation, which also affect the relative abundance of the species. The model can be tested by monitoring of caimans in vegetation units and by regression analysis.

**RESUMEN:** En Bolivia, el Programa para la Conservación y Aprovechamiento Sostenible del Lagarto (*Caiman yacare*) permite la cosecha anual de 45000 adultos machos, principalmente en los departamentos de Beni y Santa Cruz. A causa de la demanda de nuevos interesados para ingresar al programa y ante las dudas sobre el mejor sistema de asignación de cupos de lagarto, surge la necesidad de crear herramientas que permitan la gestión de este recurso. En este trabajo se presenta un modelo de predicción de la abundancia del lagarto en la Amazonia Boliviana usando unidades de vegetación como unidad espacial de estudio. El modelo se basa en la hipótesis de que existe una alta correlación entre los sistemas de vegetación existentes y la abundancia relativa de la especie. La vegetación engloba factores abióticos como ser la temperatura, precipitación, altitud, inundación, y otros, los cuales determinan la abundancia de esta especie. El modelo puede ser validado mediante el muestreo de la abundancia de lagartos en ciertas unidades de vegetación y análisis de regresión. El modelo permite predecir abundancias relativas las más próximas a la realidad.

# Molecular genetic tools to improve the selection of juvenile saltwater crocodiles (*Crocodylus porosus*) for use in genetic improvement

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**ABSTRACT:** Traditional genetic improvement programs have used performance data to estimate breeding values with little or no understanding of the underlying genes responsible for their control. Furthermore, the accuracy of the predicted breeding values relies on the pedigree structure and amount of data available. However, the development of molecular genetic technology has provided new means for aiding the prediction and accuracy of breeding values at the gene level. This improvement can be achieved by exploiting linkage between molecular markers and quantitative trait loci (QTL), known as marker-assisted selection (MAS).

QTL studies are being conducted in most livestock industries for the following reasons: 1) favourable alleles for economically important traits can be introduced into a commercial population from exotic stock using marker-assisted introgression (MAI) or repeated backcrossing; 2) selection accuracy can be improved by identifying markers linked to QTL and incorporating this information into the estimation of breeding values; and 3) the potential to increase selection intensity and reduce generation interval (because animals can be genotyped immediately after hatching). Using the linkage map presented by Miles et al. (2008; this conference), the results of the first QTL analysis in crocodilians will be presented herein.

# Monitoring populations of American Crocodiles *(Crocodylus acutus)*, in the states of Aragua, Falcon, Trujillo and Zulia, Venezuela

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**ABSTRACT**: From july 2006 and january 2008, we carried out population census and habitat evaluation of american crocodile, at nine sites located in the states of Aragua (1), Falcon (3), Zulia (4) and Trujillo (1). Crocodiles were present at all sites visited, numbering a total of 238 individuals, distributed in five size classes as fallows: class I (n=36), class II (n=121), class III (n=57), class IV (n=18) and class V (n=4). On the other hand, 22 of the capture individuals were marked and released for future monitoring purpose. Among the studied sites, rivers Chama and Torondoy and water reservoirs located at Machango (Zulia) and Agua Viva (Trujillo), are confirmed as new distribution area for the American Crocodile. Finally and as a conservation and management measure, a total of 64 American Crocodiles raised in private farm and zoos, were released in both: a Wildlife Reserve (Falcon) and a Wildlife Refuge (Zulia) located in western Venezuela.

**RESUMEN:** Se censaron caimanes y se evaluaron sus hábitat en nueve localidades entre julio del 2006 y enero del 2008 en los estados Falcón (3), Aragua (1), Zulia (4) y Trujillo (1). Se detectó la presencia de esta especie en todas las localidades. El total de cocodrilos observados fue de 238 clasificados en: 36 Clase I, 121 Clase II, 57 Clase III, 18 Clase IV y 4 Clase V. Se obtiene por vez primera información presencial de la especie en los ríos Chama y Torondoy y en los embalse Machango y Agua Viva, datos de una sospechada distribución histórica o anecdótica de *C. acutus*. 22 ejemplares fueron capturados en las localidades evaluadas, luego marcados y liberados. Adicionalmente se reforzaron algunas poblaciones en los estados Falcón y Zulia al liberar 64 caimanes provenientes de un centro de cría en cautiverio en el estado Guárico y del Zoológico de Maracaibo en Zulia.

# Morphometric Analysis in Embryos of Black Yacare *Caiman yacare* (Crocodylia, Alligatoridae)

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**ABSTRACT:** In embryology it is important to establish a descriptive system of classification of stages of embryo development that serves to correlate the different phases in a temporary scale and under specific conditions of incubation (temperature and humidity). At the same time, it is useful evaluate the existence of biometric relationships that, together with the system of stages, could serve to develop a tool that consider the precise moment of the oviposition. The objective of this work is to formulate a method base for the taking of morphometric measures and to analyze the growth patterns. For it, 33 embryos collected in the Argentinean province of Chaco (at 26°43´39,1´´S; 59°03´27,4´´W) were used. Each embryo stage was determined under a dissecting microscope, 18 morphometric measures were taken with a digital caliper (± 0.01 mm). A guide instructive with photographs was designed to measure the structures. We used the Statgraphics Centurion XV software to describe the growth patterns, correlating the morphometrics measured and the different stages. The models that better adjusted to the growth of the different structures in embryos from *Caiman yacare* correspond to second order curves: $Y = ax + bx^2$ where "Y" is the morphometric measure and "x" is the stage.

**RESUMEN:** En embriología es importante establecer un sistema descriptivo de clasificación de estadios de desarrollo embrionario que sirva para correlacionar las diferentes fases en una escala temporal y bajo condiciones especificas de incubación (temperatura y humedad). Al mismo tiempo, resulta de utilidad evaluar la existencia de relaciones biométricas, que junto con el sistema de estadios, sirva para desarrollar una herramienta que permita estimar el preciso momento de la ovipostura. El objetivo de este trabajo es crear un método base para la toma de medidas morfométricas y analizar los patrones de crecimiento. Se utilizaron 33 embriones colectados en la Provincia del Chaco, Argentina (26°43´39,1´´S; 59°03´27,4´´W). Se determinó el estadio de los embriones bajo la lupa, se tomaron 18 medidas corporales con un calibre digital (±0,01 mm). Se confeccionó una guía-modelo para medir las estructuras acompañadas de fotografías explicativas. Empleando el software Statgraphics Centurion XV se buscó describir patrones de crecimiento correlacionando las variables morfométricas medidas y los diferentes estadios. Los modelos que mejor se ajustaron al crecimiento de las diferentes estructuras en embriones de *Caiman yacare* fueron los correspondientes a curvas de segundo orden: $Y = ax + bx^2$ donde "Y" es la variable morfométrica y "x" es el estadio.

## INTRODUCTION

In embryology it is important to establish a descriptive system of classification of stages of embryo development that serves to correlate the different phases in a temporary scale and under specific conditions of incubation (temperature and humidity) (Ferguson, 1987). At the same time, it is useful evaluate the existence of biometric relationships that, together with the system of stages, could serve to develop a tool that consider the precise moment of the oviposition. Previous works exist that describe the reproductive biology and embryology of crocodiles (Ferguson, 1985), others analyze the patterns of ossification in the skeleton of *Alligator mississippiensis* (Rieppel, 1993) or the sex determination by temperature for the same species (Lance & Bogart , 1994).

On South American caimans many works refer to *Caiman latirostris*, dealing with effect of incubation temperature in the sex determination and survivorship (Piña *et al*., 2003), the size at hatching (Piña *et al*., 2007), relationships between the period of incubation and the embrionary development at three different temperatures (Donayo, 2002), and one description of embryonic stages, considering the age of embryos with unknown date of ovoposition (Iungman *et al*., 2005). Much less work refers to the black jacare, *C. yacare*. For this reason, our objective is to formulate a base method for taking of morphological measures and to analyze the growth patterns in this species.

## MATERIALS & METHODS

Thirty-three embryos of *Caiman yacare* were collected in the Argentinean province of El Chaco (26° 43´ 39.1´´ S; 59° 03´ 27.4´´ W). Each embryo stage was determined under a dissecting microscope following Ferguson (1987), and 18 morphometric measures were taken with a digital caliper (± 0.01 mm). The total length, snout-vent length, and tail length was first verified with the aid of a thread, then the resultant distance was measured on the caliper. A guide instructive with photographs was designed to measure the structures (Table 1, and Figures 1-3).

**Table 1.** Morphometrics in *Caiman yacare* embryos.

| | |
|---|---|
| 1 Total length | Measured from the tip of snout to the end of the tail. (Figure 1) |
| 2 Snout depth | From the middle of nose to the inferior border of the jaw, transversal to the snout length. (Figure 1) |
| 3 Snout length | From the most anterior limit of the eye to the tip of the snout, parallel to the mouth border. (Figure 1) |
| 4 Jaw length | From the tip of jaw to the posterior angle of it. (Figure 1) |
| 5 Smaller diameter of the eye | (Figure 1) |
| 6 Greater diameter of eye | (Figure 1) |
| 7 Arm length | Taken on the right arm from the shoulder to the elbow. (Figure 1) |
| 8 Forearm length | From the elbow to the carpus. (Figure 1) |
| 9 Hand length | From the carpus to the tip of the second finger. (Figure 1) |
| 10 Thigh length | From the groing to the knee, on the right thigh. (Figure 1) |
| 11 Tibial length | From the knee to the ankle, on the right leg. (Figure 1) |
| 12 Foot length | From the ankle to the third toe. (Figure 1) |

| | |
|---|---|
| 13 Nostril-eye length | Straight distance between the nostril and the eye.(Figure 2) |
| 14 Snout-ventlength | By ventral, from the tip of the snout to the anterior limit of the vent. (Figure 2) |
| 15 Tail length | By ventral, from the end of the vent to the tip of the tail. (Figure 2) |
| 16 Armpit -groin length | In straight line, on the right side of the embryo. (Figure 2) |
| 17 Head width | On a tranversal plane, behind the eyes. (Figure 3) |
| 18 Head length (excluding the snout) | From the anterior border of the eyes to the beginning of the neck. (Figure 3) |

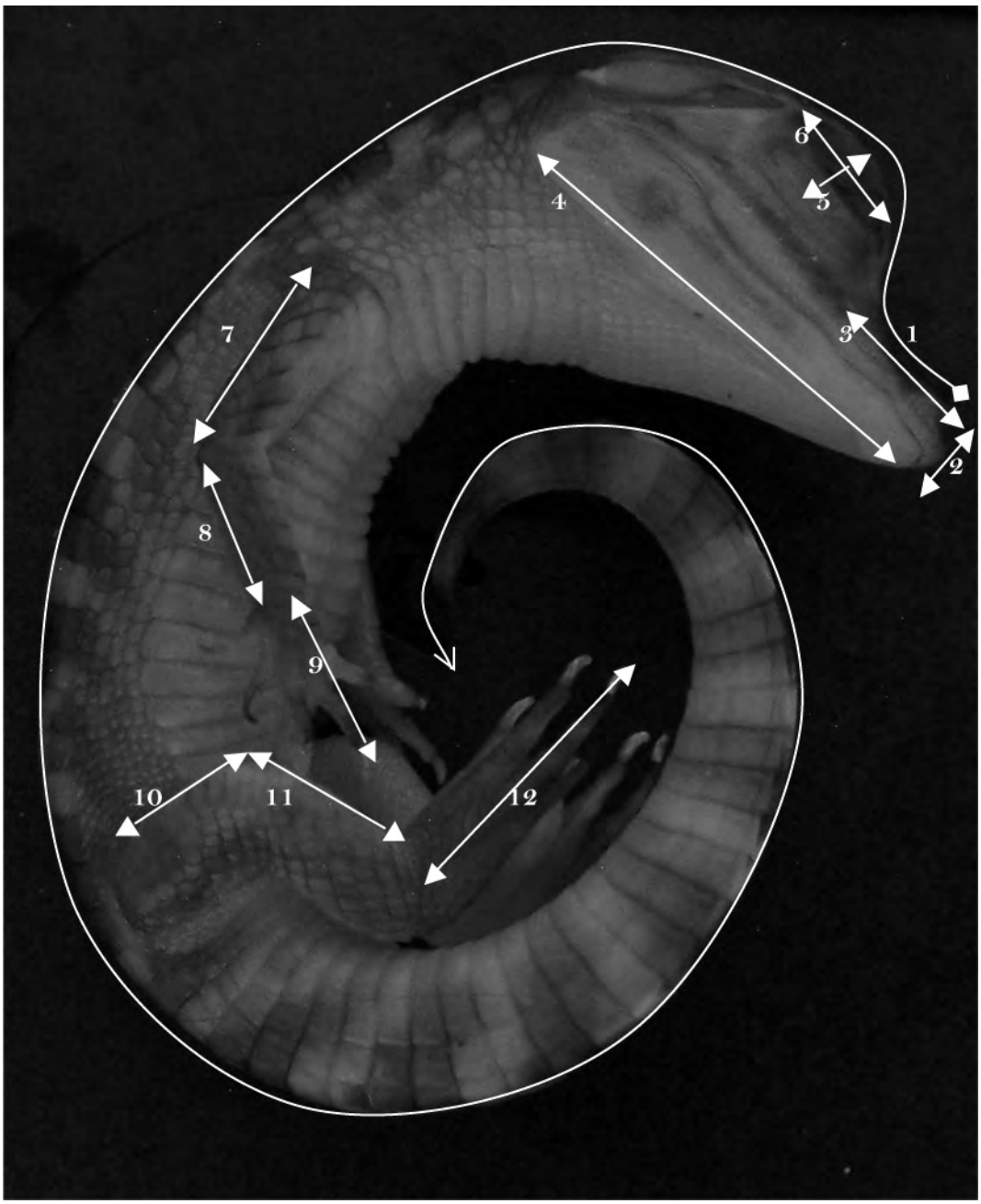



**Figure 1**.

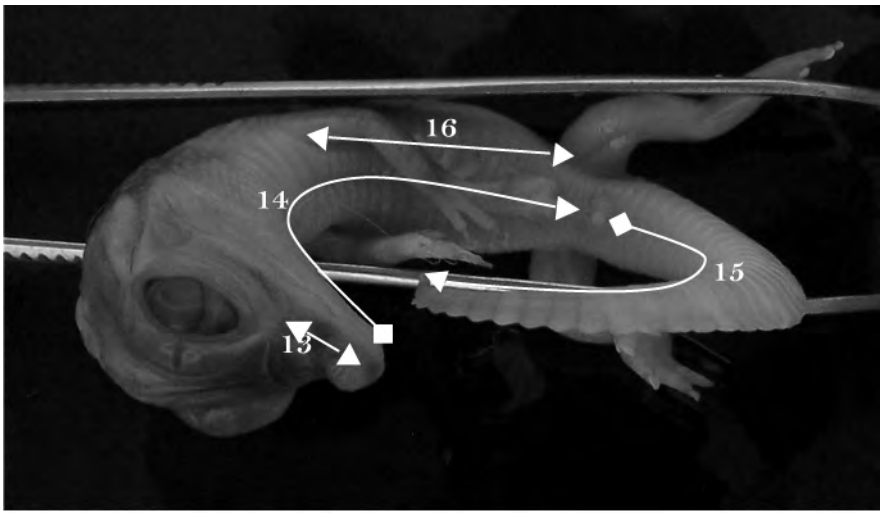



**Figure 2.**

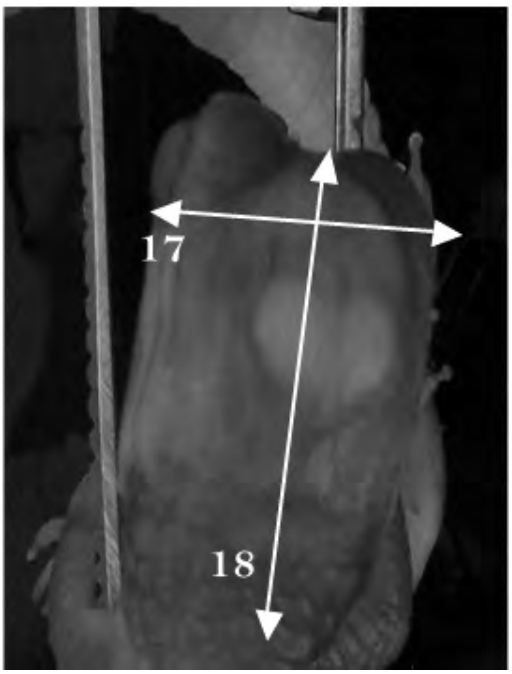



**Figure 3.**

The Statgraphics Centurion XV software was used to describe the growth patterns, correlating the morphometrics measured and the different stages.

## RESULTS & DISCUSSION

Figures 4-7 show the model that better adjusted to the growth of the different structures analyzed. These follow to second order curves: $Y = ax + bx^2$ where "Y" is each morphometric measured, and "x" the stages. Table 2 shows the values of $R^2$ obtained to describe the relationship between the morphometrics and the stages. For each $R^2$ showed ANOVA values of $\mathbf{p}<0.05$, suggesting a statistically significant relationship between the measures and the stages.

**Table 2.**

| Morphometric measure | $R^2$ (%) |
|---|---|
| Total length | 98.10 |
| Armpit -groin length | 96.83 |
| Snout-vent length | 98.08 |
| Tail length | 97.82 |
| Head width | 97.99 |
| Head length (excluding the snout) | 99.06 |
| Nostril-eye length | 94.98 |
| Smaller diameter of eye | 96.64 |
| Greater diameter of eye | 98.23 |
| Jaw length | 98.12 |
| Snout length | 97.11 |
| Snout depth | 94.91 |
| Arm length | 95.32 |
| Forearm length | 97.19 |
| Hand length | 99.54 |
| Thigh length | 94.69 |
| Tibial length | 98.03 |
| Foot length | 97,21 |

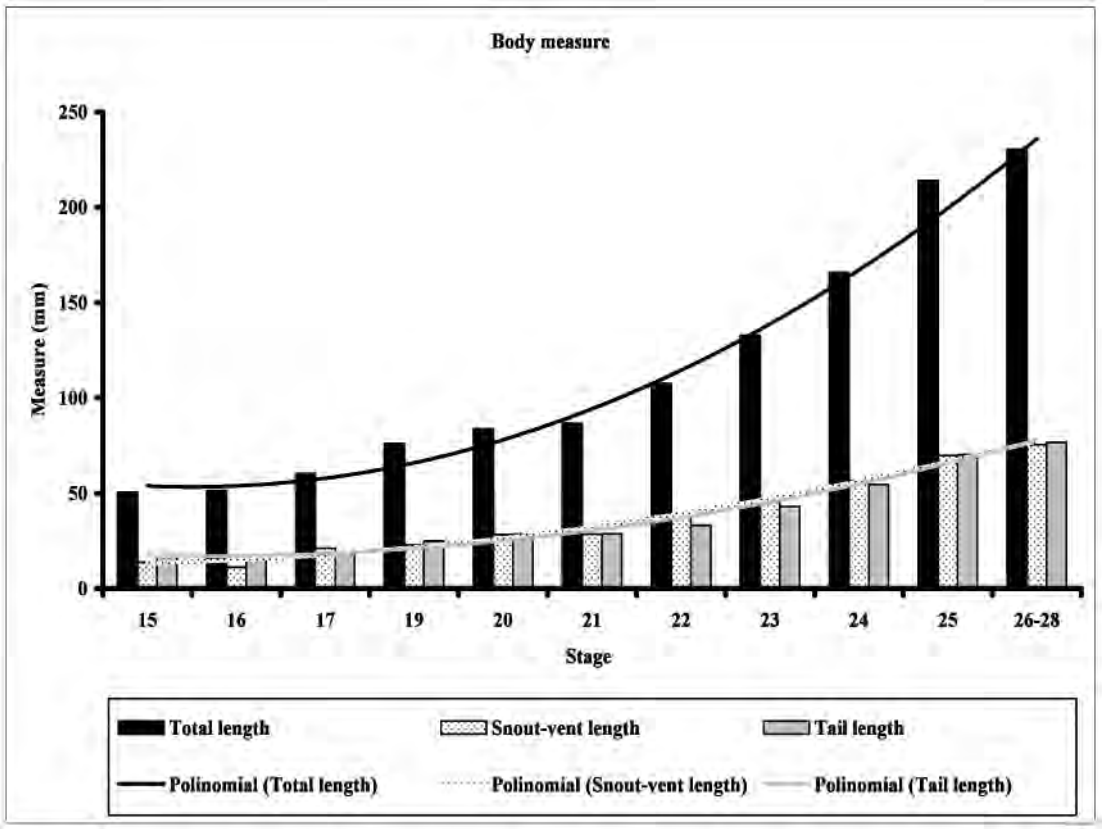



**Figure 4.**

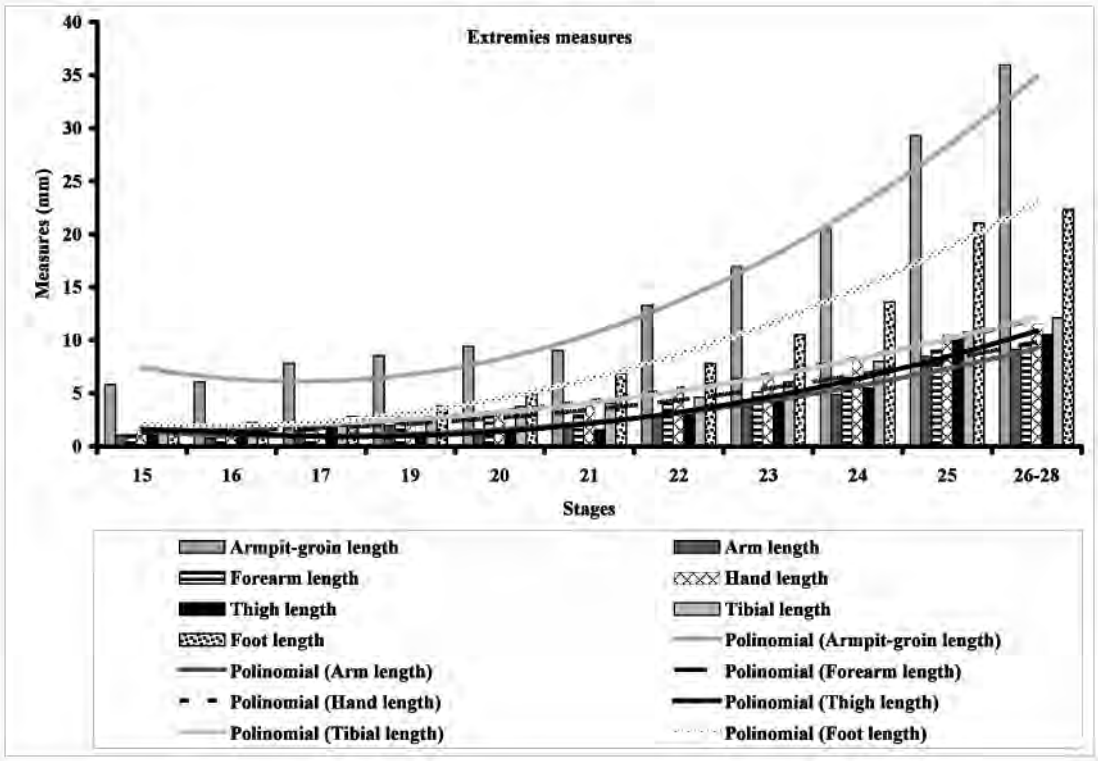



**Figure 5.**

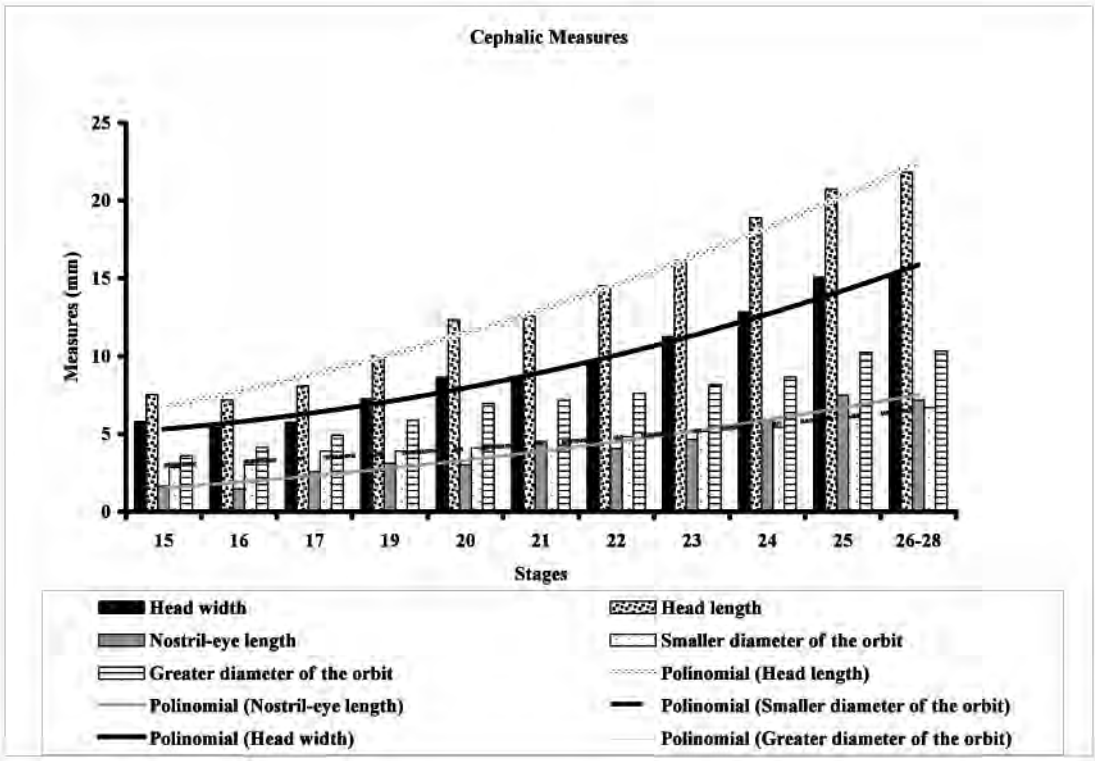



**Figure 6.**

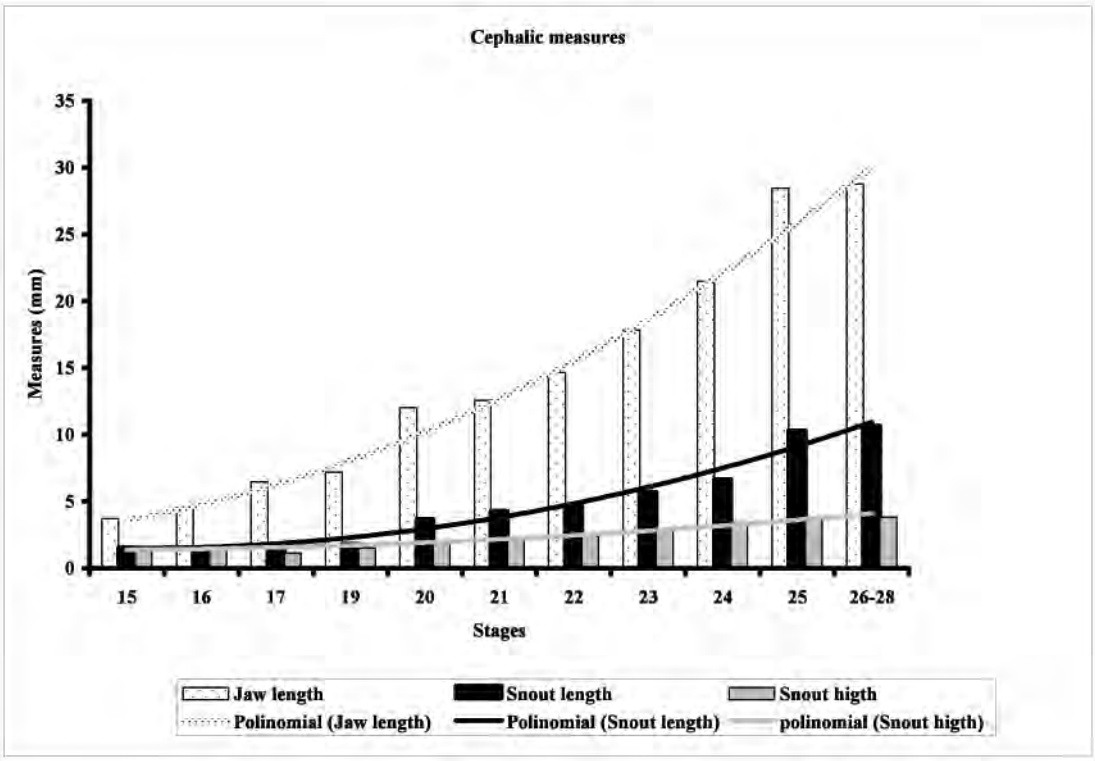



**Figure 7.**

Development of the reptilian embryo *per se* involves two distinct but related processes. One is the differentiation, *i. e.* the origin of tissues and organ systems. The other is growth, *i. e.* the increase in the size of the embryo (Andrews, 2004). The basic events during the organogenesis phase happen in the first half of development (Ferguson, 1987), which corresponds to the 35th day after oviposition (stage 21) for *Alligator mississippiensis* (Ferguson, 1985), and to 30-32th day (stage 21) for *Caiman latirostris* (Iungman, 2005). Variations in the relative sizes and proportions of structures such as the tail, snout, limbs and others, appear principally during the second half of development (Ferguson, 1985). This seems be the rule also for *C. yacare*, which shows (Figs. 4-7) an increment in the growing at beginning of the stage 21.

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Piña, C., A. Larriera, M. Medina & G. J. W. Webb. 2007. Effects of incubation temperatura on the size of *Caiman latirostris* (Crocodylia: Alligatoridae) at hatching and after onePansteatitis in Nile crocodiles (Crocodylus niloticus) associated with fish die-offs in the Loskop Dam, South Africa

Rieppel, O., 1993. Studies on skeleton formation in reptiles. V. Patterns of ossification in the skeleton of *Alligator mississippiensis* Daudin (Reptilia, Crocodylia). Zoological Journal of the Linnean Society, 109: 301-325.

# Pansteatitis in Nile crocodiles (*Crocodylus niloticus*) associated with fish die-offs in the Loskop Dam, South Africa

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**ABSTRACT:** Nile crocodile (*Crocodylus niloticus*) numbers have been declining in the Loskop Dam, South Africa, over the last decade. The quality of water flowing into the dam is generally poor and is known to cause regular fish die-offs at the inflow area. During 2006 a clinically ill mature crocodile was caught alive and a necropsy was performed after it was euthanazed. Typical lesions of steatitis were observed in all fat depots in the carcass. Following a large fish die-off in 2007 several crocodiles were observed to be affected. These crocodiles were reluctant to move on land or could not swim properly. Further necropsies were performed and similar lesions of steatitis were observed. As the affected fat hardens, animals become partially immobile and we suspect that the clinically affected animals die of exposure because they are less mobile or they drown because they cannot swim properly.

The predisposing factors, pathogenesis and lesions will be discussed and illustrated. This report should serve to alert veterinarians and other scientists to the fact that the consumption of rancid fish over long periods (weeks) by crocodiles may cause pansteatitis and subsequent mortality.

# Pathological Investigation of Runting in Farmed Saltwater Crocodiles (*Crocodylus porosus*) in Australia.

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**ABSTRACT:** Runting in farmed saltwater crocodiles (*Crocodylus porosus*) is a major cause of mortality marked by poor growth and emaciation. The purpose of this study was to perform a thorough pathological investigation. Forty animals (5-7 months old) were selected for the study over two years (twenty each in 2005 and 2007). Each group consisted of ten runts and ten clinically healthy ("normal") crocodiles. The study included full post-mortems, general bacterial culture, faecal parasitology, standard diagnostic haematology and serum biochemistry, histological evaluation of an extensive range of tissues, and, in the 2007 group, serum corticosterone. There were no differences with respect to bacterial culture or faecal parasitology. Haematological and serum biochemical findings in runts included marked non-regenerative anaemia (mean PCV 13% runts, 20% normals) and hypoalbuminaemia (mean albumin 10.9g/L runts, 17.6g/L normals), likely secondary to cachexia (prolonged poor-doing). Runts also had significantly lower serum phosphorus (1.0 mmol/L) than normal crocodiles (1.6 mmol/L), which, along with decreased growth plate activity evident histologically in runts, likely reflects poor growth. Histologically, there was severe lymphoid atrophy, most notably involving the thymus and tonsils. The adrenal glands in runts appeared relatively active whilst serum corticosterone levels were higher (mean 18.8 ng/ml runts, 8.3 ng/ml normals) suggesting increased stress levels.

# Phenotypic plasticity of the crocodile lingual salt glands in response to salinity

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**ABSTRACT:** The estuarine crocodile, *Crocodylus porosus* is found inhabiting water of varying salinity, from freshwater to hypersaline. In salinities, hyperosmotic to their plasma, estuarine crocodiles are understood to excrete excess salt via the lingual salt glands to maintain osmoregulatory homeostasis, although the functional significance salt glands in salt and water balance is debated. Here, we examined the morphological and functional phenotypic plasticity of the lingual salts to environmental salinity. We hypothesised that in crocodiles acclimated to freshwater environments, gland size and ion transport capacity would be reduced, and conversely in animals chronically exposed to hyperosmotic salinities they would demonstrate a greater capacity for salt excretion. Juvenile *C. porosus* were acclimated to freshwater and 70% seawater for six months, after which we compared between the two treatments, the morphology of the glands, ion transporters, and maximal excretory rates for NaCl. *C. porosus* shows a highly adaptive phenotypic response to increased environmental salinity which is functionally significant.

# Population diagnosis and some reproductive aspects of "Caimán Aguja" (*Crocodylus acutus*) in Bahía Portete, La Guajira peninsula, Colombia

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**ABSTRACT:** The current research corresponds to the first stage of the *Crocodylus acutus* conservation program in Bahía Portete - Colombia, developed by the company CARBONES DEL CERREJÓN LIMITED.

The population of the *C. acutus* is ubicated on three fragments of mangrove swamp. 70 individuals were observed, and 31 animals were captured and marked with two identification systems. In 50,8 km (31,7 miles) traveled across, a density of 1,37 individuals per kilometer were estimated. In the mangrove swamp area, 0,054 individual per hectare with a proportion of sex of 1:1 were also determined. The population is composed by hatchlings (<30cm) 10%, juveniles (31-90cm) 44,29%, subadults (91-180cm) 24,29% and adult class (>180cm) 21,42%; which is typical distribution of a population that has been subdued to overexplotation. According to the relative fatness index, the physic condition of these animals is healthy. Three nesting areas were identified, confirming that the reproductive period is between March and September. Ten nests were registered, finding a litter size of 26,7 ±11.35 eggs, which largest diameter was 71,63±3,11mm, and shortest diameter of 44,61±2,17mm, and an average weight of 76,65±8g; fertility rate 85,2%, embryo mortality rate 13,48%, indicating a high reproductive capability. Incubating period lasts 8-9 weeks aproximmately. Factors that affect litter survival are: scarcity of proper nesting areas, predation by *Procyon cancrivorus*, flooding and plundering by the inhabitants. According to the biotic potencial, it is estimated that the maximum time for its recovery is from 4 to 5 years, under nonextration conditions.

**RESUMEN:** La presente investigación constituye la primera fase del programa de conservación del *Crocodylus acutus* en Bahía Portete- Colombia, adelantado por la empresa CARBONES DEL CERREJÓN LIMITED.

La población de *C. acutus* se ubica en tres fragmentos de manglar. Se observaron 70 individuos y se capturaron 31 animales y se marcaron con dos sistemas de identificación. En 50,8km recorridos se estimo una densidad de 1,37ind/km. En el área de manglar de 0,054ind/há con una proporción de sexo 1:1. La población esta compuesta por neonatos (<30cm) con un 10%, juveniles (31-90cm) con 44,29%, subadultos (91-180cm) con 24,29% y la clase adulta (>180cm) con 21,42%, características distribucionales propias de una población sometida a sobre explotación. Según el Índice de Gordura Relativa la condición física de estos es saludable.

Se identificaron 3 áreas de anidación, confirmándose que el periodo reproductivo es de marzo a septiembre. Se registraron 10 nidos con un tamaño de camada de 26,7 ±11.35 huevos, con un diámetro mayor de 71,63±3,11mm, un diámetro menor de 44,61±2,17mm y un peso

promedio de 76,65±8g; la tasa de fertilidad de 85,2%, mortalidad embrionaria de 13,48%, indicando una alta capacidad reproductiva, el período de incubación dura aproximadamente 8-9 semanas. Los factores que afectan la supervivencia de las camadas son: escasez de áreas adecuadas para la anidación, depredación por *Procyon cancrivorus*, inundaciones y saqueo por los pobladores. De acuerdo a su potencial biótico, se estima que el tiempo máximo para su recuperación es de 4 a 5 años, bajo condiciones de no extracción.

**Key words:** *Crocodylus acutus*, population density, reproduction, wild population

## INTRODUCTION

Worldwide, *Crocodylus aucutus* (Cuvier 1807) is currently recognized as “endangered” according to the criteria established by the UICN (2003) and listed in the Index I of the CITES, exempting Cuba (anonym 2004). In Colombia the specie is also ranked as “endangered” (Mora-Castaño 2002), due to factors such as indiscriminate hunting prior to 1960 (Medem 1981), the habitat degradation, has contributed to the decrease in the natural population which is actually fragmented into subgroups of population (Rodriguez 2000), among this subgroups is located the Bahia Portete.

Between July 5$^{th}$ to 20$^{th}$ and September 12$^{th}$ to 14$^{th}$ of 2007, the current state of the population was evaluated and the confirmation of the reproductive period of the *C. acutus* in Bahia Portete, as one of the objectives that constitute the first stage of the Conservation Program of the *C. acutus*. The program presents the interest from the company Carbones del Cerrejón Limited, which is aimed at the integration of the local indigenous population (Wayuu) with the conservation of the specie.

## MATERIAL AND METHODS

Study area: Bahia Portete (Fig. 1) is the biggest of a series of Bahias located in the northern sector of Colombia, it is located in the town of Uribia and communicates with the Caribbean Sea, with an approximate extension of 12.793ha, and it is located between 12°16’48” N and 72°02’21” W (Anonym 2005). The region presents an average annual precipitation of 451mm, with the values between the 50-1300mm. The area is very dry since early December until mid-April, after two months of moderate rainfall, continues a dry season between July and August and finally a winter season between September and November, this being the main and sometimes the only rainy period, which represents over 60% of annual rainfall. It does not receives and input of fresh water directly from the continent, except for runoff from small streams formed during the rainy seasons

According to the structural and functional classification of mangroves, the mangrove formations of Bahia Portete, are considered as mangrove edge, framed within the low type, highly branched, leading to the formation of very narrow strips, under 50m, presents dry conditions and saline areas marked with water deficit, with a predominance of *Rizhophora mangle* and *Avicennia germinans*. In general, this ecosystem is behaving like a forest in recovery, showing a sign of good health, the process of succession is recognized by the large number of enclaves or islands of *R. mangle* colonizing new areas.




**Figure 1.** Study area. Mangrove areas in Bahia Portete, Salinaru Sector, Ian Sector, Wasinsay Sector and Portete Sector.

Recognition and establishment of transects. - The area was divided into six sectors and they in turn locations, nominated according to the indigenous place names. The mangrove areas were measured using Satellite image of true color of Google Earth Plus. The tours were conducted by boat and walking, to establish routes within the sampling channels, lagoons, mangrove and inland beaches. During this inspection were identified places of transit, sunbathe, nesting sites and caves. These tours were conducted between 7:00 and 15:00 hours (Figure 2).

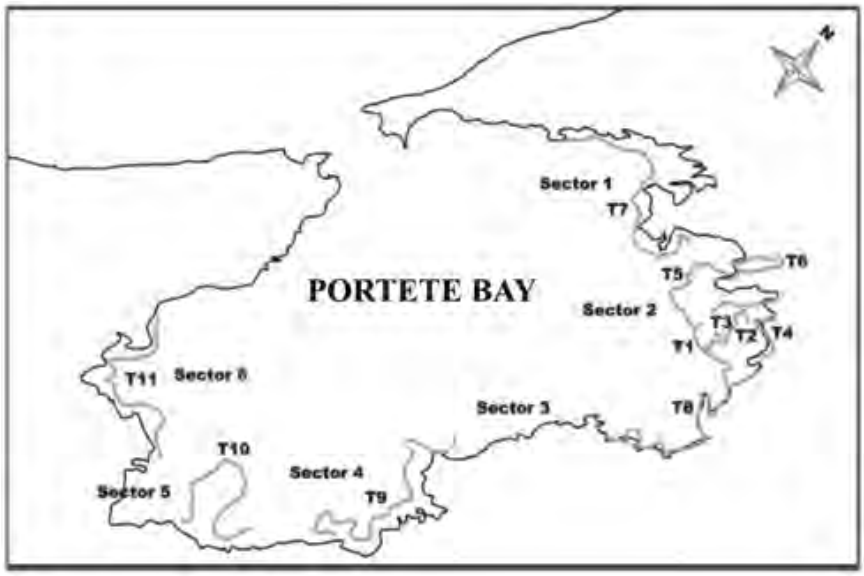



**Figure 2.** Transects established in monitoring the population of Cayüshi in Bahia Portete.

**Night counting, catch and identification systems. -** The "Counts night with lamps," methodology was used, standardized by the Ministry of Environment (Martinez 1994), these transects (11 in total) were conducted between 18:00 and 04: 00 hours, 2.000.000 lumens lamps and hands free 150.000 lumens were used. At the time of the sighting, the time,

number of individuals were registered, as well as the environmental variables of the air temperature and relative humidity with a digital thermo-hygrometer, water temperature and salinity levels with a refractometer (Hanna Instrument), the pH of the water with a digital meter (0.1 watt Hanna Instrument) and features of the site observation and / or capture.

The class structure proposed by Thorbjarnarson & Platt (2000) was used; Class I (hatchlings) under 30 cm, Class II (juveniles) between 31 and 90 cm, Class III (subadults) between 91 and 180 cm and class IV (adults) larger than 180 cm, by the presence of a female pregnant with LT of 180cm.

During the catches the biometric features of Total Length (TL), Snout-Vent length (SVL), measured from snout to the tip of the middle part of the sewer), carried out with tapes (± 0.5 cm) were recorded, the weight with pesolas of 300 and 500gr, 25 and 250 kg, for the evaluation of the physical conditions of the animals Concerning the Relative Fatness Index (RFI, figure 3) was employed, proposed by Seijas *et al*. (2003) for *Caiman crocodilus crocodilus*, where $W_R$: equals Real Weight, $W_E$: Expected Weight, a: and b: are constants obtained from regression analysis of data on the length and weight of the animals in obtaining the Expected Weight (WE).

$$RFI = \frac{W_R}{a \times TL^b}$$

**Figure 3.** Index Formula of Relative fatness

The sex determination was conducted by direct palpation of the gutter and use of claw and/or forceps. Individuals were marked with two identification systems: an electronic identifier (Micro chips brand AVID) and the amputation of their single and double caudal whorls on the tail, the latter suggested by the Ministry of Environment (Anonymous 2000), each whorl successive from the simpler region single caudal whorls is assigned a number from the series 1, 2, 4, 7, 10, 20, 40, 70,100, 1000 ... In this work the tens and hundreds are marked in double whorls as follows: in the caudal whorls of the right row the dozens and to the left the hundreds, and in the simple caudal whorls the units. The observation sites and captures were georeferenced using GPS (Garmin 60CSx).

The intensive search for nests was carried out between 7:00 and 15:00 pm. These were georeferenced using a GPS (Garmin 60CSx). Appraising the excavations according to their condition, taking into account the following criteria: good nest (with eggs), drowned nests (embryonic death by floods), old nest (used in previous posture periods), abandoned nest (with signs excavation and were not used), preyed nest (with signs of having been preyed), plundered nest (extraction of eggs by humans) and hatched nest (in those that became apparent signs of hatching). In active nests were reported, length, width and depth, distance at which these were at the edge of the mirror of water, and by triangulating the height of this with respect to the level of the mirror of water, also were recorded: temperature and relative humidity, identification of close vegetation and distance to the nest.

Each active nest was object to record their litter size, major and minor diameter of eggs with a type vernier caliper (Electronic Digital Caliper) and Weigt (Balance of 200g), the embryonic state (fertility, infertility and embryonic death), and physical conditions of the

egg (fractured or broken) and the length of embryonic development with the assessment of an opaque bandwidth (Ferguson 1995, In Gutierrez and Rodriguez in 1993).

## RESULTS

**Population density .-** We traveled a total of 50.8 km, having observed a total of 70 individuals in 9 transects, presenting an average of 7.7 ± 3.5 animals daily and a density of 1.37 ind/km, transects 1 and 11 were not sampled as there is no evidence of the presence of individuals during the daylight inspection (Fig. 2).

Individuals of *C. acutus* were observed in 3 of the 6 sectors, more accurately in the sectors of Ian, Waâbpana, Wasinsay, Turruli, Wayetalo and Kupterra.

Sector 2 (locality if Ian): It covers an area of about 500ha. Characterized by having a series of internal channels and not been easily accessible by boat, providing protection to animals of fishing activities (Figure 2). In this sector a density area of 0.114 ind/ha was founded. (n: 57, chart 1)

Sector 3 (localities of Waâbpana and Wasinsay): Between these two locations is a patch of mangrove of 20ha, which posses interior channels and clayish soil, is a zone of high floods and on its inner edge lies a patch of dead mangrove (Figure 2). In this sector a density area of 0.00793 ind/ha was founded (No: 8, table 1).

Sector 4 (localities of Turruli, Wayetalo and Kupterra or Puerto Portete): A thin patch that is growing and has a navigable interior channel, wide and deep, the inner edge of the mangrove is sandy, tall and suitable for nesting the mangrove area covers an area of 630ha (Figure 2). In this sector a density area of 0.400 ind/ha was founded (No: 5, table 1).

Sector 1 (localities of Los Cocos, Kashiara, Achii, Savaincle, Salinaru, Utûana and Siluru), Sector 5 (town of Puerto Nuevo) and the Sector 6 (locality of Media Luna): these have in common being thin strips of mangroves and in growing process, no interior channels, the beaches and the internal border of the mangrove are clay soil (Figure 2). No evidence of utilization from the *C. acutus* was founded in any of these sectors.

**Table1.** Densities per area of *C. acutus* mangrove.

| Mangrove | Area(ha) | Nº de Indiv | Ind./ha |
|---|---|---|---|
| Salinaru | 140 | 0 | 0 |
| Ian | 500 | 57 | 0.11400 |
| Wasinsay | 20 | 8 | 0.40000 |
| Puerto. Portete | 630 | 5 | 0.00793 |
| **Total** | **1290** | **70** | **0.05420** |

Structure of the population - Of the 70 individuals that were spotted, 31 were captured, of these, 29 were marked with caudal whorls and from this group 19 were marked with electronic identifiers. The 70 individuals were distributed as follows: 10% hatchlings, 44.29% juveniles, 24.29% sub-adult and 21.42% adults (Fig. 5). The captured: 10% neonates, 50% juveniles,

45.83% sub-adults and 4.16% adults (Fig. 6). The classification structure is characteristic of a population subject to overhuting (Figure 7, d.) Acording to the class structure proposed by Velasco and Ayarzagueña (1995) submitted for wild populations of *Caiman crocodilus*.

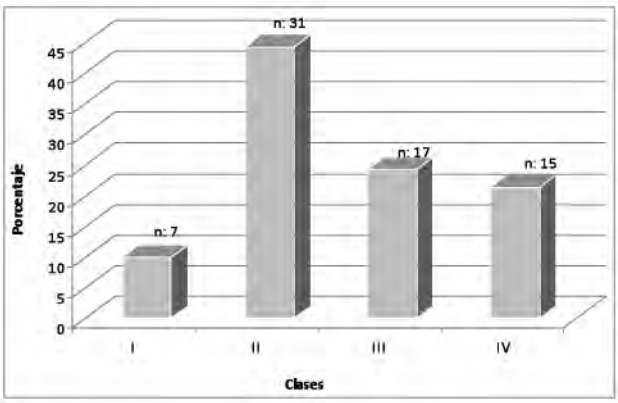



**Figure 5.** Distribution of all classes of individuals observed in the population of *C. acutus* in Bahia Portete

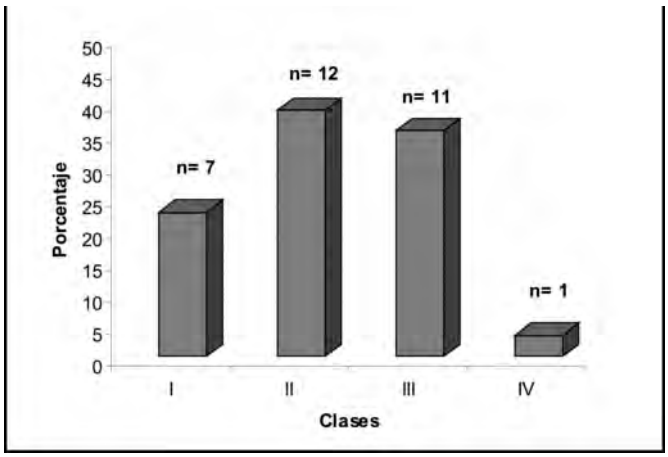



**Figure6**. Distribution of class *C. acutus* captured in Bahia Portete

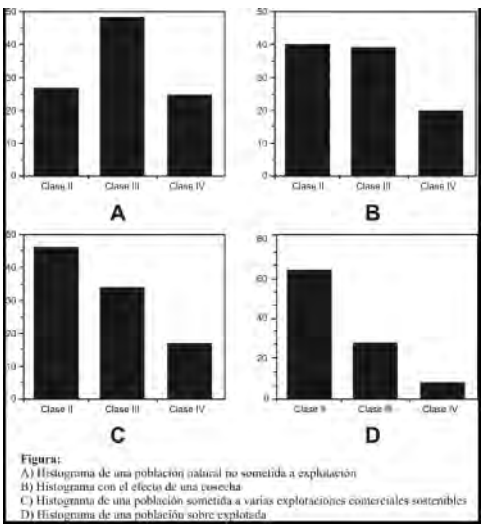



**Figure 7.** Class structure proposed by Velasco and Ayarzagueña (1995) from a wild population of *Caiman crocodilus* in Venezuela.

The catch had a sex range of 1:1, with 50% of females and 50% males. The distribution of sex in classes presented for the case of a female 41.66% in Class II, 50% and class III, and only 8.3% in Class IV; in the case of the males 58.33% in class II, 41.66% in class III and there were no captures for class IV (Fig. 8).

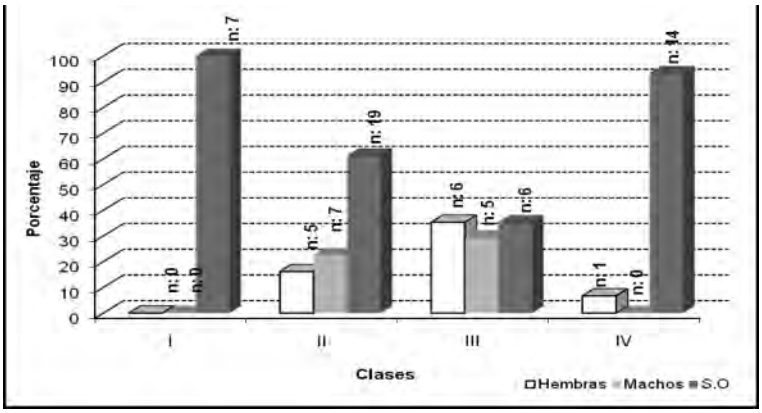



**Figure 8.** Distribution of sexes in the structures of classes of people captured in *C. acutus* in Bahia Portete (S.O = single eyes).

**Estimating the physical conditions of individuals. -** The animals presented a range of TL between 25,8 and 200 cm and weights between 300 and 30.000 g. The Weight-Length relation is defined by the following equation (Equation 1), with a correlation coefficient of r : 0.97 and R2 95.5%.

$$W_E: 0.00191633\ TL^{3,07563} \quad \text{(Equation 1)}$$

Where the rate of obesity corresponds to the equation 2

$$RFI = \frac{W_R}{0{,}00191633 \times TL^{3,07563}} \quad \text{(Equation 2)}$$

With a Relative Fatness Index average of 1,03, indicates a healthy population. The animals showed no signs of mutilations, scars or obvious signs of disease (Fig. 11).

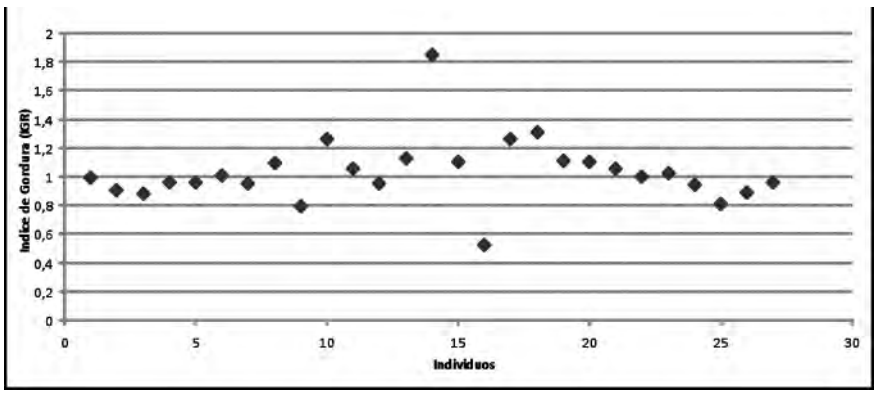



**Figure 11.** Relative fatness index of individuals caught in Bahia Portete.

**Hours of sighting.-** the population began their evening activities around 18:00 hour until the 4:00 hours, being the time range between 20:00 and 1:00 hours, the most active and the peak of maximum activity at 22:00 (n: 17) hours.

**Sighting locations. -** The largest number of observations were submitted among the vegetation with a 84.28% (n: 59) and a 15.71% (n: 11) located in open waters, there were no animals on land. With regard to the depth of water, largest number of sightings were sighted, in shallow waters with a 91.43% (n: 64) and an 8.57% (n: 6) in deep water.

**Environmental variables and physical-chemical registered .-** The animals were among the 26 to 32 ºC ambient temperature and a single individual observed during the day (38 degrees ºC), the largest number of animals were found among the 27-30 ºC (n: 41). The values of relative humidity were recorded among 61% to 84%, for the individual caught in the day a value of 40% was registered. Individuals were observed at a superficial temperature of the water between 26 to 30 ºC.

**Nesting area distribution. -** 3 nesting areas were identified. Two islands located in Sector 2 and in the sand dunes located in sector 4.

Pioyoi Island is the largest and covers an area of 1,0689 ha (without the flood peak), presents characteristics of the subxerofítical dry forest vegetation, with cacti, scrubby trees and low, the soil is composed of burdensome material and only in 0.0003 ha sandy material. On this island 8 nests were located, with a density of 7.48 nests/ha, of which 6 were found in the sandy substrate to form a communal nesting area, the remaining nests were located in other parts of the island (Table 2 ).

Espirol Island is divided by two small portions of mangrove and for greater clarity known as Espirol 1 with an area of 0.0588 ha and Espirol 2 with 0.2819 ha (areas calculated without the flood peak). This just like Pioyoi, shows characteristics of the subxerofítical dry forest vegetation, the soil is composed of burdensome material. In Espirol 1 no active nests were located and in Espirol 2, 1 active nest was located, with a density of 3.50 nests/ha (Table 2).

The third corresponds to the sand dunes (located in sector 4) formed behind the mangrove, these are colonized by *Batis maritima* and have a height of about 2 m of sand-clay material, *R. mangle* which forms the edge provides shade for nests, the area behind the dune is a flood zone. Here, 1 nest was located. The area of sand dunes could not be determined.

**Table 2.** Clutches found in the areas of search in Bahia Portete.

| Location | Area (ha)e | Activ Nests | Nest under Construction | Old Nest | Drowned Nest | Destroyed Nest | Total | Nests Density/ha |
|---|---|---|---|---|---|---|---|---|
| Pioyoi Island | 1.0689 | 8 | 3 | 5 | 0 | 2 | 18 | 7.48 |
| Isla of Espirol 1 | 0.0588 | 0 | 4 | 1 | 0 | 0 | 5 | 0 |
| Island of Espirol 2 | 0.281 | 1 | 2 | 1 | 0 | 0 | 4 | 3.5 |
| Dunes * | | 1 | 0 | 0 | 1 | 0 | 2 | |
| **Total** | | **10** | **9** | **7** | **1** | **2** | **29** | |

* It could not be established

**Characteristics of the nests.** A total of 29 digs were located, 10 of these presented postures which correspond to a 34.48% (Table 2), 31.0% were found evidence of construction activity, 24.13 % corresponded to old nests, 3.44% to drowned in nests and 7.83% destroyed nests by activities of other females.
The ten nests showed the an average size 62.7 ± 31.21 cm long (Table 3), a width 63.8 ± 41.31 cm and depth to bottom of clutch 31.5 ± 12.06 cm, in the last six nests, for been located in a communal nesting area. The registration of the dimensions of the communal nest shows a bias in the values due to the closeness between them, which impeded the certain determination of the limits

**Table3**. Biometric characteristics of the nests in which they recorded the existence of eggs of *C. acutus* found in Bahia Portete.

| Nest Nº | Location | Length | Width | Depth |
|---|---|---|---|---|
| 1 | Pioyoi | 103 | 93 | 41 |
| 2 | Pioyoi | 58 | 53 | 41 |
| 3 | Pioyoi | 71 | 86 | 50 |
| 4 | Espirol 2 | 128 | 160 | 41 |
| 5 | Pioyoi | 43 | 38 | 23 |
| 6 | Pioyoi | 58 | 53 | 20 |
| 7 | Pioyoi | 38 | 34 | 20 |
| 8 | Pioyoi | 62 | 70 | 40 |
| 9 | Pioyoi | 35 | 26 | 20 |
| 10 | Wayetalo | 31 | 25 | 19 |
| | **Average** | **62.7** | **63.8** | **31.5** |
| | **DS** | **31.2198299** | **1.31128** | **12.0669** |

A 100% of the clutches are under shade most of the day, located between 20 to 100cm from the base of the nearest bush, 8 nests beneath a *Cesalpinacea sp*, a nest under an *Astronium sp (Anacardiacea)* and one below a *R. mangle*.

The direction of the actives nests was to the West for 60% of cases, 20% to the East, 10% to the North and 10% to the South.

The nests showed temperatures between 31.8 °C and 36.8 ºC with an average of 34.13 ± 1.58 ºC and relative humidity between 31% and 56% with an average of 44.1 ± 9.12%. The temperatures recorded during the environmental assessment of the nests were between 32 and 41.3 ºC with an average of 35.62 ± 3.37 ºC and relative humidity of the environment was between 32 and 45% with an average of 38.8 ± 4.8 % (Table 4).

**Table4**. Characteristics of temperature and relative humidity in the nests of *C. acutus*.

| Nest Identification Nº | Environmental Temperature | Environmental Humidity | Nest Temperature | Nest Humidity |
|---|---|---|---|---|
| 1 | 41.3 | 32 | 31.8 | 56 |
| 2 | 32 | 42 | 32.6 | 56 |
| 3 | 32 | 42 | 33.1 | 56 |
| 4 | 37.6 | 41 | 33 | 41 |
| 5 | 36.5 | 45 | 33.5 | 44 |
| 6 | 32 | 42 | 34.4 | 44 |
| 7 | 32 | 42 | 35.7 | 41 |
| 8 | 37.1 | 34 | 34.9 | 35 |
| 9 | 37.1 | 36 | 36.8 | 37 |
| 10 | 38.6 | 32 | 35.5 | 31 |
| **Average** | **35.62** | **38.8** | **34.13** | **44.1** |
| **DS** | **3.37** | **4.8** | **1.58** | **9.12** |

Through the evaluation of the embryonic band of development were estimated the probable dates for the clutches, like this: clutch number 1 presented a developmental band of approximately 18 days and it would be June 28, 2007 the probable date of position (Figure 12c); clutches number 2-5 and 10 showed a band of embryonic development of two weeks' stance setting the likely date between 1 and July 2, 2007; clutches number 6-9 showed a band of two weeks' positions , even though the day July 13 the position had not taken place in the afternoon, the probable dates of positions are between July 13 2007 in night time and July 18 in the morning hours (Figure 12b). It is estimated the last week of June as the possible start of the positions of *C. acutus* in Bahia Portete.

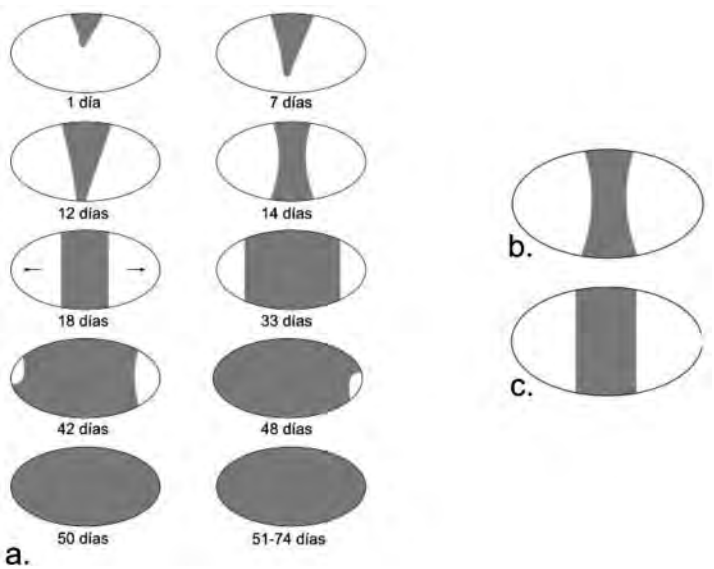



**Figure12.** a) Development of the opaque band under artificial incubation of *C. acutus* eggs with temperature conditions of 32.5 ºC and relative humidity of 99% (Mejia F. com Pers.). Characteristics of different nests of *C. acutus* found in Bahia Portete, evaluated according to Ferguson (1985 in Gutierrez and Rodriguez 1993): b) Nests. No 2-10 and c) Nest No 1.

**Characteristics of the Clutches -** The clutch size showed an average of 26.7 ± 11.35 eggs. The evaluation of the viability of the eggs showed that 95.5% of the eggs were fertile, 77.9% variables, 4.5% infertile and 17.6% showed embryonic mortality (Table 5). The eggs length average diameter 71.69 ± 3.11mm, a width average diameter of 43.64 ± 2.17mm and average weight of 77.89 ± 8g (Table 6).

**Table 5.** Viability of the litter of active nests in Bahia Portete.

| Nest Identification Nº | Location | Date | Nº of Eggs | % Viable Eggs | % Infertile Eggs | % Embryonic Mortality |
|---|---|---|---|---|---|---|
| 1 | Pioyoi | 11-jul | 30 | 73.3 | 0 | 26.7 |
| 2 | Pioyoi | 13-jul | 25 | 48 | 12 | 40 |
| 3 | Pioyoi | 13-jul | 28 | 82.1 | 3.5 | 14.4 |
| 4 | Espirol 2 | 13-jul | 10 | 60 | 0 | 40 |
| 5 | Pioyoi | 14-jul | 41 | 68.2 | 19.51 | 12.19 |
| 6 | Pioyoi | 19-jul | 42 | 95.3 | 0 | 4.7 |
| 7 | Pioyoi | 19-jul | 20 | 60 | 0 | 40 |
| 8 | Pioyoi | 19-jul | 27 | 81.5 | 0 | 18.5 |
| 9 | Pioyoi | 19-jul | 35 | 97.1 | 0 | 2.9 |
| 10 | Wayetalo | 19-jul | 9 | 100 | 0 | 0 |
| | **Total** | | **267** | | | |
| | **Average** | | **26.7** | | | |
| | **DS** | | **11.35** | | | |

**Table 6.** Biometrics of the eggs.

| Nest Identification Nº | Clutch size | Egg Length Average | Egg Width Average | Weight Average |
|---|---|---|---|---|
| 1 | 27 | 74.86 | 44.34 | 85.3 |
| 2 | 18 | 68.97 | 45.82 | 80.23 |
| 3 | 24 | 67.4 | 41.17 | 62.52 |
| 4 | 6 | 74.34 | 42.96 | 77.6 |
| 5 | 41 | 70.81 | 45.63 | 79.15 |
| 6 | 42 | 71.73 | 44.9 | 82.58 |
| 7 | 20 | 70.51 | 39.57 | - |
| 8 | 23 | 77.82 | 46.23 | - |
| 9 | 34 | 70.81 | 43.53 | - |
| 10 | 9 | 69.73 | 42.31 | - |
| **Total** | **244** | | | |
| **Average** | | **71.698** | **43.646** | **77.896667** |
| **DS** | | **3.11331** | **2.17069** | **8.0036009** |

**Factors affecting the survival of the nests.-** four factors that affect the survival of nests were identified, the first and most important is the shortage of areas suitable for nesting by the silvicolous use of the mangrove, the second is the predation from the *Procyon cancrivorus* specie; the third, are floods and the fourth but no less significant is the looting by the indigenous inhabitants of the area.

**Births .-** During the visits from 12 to Sept. 14, 2007, the emergence state of all clutches was verified, egg shells were found around the nests and in the adjacent mangrove area, apparently they had been allegedly removed by the females to help out the hatchling, proving the birth of the clutches and the help received from the females during hatching. We found no evidence of embryonic mortality after the first revision, apparently all of the fertile eggs were born (n: 208). On the Island Espirol 1 were the nests under construction were located, no stance was realized.

7 hatchlings were captured near the nesting areas, 5 of those with complete healing of the navel, which gives an age of approximately two weeks, the remaining 2 had an age of about a week, placing births in the first week of September.

The hatchlings had a TL between 25.8 and 31.8 cm and an average of 29.62 ± 1.19 cm in SVL between 12.2 and 15.5 cm and an average of 14.41 ± 1.12 cm and weighed between 39.6 and 72.1 g and an average of 61.91 ± 10.42 g. They were captured between the neumatophorus of *A. germinans* in an area that had a depth of about 5 to 20cm, with an abundance of food represented in fingerlings of various species. The hatchlings showed a brown color darker than the color of the adult.

The hatchlings presented a RFI of 0.954, which indicates that these individuals were in relatively good health (Fig. 13).

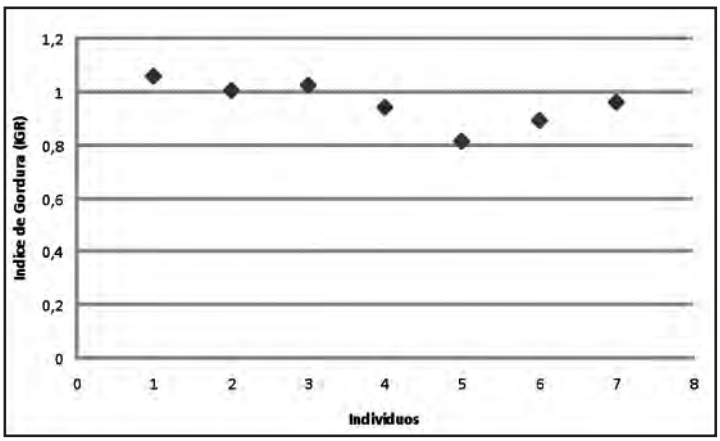



**Figure13.** Relative Fatness Index of hatchling captured in the island Pioyoi.

**Reproductive period.-** Taking into account the evaluation of the embryonic band of development and the probable dates of onset, the reproductive period for the specie *C. acutus* in Bahia Portete was estimated between March and October, where the courtship and mating would be between March and May, the oviposition by mid-June until mid-July and finally hatching is presented with the onset of the rains in September and culminates in October (Fig. 14).

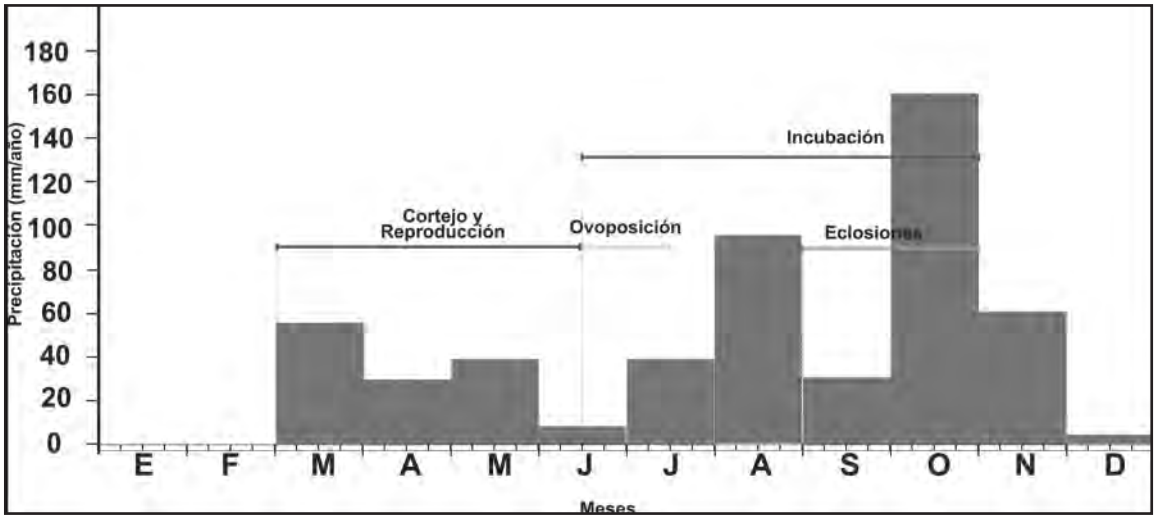



**Figure14.** Graphic of the reproductive period observed for the *C. acutus* in Bahia Portete in 2007, according to rainfall.

## DISCUSSION

The mangroves which are habitats to most individuals have the greatest area, inland canals, places to sunbathe, for nesting and inland lakes where they feed.

The crocodile census of Colombia between the years 1994-1997, presented as a result 0,47 ind/km in a transect of 21km, with a population made up of 10 specimens (juveniles and sub-adults) with sizes of inferior than 180 cm (Rodriguez 2000). Abadia and Drew in 1981 reported a low population because the population is subject to overexploitation, the same author in 1993 ensures that the population is in recovery and estimating population size of 140 individuals based on the finding of 9 structures, nest under construction, in 1998 conducted a monitoring and taking into account his previous reports concluded that the population remains stable, however the basic information collected is not statistically significant for the conclusion. The density founded during this study is higher than those reported previously for this area but it cannot be assumed that the population is in recovery, since previous studies do not report a database detailed enough to clearly compare, evaluate and infer about the development of population density far less about its spatial and temporal variation.

The area used by *C. acutus* is 35.58% of the total mangrove area (1290ha), with 831ha in which there were no animals and that have the potential to be used by individuals or the non-use of these areas is due to the existence of anthropic activity.

Most part of the population is concentrated among the youth and sub-adults, it seems that the adult class is being decimated by a selective approach; Rodriguez (2000) comes to similar conclusions, by reporting that this population was subjected to a sustainable harvest, the removal of the adult class in Bahia Portete can be linked to meeting the food needs of the local indigenous population. But a population dominated by juveniles may indicate that the population or the portion affected is recovering from the overexploition that occurred in previous years (Seijas 1986, Llobet & Seijas 2003, En Grajales Garcia *et al*. 2007).

The catch had a sex ratio of 1:1, equal to those reported in other populations (Kuslan & Mazzotti 1989, Platt & Thorbjarnarson 2000 and Cedeño-Vasquez, 2002, En Grajales Garcia *et al.* 2007) unlike those found in Window (Mexico) by Garcia-Grajales (2007), with a ratio of 3 males for every female.

The physical conditions of the individuals captured were healthy; there were no signs of disease or infection, no scarring or mutilation. The conditions under which the animals were can be influenced by the time of reproduction and climatic conditions.

Abadia (1981) reports two areas of nesting in Bahia Portete, which are the islands known as Pioyoi and Espirol 2, in this work he identified a new nesting area for the zone (dunes in sector 4), which suggests the possibility that there are other nesting areas which have not yet been identified, that is why it is necessary to carry out further searches.

The presence of a small sandy portion in the area of the Island of Pioyoi for the development of nests has resulted in the existence of a communal nesting area, which increases the possibility of the destruction of nests, aggressive encounters between female and partial loss Egg by breaks.

Near the Island of Pioyoi there is an area with characteristics similar to islands, which are being used by the settlers as grazing area (cows, goats and goats) and for extraction of plant material (wood), an area that is being used by animals as a zone to obtain food (in adjacent mangroves) and could have been used by animals for nesting in the recent past, this area can fit into a future with a proper handling for this purpose.

The nests in Bahia Portete were found at a depth of ≥ 19cm, at a distance from vegetation of 0.20 m to 1m, where 60% of these were in direction to the West, conditions that despite the climatic characteristics (desert), and low vegetation of the place, it provides the clutches the necessary protection against direct radiation from the sun's rays in the morning and midday, when the sun's rays are more incidents.

The ecology of the *C. acutus* nesting in Bahia Portete requires further study, involving fluctuations of temperature and relative humidity during the day and night, to know their relationship to the development of the incubation period.

In Bahia Portete an clutch average of 26.7 ± 11.35 eggs was obtained, a 95.50% fertility, an egg lenght average of 71.69 ± 3.11 cm, a egg width average of 41.66 ± 3.18 cm and an average weight of 77.89 ± 8.00 g, nesting females showed a total length greater than 180cm. Casas-Andreu (2003) reports for Jalisco an average eggs for clutches of 2.9 ± 13.2 eggs, a greater average diameter of 74.6 ± 4.2 mm, lesser diameter of 45.5 ± 2mm and a weight of 93.1 ± 10g, and a fertility of 81 ± 21%, where the population of nesting females were between 225 and 305cm in length, asserting that the relationship between the size, weight and fertility of eggs is directly proportional the size of the female; Mejia (pers.) reports that in breeding farms the females with sizes of less than 225cm are synonymous with small litters, small eggs and infertility increased to 18%, which would suggest that breeding females in Bahia Portete are phenotypically small. This may be related to the fact that for a long time the population of *C. acutus* has been subjected to extraction, where the adult class is the most affected, especially male individuals because of their larger size, giving

to young males to reproduce at reduced age or eliminated intraspecific competition between adults and sub-adults

Given the above, it is expected that individuals who are reproducing have similar sizes, so it is possible that this selection has eliminated those elements of the population who are phenotypically larger in size.

In Bahia Portete a short reproductive period has been registered, compared with those recorded in the closed-loop farms in the department of the Atlantic (Colombia), which began in December with the mating, the positions starting in the last week of January, Continuing the months of February, March and until the first week of April, where births occur between April and June.

The short reproductive period in Bahia Portete, may be due to factors such as, the short incubation period due to climatic conditions, mainly the occurrence of rains and the unbalanced structure of the groups that have an impact on the organization of the hierarchy and dominance, linked to the size of the animal, presenting a possible homogeneous distribution of animals within the area of mangroves, increasing the likelihood of a meeting of individuals over the age of reproduction thus reducing the time for courtship and mating.

Bahia Portete infants showed LT average (29,6 ± 1,91cm) and average weight (61.91 + 10.41 g) higher than those reported by Arzuza in 2000 (LT Avg 24.16 ± 2.53cm and Weight Avg 51.76 ± 9.88g) and Perez & Escobedo in 2005 (LT Avg 24.54 ± 0.98cm and Weight Avg 46.13 ± 4.28g) for newborn individuals, however lies in the size ranges and weight reported by Brazaitis (1973, in Sanchez 2001) with LT 25-30cm and weights between 40-70g, Alvarez del Toro (1974, Sanchez in 2001) with LT between 25-30cm and Medem (1981, Sanchez in 2001) with LT between the 25-30cm between 40-70g for the species. The total longitudes of newborns in the Bay may be due to the time of birth of the animals, from about 4 to 15 days of birth. The state of thinness found in infants may be because they were still absorbing the yolk and developing strategies to catch food.

The identification of factors affecting the survival of the litters, is of great importance when it comes to implementing management plans that share the conservation of a specie, especially when one of these factors comes directly from the use of the resources specially by the settlers , Which in our case in particular is an indigenous population, hence to achieve it is of utmost importance to perform a work of socialization and awareness to people about the importance of conservation, creating economic alternatives to provide welfare to population derived from resource conservation.

## CONCLUSIONS

- It confirms the reproductive period of *C. acutus*, which covers the period from April to October
- To *C. acutus* in Bahia Portete found a density 1.37 ind/km in a total route of 50.8 km.
- A low density of individuals by area of mangrove was determined (0.05420 ind/ha).
- The status of the population of Bahia Portete presents characteristics by distributional classes of a population subjected to exploitation.
- It is not clear what the conditions of selection that makes the residents to use the

species are, the same for the routes or destinations that the products have.

- The proportion of sexes was a 1:1 relationship, one female for every male, for the population of Bahia Portete
- Trapped animals showed healthy physical condition (weight, vitality and free from external pathology).
- The average litter size was 26.7 eggs. Fertility rate of 95.5% and 17.60% of embryonic mortality which indicates a high reproductive capacity of the species in this environment.
- A new nesting area was identified and there is the probability of locating other areas with similar characteristics on which nesting might be occurring, and must be located for protection.
- Nesting areas present a pastoral use, an activity on which there is yet to establish a standard for the conservation of these areas, an example of this is the area known as Toloi, which found signs of being used by *C. acutus* in a not-too-recent past as a nesting site.
- The registration of births presented to the hatch during the second period of rain during the month of September.
- *C. acutus* represents for the local indigenous population a component of the diet product of opportunist hunting, determining efforts in the valuation of the appeal by the Wayuu community and its participation in any management plan for conservation of this species, given the importance of mangrove ecosystem for the villagers and Cayüshi.

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# Population Study of *Caiman yacare* in the Province Ángel Sandóval (San Matías region), Santa Cruz, Bolivia

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**ABSTRACT:** In the northern zone of influence in the area of integrated management San Matías, Santa Cruz, Bolivia, counts in the night were conducted on Yacare Caiman (*Caiman yacare*) in the months of December 2004, May and June 2005, sampling 91 waterbodies, 48 ponds, 13 curiches, 9 lakes, 14 small lakes, 6 rivers and 1 stream. The results obtained show greater abundance (indiv/Km) in small lakes < 1Km, curiches and ponds, while the greatest abundance of Yacare of the Class IV occurred in rivers, small lakes and ponds. The population's structure showed a bigger number of individuals of the classes II and III. In conclusion a low number of individuals of the class IV (adult for optimal utilization) was observed possibly by the time when the counts were performed (season high water where they are scattered), in addition to agreement to interviews with local residents, reported that the area is the subject of an intensive harvest.

**RESUMEN:** En la zona norte de influencia y el Área de manejo integrado San Matías, Santa Cruz Bolivia, se realizaron conteos nocturnos de lagarto (*Caiman yacare*) en los meses de diciembre del 2004, mayo y junio del 2005, muestreándose 91 cuerpos de agua, 48 estanques, 13 curiches, 9 lagunas, 14 lagunetas, 6 ríos y 1 arroyo. Los resultados que se obtuvieron muestran una mayor abundancia (indiv/Km) en lagunetas, curiches y estanques; mientras que las mayores abundancias de lagartos de la Clase IV se registraron en ríos, lagunetas y estanques. En cuanto a la estructura poblacional del lagarto en el área de estudio corresponden en mayor número a las Clases II y III. En conclusión para la zona de estudio se observa una baja cantidad de individuos de la categoría IV (adultos óptimos para el aprovechamiento) posiblemente por la época en la que fueron realizados los conteos (temporada de aguas altas donde se encuentran dispersos), además de acuerdo a las entrevistas con pobladores locales, informaron que la zona es objeto de una intensa cosecha.

# Production of Superoxide Ions by Leukocytes of the American Alligator (*Alligator mississippiensis*)

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**ABSTRACT:** We used WST-1, a tetrazolium salt which can be reduced to a water-soluble formazan compound with high molar absorptivity at 438 nm, to probe the production of superoxide by alligator leukocytes. Incubation of alligator whole blood with WST-1 resulted in a time- and concentration-dependent increase in absorbance of the plasma at 438 nm. The reduction of WST-1 was inhibited in a concentration-dependent manner by superoxide dismutase, an enzyme that catalyzes the reduction of superoxide to peroxide, confirming that the reduction of WST-1 was due to the presence of superoxide. Treatment of whole blood with NBT resulted in the staining of only heterophils and macrophages, showing that the production of superoxide is due to the presence of leukocytes. It is interesting to note that the production of superoxide by the alligator leukocytes required no external stimulation while human leukocytes must be stimulated with an immunological challenge before producing superoxide.

# Program for rescuing and protecting American crocodile's eggs from human predation with participation of indigenous people and communities in Zulia state, Venezuela

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**ABSTRACT:** We have been working to capacitate and to achieve participation of local communities, Barí Indigenous people and landowners from part of Maracaibo lake basin in a conservation program for the American crocodile (*Kanta* in Barí dialect) around their living areas (basins of rivers Negro, Santa Rosa and Aricuaizá). These people supported by specialists (Crocodile Specialist Group of Venezuela) could search for nest and collect the eggs, which will be incubated in a safe place until hatching. In this way illegal commercialization of eggs could be diminished. The hatchlings produced will be raise in captivity and then used in programs to restore and reinforce populations of the species.

**RESUMEN:** Se ha estado trabajando en capacitar y lograr la participación de comunidades locales, indígenas Bari y hacendados en parte de la Cuenca del Lago de Maracaibo para que ejecuten anualmente acciones de conservación relacionadas con el Caimán de la Costa (*Kanta* en dialecto Bari) en las cercanías de sus espacios de vida (Cuencas de los ríos Negro, Santa Rosa y Aricuaizá). Ellos, apoyados por asesores y especialistas en el área (Grupo de Especialistas en Crocodilos de Venezuela) podrán buscar y recoger los huevos de la especie e incubarlos hasta su nacimiento. De esta forma se busca disminuir la comercialización ilegal de los huevos. Los cocodrilos nacidos serán criados en cautiverio y usados luego en programas de restauración y fomento poblacional de la especie.

# Public Opinions, Attitudes, Risk Perceptions, and Knowledge of Alligators in Florida

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**ABSTRACT**: As American alligator (*Alligator mississippiensis*) populations in Florida have recovered from depressed levels in the 1960's, human-alligator conflicts have increased. Maintaining populations of potentially dangerous wildlife species at levels consistent with human desires can be a challenge. The Fish and Wildlife Conservation Commission's Alligator Management Program (FWC) recently sought input from stakeholder groups and received conflicting feedback. Some suggested that alligator populations are excessive while others believed that populations are below desirable levels. Although informal, this survey highlighted the need for a more sophisticated examination of Floridians knowledge and opinions. FWC has previously conducted surveys of public attitudes about alligators in 1976 and 1996, and these can provide information against which to measure changes in public attitudes and knowledge over time. The purpose of this study is to determine current public knowledge, attitudes and risk perceptions about alligators, to assess changes in these characteristics since 1976 and 1996, and to determine if regional differences in these exist within the state.

# Social component of the Management Plan for Spectacled Caiman (*Caiman yacare*) in the Protected Area of San Matías, Santa Cruz – Bolivia

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**ABSTRACT:** The Natural Area of Integrated Management (ANMI) San Matías is located at East of the Department of Santa Cruz; it is the second bigger protected area of Bolivia with a total of 2.918.500 hectares, containing a varied dispersed social geography in their surface. To elaborate a Management Plan of Spectacled Caiman for the ANMI San Matías, which should be concerted and approved by the direct beneficiaries (indigenous communities and cattle ranches) 32 communal workshops, 2 regional workshops, 2 workshops with cattlemen were made, plus diverse meetings with institutions and local organizations. The development of the management plan was based on the respect to the communal organizations, the local actor's leading role in the taking of decisions, strengthen of the organizations and institutions for the integral and adaptive management of the resource and increment of revenues by means of a bigger role of the direct actors (communards and cattlemen) in the productive chain. Despite of the complex human mosaic of the ANMI San Matías, extension of the area, difficulty of accesses and scarce advance in the legal tenure of lands, it has been possible to concert in a participative way the administration system for the conservation and use of the Spectacled Caiman and to plan the first harvest for the year 2008.

**RESUMEN:** El Área Natural de Manejo Integrado San Matías se encuentra ubicada al Este del Departamento de Santa Cruz; es la segunda área protegida más grande de Bolivia con un total de 2.918.500 ha. albergando una variada geografía social dispersa en su superficie. Para elaborar un Plan de Manejo de Lagarto para el ANMI San Matías (PML), concertado y aprobado por los beneficiarios directos (comunidades indígenas y estancias ganaderas) se efectuaron 32 talleres comunales, 2 talleres regionales, 2 talleres con ganaderos y diversas reuniones con las instituciones y organizaciones locales. El desarrollo del PML se basó en el respeto a las organizaciones comunales, protagonismo del actor local en la toma de decisiones, fortalecimiento de las organizaciones e instituciones para el manejo integral y adaptativo del recurso e incremento de ingresos mediante un mayor protagonismo de los actores directos (comunarios y ganaderos) en la cadena productiva. A pesar del complejo mosaico humano del ANMI San Matías, extensión del área, dificultades de acceso y escaso avance en el saneamiento de tierras, se ha logrado concertar de manera participativa el sistema de gestión para la conservación y aprovechamiento del Lagarto y planificar la primera cosecha para el año 2008.

# Spectacled Caiman (*Caiman yacare*) Integrated management plan in the Tacana III TCO (Original Communitarian Territory), aimed for the conservation and sustainable use of the natural resources of the indigenous people in Beni Department, Bolivia.

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**SUMMARY:** The management plans represent an alternative for improving the National Program of Conservation and Sustainable Use of Spectacled Caiman (PNASL) in Bolivia, they become essential tools for generating shared-responsibilities, giving active participation to the indigenous people and representing an opportunity for obtaining permanent information. In the context of the National Program of Sustainable Biotrade, the 'Asociación Boliviana de Conservación', Central de Pueblos Indigenas del Beni, and the Organización de Comunidades Indigenas Tacanas worked together to establish the bases of the sustainable management plan of *C. yacare* in the Tacana III TCO, member of the PNASL since 2004. Between December 2006 and February 2008, biological and social activities combined to elaborate a concerted Management Plan for the spectacled caiman in the Tacana III TCO. An integrated management proposal generated from a local perspective, that includes harvesting and ranching activities with an adaptive management view, focused in the conservation and sustainable use in different levels (economical, ecological, and social). This process gives interesting experiences to be used in the PNASL and new options for the sustainable development and integrated communal management of the indigenous natural resources.

# Status of American Crocodiles, *Crocodylus acutus*, in Santa Rosa and Las Baulas National Parks, Guanacaste Costa Rica

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**ABSTRACT:** *Crocodylus acutus* populations in Costa Rica are reported as healthy by the International Union for the Conservation of Nature and Natural Resources (IUCN) Crocodile Specialist Group (CSG), however little census work has been undertaken. We conducted daylight surveys and nighttime spotlight surveys within Santa Rosa National Park (Nancite and Naranjo estuaries) and Las Baulas National Park (Tamarindo estuary) in 2007. The majority of crocodiles encountered were juveniles (0.5-1.0 m) and subadults (1.0-2.25 m), with few adults (>2.25 m). Encounters rates for Las Baulas National Park and Santa Rosa National Park were 1.68 and 3.39 crocodiles/km respectively. Encounter rates include all crocodiles sighted and observed over all surveys. Two nests were located in the Naranjo estuary system. Hatchlings were encountered in Tamarindo, but no nests were located. We collected tissue and blood from *C. acutus* in Santa Rosa (27 individuals) and Las Baulas (40 individuals) to investigate the conservation genetics of this species along the Pacific coast of Costa Rica. We will determine the genetic structure, extent of gene flow and relatedness in Guanacaste and the Osa Peninsula. Local population estimates and genetic information will enhance the conservation and management of *C. acutus* in Costa Rica.

# Survival and Growth of the American Crocodile (*Crocodylus acutus*) from the nests of the rivers Negro and Santa Rosa, Machiques de Perijá, Venezuela

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**ABSTRACT.** During May of 2007, 43 offspring of American Crocodile entered into Vereda del Lago Park. They were born as a result of two nests incubated *ex-situ* in a house in the Bari of Senkay indigenous community. The smallest and largest hatchlings when arrived to the Park were 230 and 300 mm (TL), respectively. Their weight was between 70 and 200g. The crocodiles are being kept in plastic tanks with a constant change of water (every 4 days). The crocodiles are been fed with cow hearts, cow kidneys, chicken stomach and fish. After 10 months in captivity, only two individuals died (95 % survival rate). The smallest and largest size reached by these juvenile crocodiles was 335 and 560 mm, respectively. They have also reached a weight in grams between 245 and 664.

**RESUMEN:** Durante mayo del 2007, 43 caimancitos ingresaron al Parque Vereda del Lago, ellos nacieron como producto de sendas nidadas incubadas *ex-situ* en una casa de la comunidad indígena Bari de Senkay. El menor y el mayor tamaño (LT) de estos neonatos al llegar fue de 230 y 300 mm respectivamente. El peso en gramos varió entre 70 y 200. Los cocodrilos se mantienen en tanques de plástico con recambio constante de agua (cada 4 días) y alimentados con corazones de res, riñones de res, estómagos de pollo y pescado. Luego de 10 meses de cría en cautiverio, únicamente murieron 2 individuos (95 % de sobrevivencia). El menor y mayor tamaño alcanzado por estos jóvenes caimanes fue de 335 y 560 mm respectivamente. A su vez han alcanzado un peso en gramos que va desde 245 a 664.

# Television documentaries: Tools for the conservation of crocodiles

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**ABSTRACT:** Are we able to invest the same efforts in the recovery and protection of the false gharial as we do, for example, with the orangutan?
How the local people who coexist with these species could be involved to participate and make possible their conservation?
One of the main aims of the Biology of the Conservation is to establish some priorities about specific places and "taxa" where effective strategies for conservation can be developed. However, most of the time, the establishment of political and financial efforts for the conservation, can only be seen in some "emblematic species ".
The Balikpapan Orangutan Survival Foundation, born under the economic protection of some TV channels of Japan has allowed, by protecting orangutans, the avoidance of the mass destruction of thousands hectares in the jungles of Borneo (Kalimantan-Timur). Currently, in the medieval fort of Ranthambore (Rajasthan) thanks to the fees, canons and taxes collected from TV's of countless countries, the reborn of the Tiger Project is again taking shape.
The reduction –or extinction- of some crocodiles in Indonesia could be a dramatic result for the health of many rivers and wetlands, but the conservation efforts have found no social or institutional response, with some exceptions.
Can the documentaries help in the protection of crocodiles?

# Verification of the Skeletochronology Technique in the Broad-snouted Caiman, *Caiman latirostris* (Reptilia, Alligatoridae)

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**ABSTRACT:** Skeletocrhonology is the name of a technique used to estimate the age in different extant and living vertebrates based on counting bone growth rings that are expressions of histological changes in the rates of osteogenesis influenced by external factors. Bone diameter growths by deposit of successive layers of new bone from the center to the external border, usually named as bone marks, that in an histological cross section looks like rings. This technique has been successfully applied on fishes, amphibians and reptiles. In crocodiles, the information about the applicability of this technique is scarce, and particularly in caimans nothing is known. To determine the actual age through this method, two main objectives must be achieved: the observation of histological bone cross sections in order to investigate the cyclic growth represented by bone marks, and to determinate if each growth mark corresponds exactly to one year old of the specimen. In the present work, details of the technique and preliminary information are presented on the use of this in phalanxes of South American broad-snouted caimans for the first time, showing that *Caiman latirostris* presents bone growth rings similar in structure to those observed in other reptiles and amphibians.

# Vocalizations in juveniles of *Caiman latirostris* (Daudin, 1801) I: acoustic structure and individual discrimination.

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**ABSTRACT:** Acoustic communication is essential for social behaviors such as parent-offspring interactions. Crocodilians show a sophisticated repertoire of acoustic signals that vary within a wide range of contexts. Juveniles start to vocalize before they emerge from the egg and continue after hatching. These vocalizations usually elicit maternal care and induce crèche cohesion. The aim of the study was to describe the acoustic structure of *Caiman latirostris* juveniles' calls and to determine the possibility of individual discrimination. The analysis of 100 notes from seven individuals showed a basic acoustic structure of strongly frequency modulated sounds usually composed of a main frequency band (1036.8 ± 91.9 Hz) associated to several side-bands (range: 5-20). The note can be divided into two temporal segments: an ascending frequency modulation that continues into a descending one. Sixty percent of individuals show both parts of the signal. A Discriminant Analysis of all the vocalizations showed that individual discrimination could be attained in 89 % of the cases. These results can be related to juveniles' age, size and/or sex, to them being part of different clutches or to environmental temperature variations.

# Vocalizations in juveniles of *Caiman latirostris* (Daudin, 1801) II: acoustic structure and comparison with other crocodilians.

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**ABSTRACT**: Crocodilians show a sophisticated repertoire of acoustic signals that vary within a wide range of contexts. Juveniles start to vocalize before they emerge from the egg and continue after hatching, and these vocalizations usually induce crèche cohesion and elicit maternal care. The results of the analysis of 100 notes from seven individuals of *Caiman latirostris* were compared with published data on similar vocalizations from other eight crocodilian species. Most crocodilians share a basic acoustic structure: strongly frequency modulated notes usually composed of a main frequency band associated to several side-bands. In addition, calls can be divided into two temporal segments: an ascending modulated frequency that continues into a descending one. Not all species showed this condition. A Discriminant Analysis of the calls showed that main frequency and acoustic energy appear as the most significant variables. Furthermore, the arrangement of the species in the dispersion diagram loosely followed their phylogenetic relationships. The mentioned differences in sound characteristics could be explained by age and size of the sampled individuals for the other species, their habitat and ecology, or the context of the sampled vocalizations.

# Zoocriadero Puerto Pizarro: An alternative for conservation of American Crocodile (*Crocodylus acutus*) in Peru

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**ABSTRACT:** The American crocodile is the only member of family Crocodylidae in Peru. The Peruvian government categorized the species as critically endangered because their wild populations are decreasing by the habitat loss, illegal hunt and other anthropogenic activities. Its distribution in Peru is restricted to mangrows ecosystems in the Northwest region; however it has been reported its disappearance in the Chira River. In 1997, the Crocodile Specialist Group determines that not more than 6 reproductive couples were found in Tumbes River.

In 1996, the Peruvian government created Zoocriadero La Tuna Carranza, in Puerto Pizarro (Tumbes) for the conservation of the species. Currently this centre has 16 breeding pools and around 300 of animals, grouped according the biological and size cycle stage. Despite the deficiencies of infrastructure has been achieved the increased of the number of individuals by natural reproduction, although with some problems. Currently with the participation of Universidad Peruana Cayetano Heredia has been initiated the evaluation and health control of all the animals, and an artificial incubation program is going to be developed.

**RESUMEN:** El cocodrilo americano es el único miembro de la familia Crocodylidae en el Perú. El Estado Peruano la categoriza como una especie en Peligro Crítico debido a que sus poblaciones silvestres están decreciendo por la pérdida de hábitat, caza ilegal y otras actividades antropogénicas.

La distribución de esta especie en el Perú está restringida a los ecosistemas de manglar en la región noroeste, sin embargo se ha reportado la posible desaparición en la cuenca del río Chira. En 1997, el Crocodile Specialist Group determinó que no más de 6 parejas reproductivas estaban presentes en la cuenca del río Tumbes.

En 1996 el Gobierno Peruano crea el Zoocriadero La Tuna Carranza, en Puerto Pizarro (Tumbes) para la conservación de la especie. Actualmente éste cuenta con 16 pozas de crianza y alrededor de 300 ejemplares, agrupados según la etapa de su ciclo biológico y tamaño.

A pesar de las deficiencias de infraestructura se ha logrado aumentar el número de animales por reproducción natural, aunque con ciertos problemas. Actualmente con la participación de la Universidad Peruana Cayetano Heredia se ha iniciado la evaluación y control sanitario de los ejemplares, y se desarrollará un programa de incubación artificial.

## Current status of the species in Peru

Tumbes' crocodile or American crocodile (*Crocodylus acutus*) is the only species of the family Crocodylidae in Peru; this species is categorized in Appendix I by CITES and as Critically Endangered by the Peruvian legislation (D.S. 034-2004-AG); these because their wild populations are declining due illegal hunting and habitat loss caused by anthropogenic activities (Thorbjarnarson, 1989; Ross, 1998).

The distribution of the species in Peru is restricted to mangrove ecosystems in the Northwest region of the country, from Chira River at the South to Zarumilla River at the North (Medem, 1983) However recent evaluations reported the possible disappearance of the population in Chira River basin (Escobedo and Mejia, 2003), also in 1997 members of the Crocodile Specialist Group identified the presence of no more than 6 breeding couples in Tumbes River basin (Escobedo and Mejia, 2003).

## History of the Zoocriadero Puerto Pizarro

In 1996, the Fondo Nacional de Desarrollo Pesquero (FONDEPES), a Peruvian Government Agency created a breeding center for the species in the locality of Puerto Pizarro in the Departamento of Tumbes. This center started with an experimental module of 220 $m^2$, with pools of concrete for the animals. For this phase two goals were proposed, the first was to obtain individuals for reproduction and the other to evaluate the captive adaptation of these animals. For this reason, the center collected around 40 specimens, from different sizes and genders, captured in the wild and also some of them were donate by fishermen who captured them accidentally. In 1999, the area of the center was increased to 1800 $m^2$, and more facilities were built to complete the reproductive cycle. The center started having reproduction successful since 2002, and in 2004 the center was extended to 8 hectares, changing its name to Zoocriadero Puerto Pizarro from 2005.

## Current status of the Zoocriadero Puerto Pizarro

Currently the breeding center has 267 animals distributed in 14 pools; they are grouped according the age and size (Table 1). The items used to feed the animals are fish and chicken, and these are provided at different frequencies per week and percentages, according the total biomass of each enclosure. The neonates are feed with pellets of a mixture of small crustaceans and fish, supplemented with chicken liver.

The reproduction is not controlled; the adult females are maintained in two separate groups, each one with one adult male permanently. Despite some lack of basic services, as electricity and full-service water, the center is increasing every year significantly the number of individuals in captivity through natural reproduction; but without technical support, the center is obtaining a larger amount of male neonates, which is why an artificial incubation program is going to start this year. Other problem observed with neonates is malformations, but in very low percentages. The facilities are currently available for tourist visits.

**Table 1.** Distribution of individuals in pools according the phase of the life cycle.

| Phase | Phase duration | Nº of animals |
|---|---|---|
| Neonates | 0 – 1 year | 11 |
| Youngs 01 year old | | 40 |
| Youngs 02 year old | | 25 |
| Youngs 03 year old | 1 – 7 years | 73 |
| Youngs 04 year old | | 40 |
| Youngs 05 year old | | 45 |
| Sub-Adults | 7 – 12 years | 19 |
| Adults | 12 to more years | 14 (02 ♀ and 12 ♂) |
| **Total** | | **267** |

## Prospects for the Future

Currently with the initiative and collaboration of the Laboratory of Wildife of the Faculty of Veterinary and Zootechnics of the Universidad Peruana Cayetano Heredia, a health evaluation of the animals have been done, including physical exams, and hematological and parasitological analysis, but this is the first step for improving the management of the center. For this reason since 2007, the Universidad Peruana Cayetano Heredia and FONDEPES have an agreement to work together to develop the center and help to conserve the species.

## Current objectives

- To develop the management plan for breeding center.
- To develop a reproduction program to achieve a higher percentage of hatching and control the proportion of sexs in neonates, and increase the number of individuals in captivity for future reintroduction.
- To obtain more information of biology and ecology of the species to support an appropriate strategy for their conservation.
- To establish a health and preventive medicine program for the animals of the breeding center collection.
- To obtain more health information of the species in captivity.
- To realize a genetic evaluation of the animals of the breeding center.
- To establish environmental education programs to promote conservation of the species, especially with local people.

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# Workshop Reports

## Workshop 1: Sustainability criteria (biological, social, economic) for the success of national management programs (Perran Ross)

Participants: about 35 Working Meeting members.

Objective

The objective of the workshop was to assess the economic and social sustainability criteria to be considered to start and -achieve in time- a successful management program of crocodile species from a “national” program point of view.

Methodology

Discussion was conducted sequentially in English and Spanish with assistance and great good will and tolerance from all participants. The chairman addressed a short introduction to all participants and led a guided brainstorm in which the main ideas were considered and written; all participants were also asked to write down on individual cards the main criteria they considered crucial for the purpose of establishing national management programs. Both of them were considered by a small reviewing group who summarized the workshop in the results shown below. Despite of the short time to discuss such a complex issue, the main criteria were defined and some measurement indicators were proposed.

Results

Since sustainable Management Programs usually involve different aspects and several stakeholders, they may be considered as a chair sustained by three-legs including: Economic, Social and Environmental issues.

=> SOCIAL because many actors are involved; for instance in the Bolivian case, indigenous communities play a major role as well as peasants, cattle owners, tanneries, government, NGO’s and scientific bodies. A successful Management Program has to be considered as such by all actors.
=> ECONOMIC because it has to be fair and generate income for all actors based on transparent operations.
=> BIOLOGICAL because it has to be sustainable and verifiable;

Undoubtedly, all 3 aspects must be integrated in the development of the Program to be SUCCESSFUL.

But first of all, some basic principles should guide the establishment and operation of a Management Program:

=> Transparency
=> Participation of all Stakeholders
=> Equal Power.

If any of them is hindered by one or more actors, it would pose an obstacle to the development of the Program as a whole.

Even though each country possesses its own particularities, general applicable criteria may be considered for national management Programs, as described in Table 1.

But, while criteria are useful to describe the main Economic, Social and Biological issues involved, they are valid to evaluate success of a program only when accompanied by measurement indicators; though in some cases it may be difficult to establish concrete ones. Table 1 shows proposed measurement indicators for each criterion, which may be adjusted to the reality of each country and to the status of advancement of the Program.

**Table 1.** Economic, Biological and Social Criteria for National Management Programs

| **ECONOMIC** | **PROPOSED QUANTIFIABLE INDICATORS** |
|---|---|
| Common vision for economic development | Vision and future goals of stakeholders established. |
| Tools for economic management | Regulations and materials in place. |
| Distribution of benefits (first links of production chain) | Established distribution of benefits especially to local communities. |
| **BIOLOGICAL** | **PROPOSED QUANTIFIABLE INDICATORS** |
| Consider traditional knowledge | Knowledge provided by local actors has been translated into program guidelines. |
| Sustainability criteria must be quantifiable | Appropriate Biological and Ecological Indicators clearly developed and communicated to the actors. |
| Articulate with other management alternatives & species | Analysis of other alternatives of sustainable management (biologically-based) including their conclusions & interactions among them in order to conserve their habitat. |
| **SOCIAL** | **PROPOSED (QUANTIFIABLE?) INDICATORS** |
| Consensus among all actors establishing clear objectives | Guidelines established signed by all actors |
| Understanding of the market by all actors | Training sessions developed and records |
| Education to all actors (sustainability and win-win strategy; create a mission-oriented role) | Training sessions developed and records |
| Truthful information flow to all actors | System developed for information flow among all actors. |
| Applied to local reality | Guidelines of the Program have considered the particularities and constraints of the management areas (e.g. accessibility, cultural issues, climatic and geographic factors, etc.) |
| Assure that program does not affect social structures | Representatives of communities and representatives of all actors are clearly and legally defined, as well as their scope of action and decision power. |

Conclusions

National Management Programs are complex and to assure their sustainability, they should involve the main stakeholders as principal actors in the frame of three guiding principles of Transparency, Participation and Equal Power. It has to be considered that a Sustainability Program is not a Product but a Process, not a destination but a road to walk through.

The goal is not to comply with all criteria at the beginning but instead consider them all, try to measure them in a realistic way and adjust them in a continuous improvement process.

**Workshop 2: Local organizations in conservation and management of crocodilians (Silvia Ovando)**

Among the topics approached in the workshop, for their high social content, the participation of the indigenous sector was very significant because the Bolivian Yacare Program has a great regional representation of different indigenous communities and indigenous territories participating as key actors in the management of *Caiman yacare*.

Among the most relevant conclusions we can remark that this type of Programs should be managed in an integral way, looking for the conservation of the species, but also assuring the social sustainability, with established programs for the Fair Distribution of Benefits among all the actors of the productive chain.

Another important point to highlight, it was the indispensable coordination that should exist between the State and the civil society, with clear rules that allow a sustainable use of crocodilians, implementing national and international rules.

Finally the experiences of countries with strong participation of local organizations in the crocodile's management were highlighted, and we conclude that it is very important to consider that any management program have high-priority social particularities to be assessed.

**Workshop 3: Aspects affecting the sustainability of trade (Don Ashley)**

Don Ashley (Chairman), John Caldwell and Alvaro Velasco (Rapporteurs), Jerome Caraguel, Hideki Sakamoto, Mariana Paz, Ana Cristina Paz, Sebastian Paz, Heidi Abadia, Matthew Shirley and Laurie Cotroneo.

Points discussed:

1. Clarify CITES Guidelines for issuing:
    a. Export and Import permits for biological specimens, crocodilian parts (Blood, biopsies, etc).
    b. Stream line the re-export of manufactured items.
2. Reauthorize California for future trade in crocodile items (if the law is changed will be necessary to review the crocodile trade).
3. After many actions to eliminate the negative information shows in the airports, we

need to identify other ways to change the information displays. Evaluate using positive sustainable examples to replace the information.
4. Fully implement personal effects CITES Resolution (Some Parties reduce the exempt items and is necessary to find a mechanism to ensure that Parties apply the CITES Resolution according to the original recommendations).
5. Provide customs and port inspectors training materials on CITES and sustainable use (The Secretariat offers to all Parties a CD of customs training course).
6. Encourage research on hide and leather quality
7. Provide CSG Industry Committee updates on priorities for actions to be implemented.
8. CSG to act as a clearinghouse to ensure that trade is legal, sustainable and verifiable and to identify ways to ensure that the trade remains like this.
9. Evaluated the situation in the EU community regarding the imports of meat from wild harvest.

**Workshop 4: Management Plans as conservation tools in Latin America (Alejandro Lariera).**

Coordinated by Alejandro Larriera, assisted by Patricia Amavet, Karina Bello, Sergio Medrano and Carlos Piña.

By definition, managing is to handle, to rule, to direct something trying to achieve by mean of an intervention, a previously defined objective. So we can say that every activity aimed to conserve and/or to obtain a benefit from the crocodilians populations do constitute itself a “Management Plan”.

The sustainable use programs on wildlife

The world strategy for the conservation of nature from 1980, recommended in its article 3°, and as a relevant matter: “to ensure the sustainability of any kina of utilization of species or ecosystems”. In 1991, the document “Care the earth”, do define that sustainable use is only referred to the “utilization of the renewable resources, when the utilization level do not overpass its renewal capability”. Today the benefits of such activities are out of discussion in terms of the “economic valorization of the ecosystems”, “incentives for the conservation by the local inhabitants”, and “generation of genuine income for the producing countries”.

As we’re talking here about crocodilians, we do basically recognize three different mechanisms for its utilization and conservation (Hunting, Ranching and Farming). The strengths and weakness of everyone are discussed openly during the workshop.

Hunting

It is clearly the most economic system to produce crocodilians products.

It is the system that provide most direct benefits to the local inhabitants that used to live in contact with the wild populations.

It is the most difficult to control and also the system that more risks present if there is not an appropriate design.
The harvest in a hunting program are usually around a 5% to a 15% of the estimated adult population for every year.



Ranching

Do require investments that still are uneconomic for many abundant and low value species.

It is easy to control and also is beneficial for the local inhabitants who are responsible of the nests identification and eggs harvest.

By far, is the system that can guarantee the security for the wild populations. It is actually possible to harvest  up to the 50% of all the eggs in the wild, with no additional measures of conservation.



Farming

It is the more expensive system in terms of money investment and time.

It is easy to control, at list in productive terms, and from a commercial point of view it is the one that in some way can guarantee more accurately can predict the production level.

It is uneconomic for the great majority of the species, and clearly it is the one with less benefits offer from a conservationist approach.



Conclusions

After a productive interchange of ideas and opinions, where the strengths and weakness of every system were analyzed, it was concluded that by far, if it is well designed on abundant populations, hunting programs are the best way to produce an incentive for conservation of the ecosystems involving the local inhabitants. On the other hand, ranching programs still have a remarkable effect as incentives, when the local inhabitants gets more and more involved in other activities more that simply identifying the nesting areas. Finally, it was very clear that farming programs are the weaker ones, in terms of incentives for the conservation of the ecosystems, and that must look for different strategies in order to improve its benefits on this issue.

It was also clear that no matter which system you're planning to develop, a well designed management plan is crucial for the success of the all program, and should have a strong structure from the beginning, mainly with regard to the objectives, benefits for the ecosystem and the local inhabitants. At the same time should be based on the adaptive management strategy, in order to modify or re-direct the activities, as the information on the impact of the activity is collected.

**Workshop 5: Report of CSG Veterinary Science Group (Paolo Martelli)**

Agenda:

1. Restate broad mission of this group
2. Identify areas of veterinary science and medicine that require work
3. Examine how to best use the resource brought about by the new website to the benefit of this group and its clients

There were 25 participants, 13 of which vets, others farm managers, biologists from 10 countries

Mission of the group: “Advance crocodile veterinary medicine and science”

1. Serve clients:
    - Animals under human care: farms and zoological or educational institutions
    - Biologists and researchers that require veterinary procedures: sampling, anesthesia, surgery
    - Conservation partners and organizations: mortalities, population health status”

2. Areas of veterinary science and medicine that require work:
    - Immunology acquired and innate
    - Response to stress, monitoring, physiological effects, impact on health
    - Emerging diseases and biosecurity, including at international levels
    - Reviewing health screening in the context of reintroduction following IUCN-SSC reintroduction specialist group
    - Specific veterinary training of managers and veterinarians in various areas. Specific for crocs and adapted to the situation
    - Medical and husbandry training of the animals

3. Use of new website:
    - Veterinary procedures (general exam, sampling, medication), Samuel Martin Terry Cullen
    - Post mortem procedures and reports, Paolo Martelli
    - Histology image data base, Paolo Martelli
    - Compilation of (anecdotal) veterinary information including contra-indications and cautionary warnings: ad hoc, needs a space
    - Literature resources, Kent Vliet
    - Imaging database and techniques, Charlie Manolis
    - Anesthesia, Sam Seashole
    - Introduction techniques for new animals in captivity, Samuel Martin Terry Cullen

4. Others:
    - Good disclaimers, passwords and access Diego Forrisi
    - IP and copyright waivers for material provided to CSG site.
    - Feedback from users to allow improvement of service
    - Procedures to contact vet group members: send to CSG chairman who’ll distribute as best
    - Offer to start a once off or seasonal course dedicated to crocodile medicine in the South American region Oscar Rendon

| Name | Contact | Areas of interest/competency | Country |
|---|---|---|---|
| Carla Barriga | carlabarriga@yahoo.com | Parasites,sampling,most commons pathologies, behaviour | Bolivia |
| Adam Britton | abritton@crocodilian.com | Immobilization, immunology | |
| John Calderon | jhoncalderon@gmail.com | Chlamydia, hypoglycemia, neonatal mortality | |
| Glenn Collard | papacollard@terra.com.br | Emerging diseases caiman | Brazil |
| Terry Cullen | tcullen@gna.net | Broad interest esp. virology, nutrition prophylaxis | USA |
| Roberto Elias | relias@upch.edu.pe | Clinical, husbandry, immobilization | Peru |
| Diego Forrisi | diegoforrisi@yahoo.com.ar | Parasites, ethology, | Argentina |
| Sally Isberg | sally@crocfarmsnt.com | Production inneficiences on cocrodile farms (genetic, nutrition,health, bioscounty,,)geneticist, captive management access to saltwater crocodile resources/pedigree | Australia |
| Angie Kirby | angelamelodykirby@yahoo.com | Captivity husbandry, reproduction and conservation | USA |
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| Samuel Martin | s.martin@lafermeauxcrocodiles.com | Broad interest , technics applied to research. | France |
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| Luis Sigler | luis@dwazoo.com | Conservation medicine | USA |
| Cesar Soares | Cesar.soares@ibama.gov.br | Clinical Disease | Brasil |
| Myriam Venegas | dracocodrilo@hotmail.com | Diagnostics, stress, genetics | Panama |
| Kent Vliet | Kent.vliet@zoology.ufl.edu | Husbandry, endocrinology, parasitology, infectious disease | USA |

**Workshop 6: CSG Zoos and Community Education Thematic Group (Kent A. Vliet)**

A small group of people interested in the Zoos and Community Education thematic group met on Wednesday afternoon, June 4, to further discussions of the group's goals and strategies. The CSG ZooEd group communicates by means of a listserv that currently has about 45 members, not all of whom are CSG members. We are very interested in building membership in this thematic group and encourage anyone interested in zoos and/or education to contact me at kent.vliet@zoology.ufl.edu. This group includes a diverse group of people with diverse interests including zoo biology, captive breeding, education in all forms, community-based conservation, and aspects of human-crocodile conflict.

**Education.** Our approach to facilitating crocodilian conservation through education is to provide resources for educators relevant to crocodilian biology, "aware-raising", conservation status, sustainable utilization, etc.. We will make these available through the CSG website. We are currently collecting prepared educational materials – lesson plans, activities, exercises and data sheets, presentations, booklets, etc., that we can post for download on the site. If you have any materials that would be appropriate for this, please contact me. We will also prepare our own presentations, species accounts, image banks, posters, etc., for this site. Dr. Adam Britton has agreed to oversee the production of species account signage and posters which can be used "as is" or can be modified for individual educational uses. The educational materials collected to date are in English and related to the American alligator. We are very interested in collecting materials in other languages and for other species of crocodilians to make the site as useful as possible internationally.

**Community education** is clearly exceptionally important and the group wishes to encourage and support these activities. But how? We have not yet developed the strategies to be of most assistance. There are clearly numerous initiatives in community education being undertaken by members of the CSG. We would like to hear about these initiatives and discuss how this group could facilitate these activities.

**Zoos.** The group wants to facilitate links between *ex situ* resources in zoos and in situ crocodilian projects. We are establishing liaisons between our group and regional zoo associations to further communication and cooperation. We recognize that activities in zoos can be very effective models for the conservation of critically endangered crocodilian species, those species for which sustainable utilization is not currently feasible. Zoos can participate in fund raising activities linked to endangered crocodilians. Grahame Webb highlighted a prime example of this during one of the CSG socials at the Bolivian meeting by acknowledging the tireless efforts of René Hedegaard, from the Krokodile Zoo in Denmark, for fund raising efforts at his own zoo as well as for encouraging donations from other European zoos. One part of these efforts include establishing agreements with several European zoos to provide continued annual donations to the Mabuwaya Foundation's community-based programs for *Crocodylus mindorensis* in the Northern Sierra Madre mountains of the Philippines. We discussed many examples and strategies for fundraising that the group can encourage among zoos. So, we will work to stimulate much more of these sorts of activities within zoos world-wide as well as post many of these examples of effective fund-raising strategies on the CSG website.

We are in agreement that zoos and education are intricately intertwined with one another and that our thematic group is an appropriate grouping and should not be split into two thematic groups. We will be coordinating our various activities discussed in the meeting through the ZooEd listserv. Lastly, preliminary discussion was made of organizing an international meeting on the importance of zoos and education in crocodilian conservation in the next two years.

## IUCN/Species Survival Commission

The Species Survival Commission (SSC) is one of six volunteer commissions of IUCN - The World Conservation Union, a union of sovereign states, government agencies an non-government organizations. IUCN has three basic conservation objectives: to secure the conservation of nature, ans specially of biological diversity, as an assential fountadion for the future; to ensure that where the earth's natural resources are used this is don in a wise, equitable and sustainable way; an to guide the developement of human communities towards ways of life that are both of good quality and in enduring harmony with other components of the biosphere.

The SSC's mission is to conserve biological diversity by developing nd executing programs to save, restore and wisely manage species and their habitats. A volunteer network comprised of nearly 7,000 scientists, field researchers, government officials and conservation leaders from 188 countries, the SSC membership is an unmatched source of information about biological diversity and its conservation. As such, SSC members provide technical and scientific counsel for conservation projects throughout the world ans serve as resources to governments, international conventions and conservation organizations.

IUCN/SSC also publishes an Action Plan series that assesses the conservation status of species and their habitats, and conservation priorities. The series is one of the world's most authoritative sources of species conservation information available to nature resource managers, conservationists and government officials around the world.

IUCN
The World Conservation Union

SSC
Species Survival Commission

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BOOK REVIEW

# Investigating the Effects of Different Distance Learning Modalities on Student Academic Performance in Technology Education Programs

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Academic performance, assessment, asynchronous, blended learning, synchronous, technology education

**ABSTRACT**
The rise of online distance learning has necessitated investigations into its effectiveness across various educational contexts. This study examines the academic performance of students enrolled in technology education programs across three distinct learning modalities: synchronous, asynchronous, and blended. The study aims to determine if significant differences in academic performance exist among these modalities and how they vary across different assessment types (formative, performance-based, and examination). Forty-five students were equally divided into three groups, each experiencing one of the learning modalities. A non-parametric Kruskal-Wallis test was employed to analyze the academic performance data. The results indicated significant differences in student performance across the three learning modalities, particularly in formative assessments where blended learning yielded significantly higher scores compared to synchronous and asynchronous modalities. The superior performance in the blended learning modality was consistent across performance-based tests and examinations. Notably, the highest academic performance was observed in the students who engaged in blended learning and were assessed through performance-based tests. Asynchronous learning consistently resulted in lower performance across all assessment types. These findings suggest that blended learning may be a more effective pedagogical approach in technology education, facilitating enhanced academic performance compared to purely synchronous or asynchronous online learning environments.

## 1. INTRODUCTION

### *1.1. Overview*

Distance learning (DL) is a type of education that enables students to study from the comfort of their own homes or any location in the world. Students can choose between synchronous and asynchronous learning modalities. Nowadays students choose online classes not because they will learn much from them but for convenience which can fit into their hectic schedules.

Among the known learning modalities, the synchronous learning modality is popular among the young ones since feedback is given immediately; however, it also has its drawback, especially potential unstable internet connectivity. Synchronous learning offers real-time feedback and interaction, which enhances student engagement (Lim, 2017; Watts, 2016). Students in synchronous online classes can communicate instantly, reducing the perceived distance

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between them, their peers and the lecturer (Francescucci & Rohani, 2019). Nonetheless, synchronous distance learning requires adequate internet bandwidth. When internet connections are unstable or down, learners may lose out on critical information, unless there is an alternative way such as a backup connection. Students may become frustrated with scheduling if they are required to be online at a specific hour (Falloon, 2011), just like what other higher educational institutions are doing.

Another learning modality is the asynchronous online modality. Brierton et al. (2016) mention that with asynchronous online discussion boards, students feel more at ease and have greater freedom to express themselves. They are not under any obligation to answer immediately to queries or comments, and they have more time to consider how they would respond. Asynchronous distant learning classes can open new avenues for class development and delivery. New responsibilities for developing and delivering asynchronous distance learning courses can be identified because students and teachers can be separated in both time and space. Physical links are not needed, just a computer and the internet (Martin & Parker, 2014). Some advantages can be offered by this learning modality, but it could be difficult for learners who lack self-motivation. Students must be self-disciplined and must do what is asked of them however they can in their own learning style. Because social connections with instructors and peers are limited, learners may feel separated from the learning environment (Hrastinski, 2008).

Additional advantages of these distance learning modalities can be observed in the fact that learners' active participation can be promoted in the synchronous learning modality whereas the asynchronous online learning environments allow students to learn at their leisure (Lin & Gao, 2020). In comparison, Motteram (2011) claimed that synchronous tools are better for dealing with the social components of education while asynchronous tools are better for dealing with the academic parts. As we mentioned earlier, synchronous online learning allows students to communicate with their peers and instructors in real-time. The advantages of both online synchronous and asynchronous classes may be combined in a blended learning environment. This blended learning modality uses an application or a learning management system (LMS) platform that teaches students how to use their cellphones more intelligently. Hrastinski (2019) states that blended learning is used to describe other blends, such as integrating diverse instructional methods, pedagogical approaches, or technologies.

Based on the gathered literature, blended learning benefits both students and teachers since it enables them to be more versatile in their knowledge. This concept of blended learning is timely because there is a tremendous push in education today to have students exhibit their work online (Moore & Kearsley, 2011), ensuring that online learning will be just as effective as conventional learning in educating students.

The current study investigates synchronous, asynchronous, and blended learning modalities focusing on their effects on students' academic performance in a technology education program and also determines which modality yields better performance.

### *1.2. Objective and significance of the study*

This research study focuses on the effect of three learning forms on students' academic performance within technology education. The study intends to explore students' performance within three assessment types: formative assessment, performance tasks, and examinations. By comparing student performance across various activities, the research focuses on identifying the learning modality where the students demonstrated better performance in technology education, thereby deepening existing understanding of effective teaching methods and learning outcomes as well as related theory.

This study is significant given its contribution to informed decision-making in education, particularly in the formulation of educational policies and intervention programs aimed at ensuring that students can receive effective technology education no matter what learning modality is used. It is of utmost importance in modern-day educational settings where various learning modalities are adopted to ensure that the learning process is maintained during unavoidable disruptions such as natural calamities or disease outbreaks. The researchers also aim to improve the capacity of technology education programs to withstand the challenges of varying learning modalities while achieving equality in learning opportunities for all students.

### *1.3. Limitation of the study*

This study only examined the performance of 45 randomly selected technology education students comprising 1st year, 2nd year, and 3rd year students at a selected state university in Davao Region, Philippines, for the academic year 2021-2022. The respondent's performance was evaluated based on the formative assessment, performance task,

and examination results during the 1st semester and 2nd semester with synchronous, asynchronous, and blended learning modalities.

## 2. LITERATURE REVIEW

Distance education, as Moore (1993) noted, can be described as any situation of learning where teachers and learners are not in the same place and there is a technology that facilitates teaching. Because of this distance, there is a need to employ a number of technological devices in order to communicate as well as disseminate learning content. There are several ways in which distance learning can be put across, each of which has their own distinct features as well as pedagogical implications. Subsequently, the rapid evolution of online distance education has led to growing interest in its effectiveness across a variety of educational contexts (Allen & Seaman, 2014). Much research work has been conducted to examine the effect of learning modalities like synchronously, asynchronously and blended on academic performance (Bernard et al., 2014). On the other hand, literature exploring how effectively these modalities work in the context of technology education programs is still scarce. Consequently, this study fills in the existing void by comparing how various forms of modalities influence academic performance among students pursuing a technology education with assessment types as a source of variability.

As defined by Hrastinski (2008), asynchronous learning is a user-friendly mode of study in the sense that coursework and other course-related activities are available to students at their preferred time and speed. This mode of study is frequently characterized by advanced learning aids such as recorded lectures, web-based classrooms, and even emails without requiring the students' or teachers' physical or temporal presence (Anderson, 2008). Despite being flexible in approach, asynchronous learning may also mean that higher levels of self-control and management of time would be required from the students enrolled (Bernard et al., 2014). In contrast, learning in real-time also known as synchronous learning means the learning takes place in a classroom setting as in normal schooling (Hrastinski, 2008). Synchronous methods of instruction employ tools such as video connectivity, telephone chat, and online classrooms thus allowing participants to share ideas and carry out tasks at the same time (Simonson et al., 2006). Synchronous learning can help build a community of learners and allows immediate questions and discussions, however, it is usually structured such that everyone is called to be present at the same time which may reduce flexibility (Bernard et al., 2014).

Blended learning as Garrison and Vaughan (2008) contend encompasses some attributes of both synchronous and asynchronous modes of learning. This type of learning model is intended to use the benefits of both methods by incorporating traditional learning and conducting classes over the internet (Graham, 2006). Blended learning can allow for customization and flexibility but at the same time making it possible for interaction and sharing of thoughts at some points. Planning and integration of courses and other activities, which may involve mixed learning environments, must be done to facilitate the smooth functioning of blended learning (Osguthorpe & Graham, 2003).

Student academic performance is one of the important educational outcomes; it is often quantified by means of tasks that assess knowledge, skills, and understanding (Allen & Seaman, 2014). The level of academic achievements is affected by student engagement, motivation and learning strategies, instructional design (Means et al., 2010). Assessment of student academic outcomes achieved by various learning techniques is important in evaluating the usefulness of the techniques and technologies used in the process of learning (Bernard et al., 2014).

Technology education refers to the understanding of technological tools and processes including their relevance in the society of the learner (International Technology Education Association, 2000). It includes a focus on basic skills of problem-solving, critical thinking, and design, but in relation to technology (Sanders, 2009). Considering the rapid adoption of technology in all fields, technology education is an important aspect in ensuring a student is ready for the job market of the 21st century (National Research Council, 2012).

## 3. MATERIALS AND METHODS

The study employs a quasi-experimental design using pre-assigned groupings to establish a cause-and-effect between independent (modalities) and dependent (score) variables. The study investigates and compares the students' performances across the three learning modalities. The respondents of the study were students enrolled in technology education programs at a selected state university in Davao Region, the Philippines in the 1st and 2nd semester of the 2021-2022 academic year. The study consists of a sample of 45 students selected randomly from the said target

population; 15 student respondents were selected from synchronous modality, 15 student respondents from asynchronous modality, and 15 student respondents from blended learning modality (please see Appendix 1, Appendix 2 and Appendix 3 for the data score for each modality).

The learning platform used for the synchronous learning modality was Google Meet while the asynchronous learning modality made use of the University Virtual Environment (UVE) and Messenger. The Blended learning modality involved Google Meet, University Virtual Environment (UVE), Messenger, and Google Forms.

Moreover, the study used stratified random sampling with an equal allocation among year levels. After stratifying the target population, the researchers randomly selected a sample of 15 from each learning modality. The goal of stratified random sampling is to eliminate the possibility of human bias in selecting cases for inclusion in the study.

Furthermore, to establish the differences in academic performance among the three learning modalities, data were subjected to a normality test using first, an exploratory analysis with stem and leaf plot for the visual investigation of the distribution of the data and then statistical method using Shapiro-Wilk test for testing the normality of the data. If data is normally distributed, a one-way analysis of variance will be carried out. Subsequently, if significance exists, all pairwise comparisons will be tested using Tukey HSD test. On the other hand, if data is normally distributed, a nonparametric Kruskal Wallis test will be conducted; and if significance exists, all pairwise comparisons will be tested using median test. SPSS software version 23 (trial version) was used for the statistical calculations of the study.

## 4. RESULTS AND DISCUSSION

This study utilises the responses from a sample of forty-five (45) students recruited randomly from the three learning modalities namely synchronous, asynchronous, and blended in combination with three different forms of assessment including formative assessment, performance test, and examination results.

15 respondents were selected from the synchronous learning modality, 15 respondents from the asynchronous learning modality, and 15 respondents from the blended learning modality. Exploring the distribution of the data, the results show that formative assessment synchronous, performance blended, and examination blended were not normally distributed since their p-values are all less than 5% which means the hypothesis that they are normally distributed was rejected. Meanwhile, the remaining sub-categories were normally distributed as shown in Table 1. As for the sub-categories that were not normally distributed, a transformation was required. However, it was impossible to guarantee their normality, so rather than doing transformations, we adopted a nonparametric method which, though less efficient, does not require normality assumptions.

*Table 1. Normality of the Data of each Modality (Shapiro-Wilk Test)*

| Type of Assessment | Modalities | Shapiro-Wilk | | |
|---|---|---|---|---|
| | | Statistic | df | p-value |
| Formative assessment | Synchronous | 0.876 | 15 | 0.041* |
| | Asynchronous | 0.937 | 15 | 0.347 |
| | Blended | 0.886 | 15 | 0.057 |
| Performance test | Synchronous | 0.923 | 15 | 0.214 |
| | Asynchronous | 0.904 | 15 | 0.111 |
| | Blended | 0.801 | 15 | 0.004* |
| Written Examination | Synchronous | 0.884 | 15 | 0.054 |
| | Asynchronous | 0.939 | 15 | 0.375 |
| | Blended | 0.821 | 15 | 0.007* |

**significant at 5 % level*

Using the Kruskal Wallis test, the analysis revealed that regarding formative assessment, there was a significant difference between the mean values of the respondents' scores across the three types of modalities (Chi-square=9.878, $p<0.05$). Similar results (highly significant) were also observed for the performance test (Chi-square=29.126, $p<0.01$) and the written examination (Chi-square=12.127, $p<0.01$) (Table 2).

Since all modalities observed significantly different students' performances among the three types of assessments, it can be seen in table 2 that formative assessment in the blended modality dominated in terms of their mean rank followed by the synchronous modality. Moreover, Picciano (2002) states that successful course completion, grades, and enhanced knowledge and abilities are all examples of how student performance can be defined and measured. Many researchers have analysed student performance using conventional evaluation and final course grades to dispute the efficiency of online learning (McFarland & Hamilton, 2005). This observation is also true for the performance test as well as in the examination. On the basis of performance comparing all assessment and learning modalities, we can see that the performance test in the blended learning modality demonstrated the best student performance in their technology education.

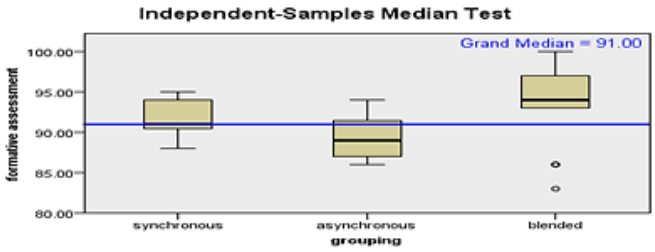
However, statistically, further investigation on which modalities have similar effects is needed using pairwise comparison median tests. Regarding the formative assessment, the results show that there was a highly significant median score among the modalities ($p<0.01$). Further analysis revealed that there were no significant differences in the medians between the synchronous and asynchronous modalities, with the median line hit both stem and leaf plots of the two modalities (see Figure 1), but both were significantly different from the blended modalities, where the median line did not hit blended modality (see Figure 1).

*Table 2. Kruskal Wallis Test for the comparison of mean ranks among the three modalities*

| Type of Assessment | modalities | Sample size n | Mean Rank | Chi-square | df | p-value |
|---|---|---|---|---|---|---|
| Formative assessment | Synchronous | 15 | 23.63 | 9.878 | 2 | 0.007* |
| | Asynchronous | 15 | 15.20 | | | |
| | Blended | 15 | 30.17 | | | |
| | Total | 45 | | | | |
| Performance test | Synchronous | 15 | 15.53 | 29.126 | 2 | 0.000* |
| | Asynchronous | 15 | 15.70 | | | |
| | Blended | 15 | 37.77 | | | |
| | Total | 45 | | | | |
| Examination | Synchronous | 15 | 21.83 | 12.127 | 2 | 0.002* |
| | Asynchronous | 15 | 15.33 | | | |
| | Blended | 15 | 31.83 | | | |
| | Total | 45 | | | | |

**significant at 5% level*

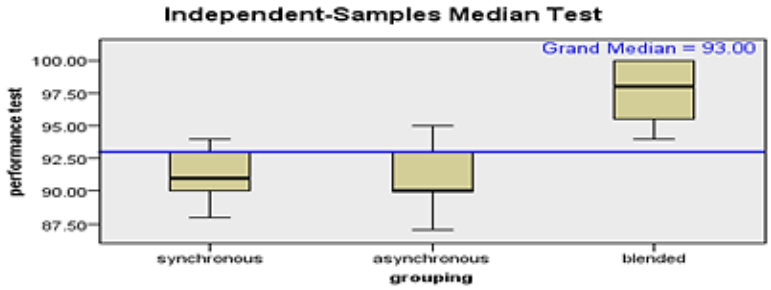
The median score in the blended modality group (94) was higher than the grand median of 91 and also both that of the synchronous (91) and asynchronous (89) modalities. The synchronous modality got the same median score as the grand median score while the asynchronous modalities the lowest median, lower than the grand median score (See Table 3 and Figure 1).




(n=45, test statistic=10.17, df=2, p<0.01)

*Figure 1. Visualisation of Pairwise comparison among modalities in the formative assessment*

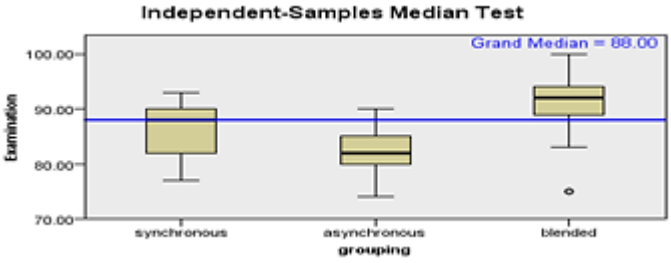
On the other hand, as for the performance test, it is shown that there was a highly significant median score across the modalities (p<0.01). However, there were no significant differences in the medians of the synchronous and asynchronous groups, both of which were significantly different from that of the blended modality (see figure 2). The median score in the blended modality group (98) was higher than those of both the synchronous (91) and asynchronous (90) modalities while the asynchronous modalities had the lowest median. The median score of the blended modality group was higher than the grand median of 93 and also those from both the synchronous and asynchronous modalities. The synchronous and asynchronous modalities had the median scores lower than the grand median score and the blended median score. The lowest median score belonged to the asynchronous modality group as seen in Table 3 and Figure 2.




(n=45, test statistic= 33.88, df=2, p<0.01)

*Figure 2. Visualisation of Pairwise comparison among modalities in the performance assessment*

Lastly, regarding written examination, the results showed that there were highly significant differences in the median score among the three modalities (p<0.01) as demonstrated by the median line (see Figure 3). Again, the median score of the blended modality group (92) was higher than those of both the synchronous (88) and asynchronous (82) modality groups while the asynchronous modalities had the lowest median. The median score of the blended group was higher than the grand median score of 88 and also higher for both the synchronous and asynchronous modalities. The synchronous and asynchronous modality groups had a median score lower than the grand median score and the blended median score. The lowest median score belonged to the asynchronous modality group as seen in Table 3 and Figure 3.




(n=45, test statistic=11.11, p<0.01)

*Figure 3. Visualisation of pairwise comparison among modalities in the examination*

*Table 3. Results of Pairwise comparison among medians of the different modalities*

| Type of Assessment | modalities | n | Median | Median compared to grand median |
|---|---|---|---|---|
| Formative assessment | Synchronous | 15 | 91 | Equal |
| | Asynchronous | 15 | 89 | Lower |
| | Blended | 15 | 94 | Higher |
| | Grand median | 45 | 91 | |

| | | | | |
|---|---|---|---|---|
| Performance test | Synchronous | 15 | 91 | Lower |
| | Asynchronous | 15 | 90 | Lower |
| | Blended | 15 | 98 | Higher |
| | Grand median | 45 | 93 | |
| Written Examination | Synchronous | 15 | 88 | Equal |
| | Asynchronous | 15 | 82 | Lower |
| | Blended | 15 | 92 | Higher |
| | Grand median | 45 | 88 | |

As seen in Table 3, it was observed that the blended modality group got the best median scores among the respondents, followed by the synchronous modality while the asynchronous modality was the worst performing, even below the grand median score. It was further observed that the best median score among the three types of assessment was in the blended performance test. Overall, the performance test got the best results among the different modalities in terms of the median score.

On the basis of our results from this particular data set, the blended learning modality for all types of assessment resulted in a very good academic performance in the technology program. Given the recent experience with the COVID-19 pandemic where direct interactions were limited, some higher education institutions are considering the replacement of total face-to-face classroom instructions with a distance online learning environment or limited face-to-face instructions. That is where blended learning modality can place itself with instruction implemented online in synchronous or asynchronous modality in combination with a face to face learning instruction, especially on the actual laboratory classes. Blended learning transcends time, space, and culture to provide numerous new options for both students and teachers. In other words, blended learning intends to consciously and effectively merge online and conventional learning to develop a unique, innovative method with its own merits (Picciano, 2006). Moskal et al. (2013) also mentioned that blended learning, as viewed by higher education institutions, is the mixture of old and new methods, and in our present practice, a combination of online classes for theories and face-to-face classes for practice among the educational institutions in the Philippines. Furthermore, Moskal et al. (2013) further suggest that there must be a better definition of blended learning so that higher education institutions can align their goals to be successful in initiating a blended learning environment.

In this manner, blended learning has been found to enhance adaptability to individuals' learning and help organizations with productive utilization of time and workforce, which has previously been supported and mentioned by Holden and Westfall (2006).

## 5. CONCLUSION

In this study, it has been figured out that the blended learning modality yielded a better result in terms of the academic performance of the students in their technology education programs even with the different types of assessments. The performance test in the blended learning modality group showed a desirable result in the students' academic performance in the technology education programs. This finding can be justified with the fact that technology education requires hands-on experience while theories and lectures can be delivered online through a synchronous or asynchronous, thus the actual performance can be best implemented in a full or limited face-to-face modality. Thus, the blended learning modality can gain its adequate role and place in the educational learning environment.

The researchers recommend that this research design should probably be replicated in more general education programs like in Applied and Pure Sciences, Engineering and Mathematics, and perhaps Arts and Social Sciences in a much higher sample size to confirm the results in the conclusion.

**Conflict of Interest:** No potential conflict of interest relevant to this article was reported.

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*Appendix 1. Score data on 15 students' Academic Performance on Synchronous Learning modality among three types of assessment*

| Student(s) | Formative Assessment | Performance Test | Exam |
|---|---|---|---|
| **1** | 91 | 90 | 82 |
| **2** | 91 | 90 | 82 |
| **3** | 89 | 88 | 89 |
| **4** | 91 | 90 | 90 |
| **5** | 91 | 90 | 77 |
| **6** | 95 | 93 | 82 |
| **7** | 94 | 93 | 88 |
| **8** | 95 | 93 | 88 |
| **9** | 95 | 93 | 82 |
| **10** | 94 | 88 | 78 |
| **11** | 91 | 91 | 90 |
| **12** | 91 | 94 | 93 |
| **13** | 88 | 91 | 90 |
| **14** | 90 | 92 | 90 |
| **15** | 90 | 91 | 88 |

*Appendix 2. Score data on 15 students' Academic Performance on Asynchronous Learning Modality among three types of assessment*

| Student(s) | Formative Assessment | Performance Test | Exam |
|---|---|---|---|
| **1** | 92 | 90 | 82 |
| **2** | 91 | 90 | 79 |
| **3** | 86 | 90 | 74 |
| **4** | 87 | 90 | 84 |
| **5** | 92 | 87 | 80 |
| **6** | 89 | 92 | 85 |
| **7** | 87 | 90 | 90 |
| **8** | 90 | 90 | 82 |
| **9** | 89 | 90 | 80 |
| **10** | 90 | 92 | 84 |
| **11** | 93 | 95 | 90 |
| **12** | 87 | 93 | 80 |
| **13** | 89 | 93 | 80 |
| **14** | 94 | 94 | 85 |
| **15** | 87 | 93 | 85 |

*Appendix 3. Score data on 15 students' Academic Performance on Blended Learning modality among three types of assessment*

| Student(s) | Formative Assessment | Performance Test | Exam |
|---|---|---|---|
| **1** | 94 | 95 | 94 |
| **2** | 93 | 95 | 95 |
| **3** | 97 | 100 | 94 |
| **4** | 97 | 95 | 92 |
| **5** | 93 | 100 | 88 |
| **6** | 96 | 96 | 94 |
| **7** | 93 | 94 | 90 |
| **8** | 100 | 98 | 92 |
| **9** | 100 | 96 | 92 |
| **10** | 100 | 97 | 92 |
| **11** | 86 | 100 | 83 |
| **12** | 94 | 100 | 75 |
| **13** | 97 | 100 | 75 |
| **14** | 86 | 100 | 92 |
| **15** | 83 | 100 | 100 |

# ITEM RESPONSE THEORY MODELING WITH NONIGNORABLE MISSING DATA

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# ITEM RESPONSE THEORY MODELING WITH NONIGNORABLE MISSING DATA

PROEFSCHRIFT

**ter verkrijging van**
**de graad van doctor aan de Universiteit Twente,**
**op gezag van de rector magnificus,**
**prof. dr. W.H.M. Zijm,**
**volgens besluit van het College voor Promoties**
**in het openbaar te verdedigen**
**op donderdag 15 december 2005 om 16.45 uur**

**door**

**Jonald L. Pimentel**

**geboren op 24 juni 1969**
**te Don Carlos, Filippijnen**

**PROMOTOR: Prof. dr. C.A.W. Glas**

**ASSISTENT-PROMOTOR: Dr. ir. J.-P. Fox**

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# 1
# Introduction

Psychometrics is a field of study connected to psychology, education and statistics. It deals with the design, administration, analysis and interpretation of tests for the measurement of psychological variables such as intelligence, aptitude, personality traits and abilities. Further, psychometrics has been used in measuring academic achievement and in health related fields, for example, to measure quality of life.

Psychometric methods have several orientations. Pioneers of psychometrics first developed classical test theory (CTT) and then more recent the item response theory (IRT). CTT can be characterized as the theory of measurement errors. The key concepts involve reliability and validity and both can be assessed mathematically. A reliable measure is measuring something consistent while a valid measure is measuring what it is supposed to measure. The major applications of CTT are item and test analyses and observed score equating. On the other hand, IRT can be characterized as a class of probabilistic models for responses of persons to test items. The main focus of this thesis will be on IRT.

## 1.1 Item Response Theory

Item response theory (IRT) is a class of probabilistic or stochastic models for two-way data, say, the responses of persons or individuals to test items. An important feature of IRT models is parameter separation, which means that the influences of the test items and persons on the responses are modeled by distinct sets of parameters. In IRT, the performance of a person (an examinee) on a test item can be explained by a set of factors called latent traits or abilities. The relationship between a person's item response and the set of traits underlying them can be described an item characteristic curve (ICC) that gives the response probabilities as a function of the latent traits. For dichotomously scored items, this curve is usually monotonically increasing. This curve specifies that as the level of the ability increases, the probability of a correct response to an item increases also.

Under unidimensional IRT models for dichotomous items, the probability of a correct response depends on the persons' unidimensional ability, say $\theta$, and the parameters that characterize the item. Popular models for items with dichotomous responses are the one,two and three normal ogive models and the one,two and three parameter logistic models namely the Rasch model (1PLM; Rasch, 1960), two parameter logistic model (2PLM; Birnbaum, 1968) and the three parameter logistic model (3PLM). For items with polytomous response, models such as the nominal response model (Bock, 1972), graded response model (Samejima, 1969) and partial credit model (Masters, 1982) are used. There are also available models that handle multidimensional cases if items appear to be sensitive to more than one ability (multidimensional IRT; McDonald 1967, Lord & Novick, 1968). Further, IRT models are available for nonmonotone items.

IRT provides a useful framework of solving a wide variety of measurement problems ranging from test construction, to reporting of test scores. Evidence of its importance can be found in the study of differential item functioning (for multiple groups) , person fit analysis, computerized adaptive testing, item banking, structural item response modeling (e.g. Multilevel IRT modeling, see Fox, 2001), test equating and the handling of missing data i.e. modeling and detect-

ing nonignorable missing data processes using IRT models (Holman & Glas, 2005). The latter application is the focus of this thesis.

The first step in applying item response theory to test data is that of estimating the parameters that characterize the chosen item response model. In fact, the successful application of item response theory depends on the availability of satisfactory procedures for estimating the parameters of the model. Estimation procedures that can be employed to obtain parameter estimates using IRT models are available using both likelihood based and Bayesian methods. Likelihood based methods are the joint maximum likelihood estimation (JML), conditional maximum likelihood estimation (CML) and marginal maximum likelihood estimation (MML) employ inferences. These methods are used in software packages as BILOG-MG (Zimowski, Muraki, Mislevy & Bock,1996), MULTILOG, TESTFACT (Wilson, Wood & Gibbons, 1991), MPLUS (Muthén & Muthén ,1998) ,OPLM (Verhelst,Glas & Verstralen,1995) and ConQuest (Wu, Adams & Wilson, 1997). The alternative method, Bayesian estimation methods, employ inferences from posterior distributions. It has been adopted to the estimation of IRT models with multiple raters, multiple item types, missing data (Patz, & Junker; 1999a, 1999b), testlet structures (Bradlow, Wainer & Wang, 1999), and models with multi-level structure on the ability parameters (Fox & Glas, 2001). The unifying theme of these applications is the use of a Markov chain Monte Carlo (MCMC) algorithm for making Bayesian inferences. Most widely used MCMC methods are the Gibbs sampler and the Metropolis-Hasting algorithm. The software packages WinBUGS (Lunn, Thomas, Best, & Spiegelhalter (2000) and MLIRT (available in the web) are some of the available software packages that employ Bayesian estimation methods in IRT.

The second step in applying item response theory to test a data is that of testing the validity of the item response models. A given item response model may or may not be appropriate for a particular set of test data, that is, the model may not adequately predict or explain the data. Hence essentially, in any IRT application, there is in a need to assess the fit of the model to the data. Model fit has two aspects: item fit and person fit. In the first case, the assumptions evaluated are differential item functioning, the form of the item response curve and local stochastic independence. Test statistics have been proposed by such authors as Mokken (1971), Andersen (1973),

Yen (1981, 1984), Molenaar (1983), Glas (1999), and Orlando and Thissen (2000). An overview is given by Glas and Suárez-Falcon (2003). Person fit statistics usually focus on the constancy of ability across the test. Examples are the person fit statistics by Smith (1986), and Snijders (2001). An overview is given by Meijer and Sijtsma (1995; 2001).

## 1.2 Missing data and Ignorability

Statistical analysis of a given data set will be more complicated in the presence of missing data. Standard methods are not directly applicable if these missing data are present. Therefore, there is a growing interest in the statistical methods that properly account for incomplete data (Little & Rubin, 1987). In behavioral sciences and educational measurement settings, for instance, the type of incompleteness that has been studied thoroughly is missing data due to incomplete designs and random missing data such as unit and item nonresponse cases. Individuals for which all responses are missing are called unit nonresponse while individuals for which only the responses to particular items are missing are as known item nonresponse cases. Huisman (1999) studied the occurrence, causes and ways to handle the statistical inferences of item nonresponses. He investigated the nature of missing data patterns and found methods to handle missing data in test items through imputations.

In literature, four common ways to handle missing data are discussed. First, there is the practice of deleting cases with missing data (listwise or pairwise) before doing the actual analyses. Dropping cases with missing values may occasionally be appropriate, but usually this approach has its hazards. The effect of such a practice will reduce the sample size, which leads to inefficient estimation, and it may lead to biased estimates if the missingness is systematic, for instance, if the missing data are correlated with the outcomes of interest. At this point, we do not define the circumstances that lead to bias precisely, this will be done at the end of this section. Besides loss of precision and introduction of bias, deletion of cases may also lower the power of statistical tests and, finally, sometimes the data are too costly to discard. Therefore, most literature discourages this practice

The second way to deal with missing data is the practice of imputation, that is, filling in the missing data with the use of imputation techniques. Examples are mean imputation, regression imputation, hot-deck imputation & multiple imputation (Little & Rubin, 1987). The third way to deal with missing data, is to ignore the missing data and estimate the model using all available observed data. The problem is that the software used must be able to handle the more complicated computations involved. Further, in some situations, which will be discussed below, this approach still leads to biased estimates.

The fourth way to deal with missing data is by explicitly modeling the mechanism that caused the missing data and incorporated this additional model into the model for the observed data. In this thesis we focus on the third and fourth methods for handling missing data in the framework of IRT models.

Above, we used the vague notion of systematic missing data and posited that this form of missingness might lead to bias in estimates. However, a precise analysis of when this actually happens is quite subtle. The problem has been analyzed by Rubin (1976). He discussed the weakest conditions on the process that caused the missing data such that it is always appropriate to ignore this process when making statistical inferences about the distribution of the data of interest. To define this ignorability principle, suppose $\theta$ and $\zeta$ are the parameters of the observed data and the missing data process, respectively. Further, suppose $D$ is the missing data indicator. In the framework of this thesis, $D$ will be a matrix with elements $d_{ik} = 1$ if for persons $i$ and items $k$ a realization $x_{ik}$ was observed and $d_{ik} = 0$ if $x_{ik}$ was missing. Then, the missing data is said to be missing at random (MAR) if the probability of $D$ given the observed data $x_{(1)}$, missing data $x_{(0)}$, the parameter $\zeta$ and, possibly, observed covariates $y$ does not depend on the missing data $x_{(0)}$, that is,

$$P(D|x_{(0)}, x_{(1)}, \zeta, y) = P(D|x_{(1)}, \zeta, y).$$

Furthermore, in a likelihood-based framework, the parameters $\zeta$ and $\theta$ should be distinct, that is, the joint space of $(\zeta, \theta)$ should factorize into a $(\zeta)$ and $(\theta)$ space. If the missing data are MAR and distinctness holds, then the missing data is said to be ignorable. So in likelihood based inferences, if the missing data are MAR then the missing data mechanism or process is ignorable. This means that we do not take into account $\zeta$ in the analysis and still the resulting estimates of our

parameters are consistent. In the Bayesian framework, the missing data mechanism is said to be ignorable if the missing data are MAR and the priors of $\zeta$ and $\theta$ are independent.

In educational measurement, it often happens that item nonresponses are nonignorable missing data. An example, for instance, is a test with a time limit condition, where examinees of lower ability do not reach the items at the end. Thus, the pattern of missingness in this case depends on the ability that is measured and hence the missing data are not generally ignorable.

## 1.3 Objectives and Outline of the Thesis

The topic of this thesis is IRT modeling in the presence of nonignorable missing item responses. The main theme of this thesis is that, apart from the observed item responses, also the variable $d_{ik}$ can be modeled by IRT.

The first part of this thesis (Chapters 2 & 3) will examine the effect in the bias of the model parameter estimates when IRT model for the nonignorable missing data is introduced in the estimation. The purpose of the inclusion of an IRT model for the mechanism that governs the missing data is to reduce the bias in the parameter estimates of the model parameters in the case of violation of Rubin's ignorability principle. Further, the reduction in bias will also be studied when the IRT model for missing data includes observed covariates. The combined model for the observed item responses and the missing data indicator is a multidimensional IRT model with two dimensions for the persons parameters: one for the observed data and one for the missing data. They are assumed to be correlated. The model parameters are estimated using the marginal maximum likelihood method (MML).

In Chapter 2, we investigate through a simulation study for both the dichotomous and polytomous case the effect in the bias of the parameters estimates. Further, the difference in precision of the parameters is investigated between including an IRT model for the missing data process with and without observed covariates.

In Chapter 3, an approach analogous to the approach of Chapter 2 is applied to data from a test with a time limit (a speeded test). The missing data indicator will be modeled using the so-called sequential or steps model (Tutz, 1990; Verhelst, Glas and de Vries, 1997). Also

here the model parameters are estimated using MML. Simulation studies are conducted to test the method, first ignoring the missing data process and second including the step model for the missing data.

In Chapter 4, two methods for deciding whether the missing data are ignorable or nonignorable in the IRT framework are proposed that are based on the splitter item technique (Van den Wollenberg, 1979; Molenaar, 1983 ). It is tested whether the item parameter estimates differ across subsets of item response data. In the first method, the observed data are split-up according to the values of the splitter item. Then, the estimated marginal distributions of the item parameters corresponding to both data sets are compared for detecting differences. In the second method, an IRT model for the observed data is extended with group specific item parameters. These extra parameters, known as Bayesian modification indices (Fox & Glas, 2005) provide information regarding item parameter differences across groups. They are estimated using MCMC, but these estimates do not interfere with the estimation of the other model parameters. Simulation studies were undertaken to illustrate the methods.

In Chapter 5, we develop a fixed effect IRT model for modeling group specific item parameters. The idea is to extend the class of binary IRT models with fixed effects. We propose a general MCMC method to simultaneously estimate all model parameters. The proposed model is used in two practical applications. First, to detect whether a response mechanism is ignorable or not using the splitter item technique and second to detect differential item functioning. Simulation studies are presented to show how the proposed model can be applied.

# 2

# IRT Models for Nonignorable Missing Data Processes

ABSTRACT: Missing data usually present special problems for statistical analyses, especially when the data are not missing at random, that is, when the ignorability principle defined by Rubin (1976) does not hold. This chapter presents a model-based procedure that handles non-ignorable missing data using item response theory (IRT). The relevant model for the observed data is estimated concurrently with the IRT model for the missing data process. As an example, the generalized partial credit model is used to model the observed data while the Rasch model is used to model the missing data process. Simulation studies for dichotomous and polytomous data are presented that show that the bias in the item parameter estimates obtained ignoring the missing data process can be removed or reduced by using the explicit model for the missing data process. It is shown that the IRT model for missing data can also include observed covariates. Using a simulation study, it is shown that the bias in the parameters can be greatly reduced when observed covariates were included in the estimation.

KEYWORDS: item response theory, latent traits, missing data, non-ignorable missing data, observed covariates

## 2.1 Introduction

In research, missing data is always a source of concern for people who are doing statistical analyses. It raises the level of complexity of making statistical inference. Many researchers, methodologist, and software developers resort to editing the data, although ad hoc edits may do more harm than good by producing results that are substantially biased, inefficient and unreliable (Schafer & Graham, 2002). One way to alleviate the bias in the item parameter esti-

mates is the identification of the variables that explain the cause of missing data. These explanatory variables are called "mechanism or process" variables. By including a model for this missing data mechanism in the estimation we can reduce or eliminate the bias (due to missingness) in our parameter estimates. Theoretically, if all the process variables associated with a particular piece of missing data can be identified and modeled accurately as controls, the impact of the missing data can be statistically adjusted to the point where it is ignorable (Little & Rubin, 1987). In practice, it is difficult to identify these process variables for all cases of missing data. However, if the given data set contains missing observations, the mechanism causing this missingness can be characterized by its variety of randomness (Rubin, 1976) as missing at random (MAR) and missing completely at random (MCAR).

Suppose $\theta$ and $\zeta$ are the parameters of the observed data and the missing data process, respectively, and $D$ is the missing data indicator with elements $d_{ik} = 1$ if a realization $x_{ik}$ was observed and $d_{ik} = 0$ if $x_{ik}$ was missing for persons $i$ and items $k$. Following Rubin's definition, missing data is said to be MAR if the probability of $D$ given the observed data $x_{obs}$, missing data $x_{mis}$, some parameter $\zeta$ and observed covariates $y$ does not depend on the missing data $x_{mis}$ that is, if

$$P(D|x_{obs}, x_{mis}, \zeta, y) = P(D|x_{obs}, \zeta, y).$$

Furthermore, the parameters $\zeta$ and $\theta$ are distinct if there are no functional dependencies, that is, restrictions on the parameter space (frequentist version) or if the prior distributions of $\zeta$ and $\theta$ are independent (Bayesian case). If these two components (MAR and distinctness) are satisfied then the missing data is said to be ignorable, otherwise the missing data are nonignorable. If MAR and distinctness hold, the missing data process is ignorable for statistical inferences, which means that we do not have take into account the distribution of $D$ and $\zeta$, yet the consistency of the estimates is not threatened by the occurrence of the missing data.

In the framework of IRT, missing data can be split into four types (Lord, 1974). The first consists of missing observations which result from a priori fixed incomplete test and calibration designs. The second consists of classes of response-contingent designs such as two and multistage testing (Lord, 1980) designs and computerized adaptive testing. These designs produce ignorable missing data, because

the design variables $D$ are completely determined by the observed responses. The third type is ignorable missing data that results from unscalable responses such as "do not know" or "not applicable", or items missing from booklets. The fourth and last type of missing data results from a nonignorable missing data mechanism. These will, for instance, occur when low-ability respondents fail to produce a response or responses as a result of discomfort or embarrassment. Bradlow and Thomas (1998) mentioned that ignoring this type of missing data process could produce bias in the parameter estimates.

Statistical inference based on the observed data when the missing data process is not ignorable in most cases leads to biased estimates of the parameters of the model. Some literature suggested remedies. One helpful proposal is to model the process that caused the missing data (Heckman, 1979), and the applications discussed below all fall in this category.

Copas and Farewell (1998) argued that nonignorable nonresponse can be explained by covariates such as a subjective measure of enthusiasm to respond. For example, it is expected that when an issue under study is sensitive, an individual may be embarrassed to give a response. In the same manner, students with a low proficiency may fail to respond to difficult items. In the framework of a medical survey, Holman and Glas (2005) report that patients with a relatively high functional status may boost the estimate of their level by failing to respond to items of a physical disability scale.

Moustaki (1996, see also Bartholomew & Knott, 1999) developed a general latent trait and latent class model for mixed observed variables. Within this framework, three methods for dealing with nonignorable missing data were proposed (O'Muircheartaigh & Moustaki, 1999; Moustaki & O'Muircheartaigh, 2000; Moustaki & Knott, 2000). In the first method for the treatment of nonresponse, the missing value is treated as a separate response category. The method includes the missing values in the analysis of the observed items to obtain information about the missing values based on what has been observed, i.e. they used the interrelationships among the items. This information is related to the attitude dimension or dimensions in which they can connect attitude with the nonresponse.

The second method to deal nonresponse is computing response propensities. The idea is to use the propensity score to weight item responses and respondents to account for item and unit nonresponse

and to obtain adjusted estimates. This response propensity method uses a logistic or probit regression which is fitted to a binary item response-nonresponse variable for the survey item of interest with a set of covariates.

The third method is to use a latent variable model with two latent dimensions, one to summarize the response propensity and the other to summarize the individual position on the dimension of interest (such as ability or attitude). As an example, O'Muircheartaigh and Moustaki (1999) used a latent variable model for the treatment of item nonresponse in attitude scales. They combined the idea of latent variable identification with the issues of nonresponse adjustment to surveys. This latent variable approach allows missing values to be included in the analysis and equally important allowed information about attitude to be inferred from nonresponse. Their method handled binary (dichotomous), metric and mixed (binary and metric) manifest items with missing values.

Working within the latter approach, Holman and Glas (2005) proposed an IRT model that allows concurrent estimation of IRT item parameters for both a model for the observed dichotomous responses and the missing data indicators. In this chapter we extend this approach to polytomous item responses. Further, we generalize the model to include covariates for the item responses and the missing data indicators. Using a simulation study, we will investigate to what extent the bias in the parameters of the observed data model can be reduced if the observed covariates are included. Both methods (the method with and without covariates) will be applied in both dichotomous and polytomous cases in order to assess the feasibility of the method.

This chapter consists of four sections and is organized in the following manner. After this introduction, the general IRT model for both the observed data and missing data are presented. The following section describes the MML estimation procedure. Finally, the last section presents the simulation studies that will apply the proposed method.

## 2.2 IRT Models

### *2.2.1 General IRT model for missing data*

Let $X$ be a two-dimensional data matrix with elements $x_{ik}$, where persons are indexed as $i = 1, ..., N$ and items are indexed as $k = 1, ..., K$. If a combination of $i$ and $k$ has been observed, then the entry $x_{ik}$ is the observation, otherwise it is equal to some arbitrary constant. We define a design matrix $D$ of the same dimension as $X$ with elements $d_{ik} = 1$ if $x_{ik}$ was observed, otherwise $d_{ik} = 0$

Using the elements of $X$ and $D$, one of our objectives is to make inferences on the individual person parameter $\theta_i$, which are potentially influenced by a latent person variable $\zeta$ representing the missing data process.

To model the missing data process, we use a Q-dimensional IRT model proposed by Reckase (1985, 1997) and Ackerman (1996a & 1996b). This model, which is in logistic form, has the probability of an observation given by

$$p(d_{ik} = 1|\zeta_i, \gamma_k, \delta_k) = \frac{\exp(\sum_q^Q \gamma_{kq}\zeta_{iq} - \delta_{k0})}{1 + \exp(\sum_q^Q \gamma_{kq}\zeta_{iq} - \delta_{k0})} \qquad (2.1)$$

where $\gamma_{kq}$ and $\delta_{k0}$ are the item parameters (discrimination and difficulty) of the missing data indicator, which we will also refer to as the missing data process.

The model (2.1) is the Rasch model (Rasch, 1960) for dichotomous items when $Q = 1$, and $\gamma_{kq} = 1$ and the two parameter logistic (2PL) model (Lord & Novick, 1968) when $Q = 1$.

When the amount of missing data is small, the appropriate model must have few parameters (like the Rasch model) to be estimable (Lord, 1983).

Another approach of modeling the missing data process is using a normal-ogive representation (McDonald, 1967 & 1997; Lord & Novick, 1968) which is comparable to the logistic approached we used above but we will not discuss it in this chapter.

### *2.2.2 Combined IRT models for missing data and observed data*

- *Combined IRT models of missing data and observed data without observed covariates*

Suppose $\theta$ and $\zeta$ are the person's latent variables related to the observed data and missing data and let $g(\theta)$ and $g(\ \zeta)$ be their densities. Let $p(x_{ik}|d_{ik}, \theta_{i,}\alpha_k, \beta_k)$ be the measurement model for the observed data. It is the probability of the response (observed) variable conditioned on the latent variable of the observed data, the design variable (missing data indicator) and item parameters. Let $p(d_{ik}|\ \zeta_i, \gamma_k, \delta_k)$ be the measurement model for the missing data indicator. It is the probability of the design variable conditioned on the latent variable and item parameters for missing data process. The general models we are using in our estimation procedure are the models described in Holman and Glas (2005). The first model which, we call the $MAR$ model, is given in likelihood form as

$$\prod_{i,k} p(x_{ik}|d_{ik}, \theta_{i,}\alpha_k, \beta_k)p(d_{ik}|\zeta_i, \gamma_k, \delta_k)g(\theta_i)g(\zeta_i). \tag{2.2}$$

It is the model that ignores the missing data process, and we ignore the model for the missing data process $p(d_{ik}|\ \zeta_i, \gamma_k, \delta_k)g(\theta_i)$ in the estimation process. The latent variables for the observed data and the missing data process are not related in the $MAR$ model.

The second model, which we call the $NONMAR$ model, is the model where missing data process is included in the estimation process. In this model, the latent variables for both the observed and missing data process $\theta$ and $\zeta$ are correlated by $\Sigma$. This model is written in likelihood form as

$$\prod_{i,k} p(x_{ik}|d_{ik}, \theta_{i,}\alpha_k, \beta_k)p(d_{ik}|\zeta_i, \gamma_k, \delta_k)g(\zeta_i, \theta_i|\Sigma), \tag{2.3}$$

where $g(\cdot)$ is the density of $\zeta_i$ and $\theta_i$. It is assumed to follow a Multivariate Normal distribution with mean vector $\mathbf{0}$ and variance-covariance $\Sigma$. Expressions (2.2) and (2.3) will be used in our procedure to make inferences on the estimation of the model parameters.

In a Bayesian framework, $g(\zeta_i, \theta_i|\Sigma)$ can be seen as a prior for the latent variables. Therefore, statistical inferences under the ignorability assumption are not justified, because the priors of the parameters modeling the observations and the missing data process are not independent. So both Bayesian estimation based on the full posterior and Bayes modal estimation integrating out part of the parameters are not appropriate.

In a frequentist framework, the argument that ignorability does not apply is more subtle. The fact that the two latent variables $\theta$

and $\zeta$ are correlated as such does not imply a functional dependence. Béguin and Glas (2001) and Holman and Glas (2005) give conditions for identification of the model. From their conclusions it follows that the basis of the two-dimensional latent space can always be transformed in such a way that both the model for the observations and the model for the missing data indicators depend on the same two latent variables. Therefore, the latent parameters of the two models are not distinct. In other words, within the framework of the model they are functionally dependent.

- *Combined IRT models for missing data and observed data with observed covariates*

The IRT model for missing data can also include observed covariates $y$. We present a model in likelihood form as

$$\prod_{i,k} p(x_{ik}|d_{ik}, \theta_i, \alpha_k, \beta_k) p(d_{ik}|\zeta_i, \gamma_k, \delta_k) g(\zeta_i, \theta_i|\Sigma, \eta, y), \tag{2.4}$$

where its components are similar to (2.3), but with an addition of regression coefficients $\eta$. The latent variable for the missing data is expressed as a linear combination of the observed covariates that is,

$$\zeta_i = \sum_{s=0}^{p} \eta_s y_{is} + \varepsilon_i \tag{2.5}$$

where we assume $y_{i0} = 1$ and $\varepsilon_i$ is the random error which follows a multivariate normal distribution with mean $\mathbf{0}$ and variance-covariance $\Sigma$.

So in (2.5) we want to model the components $\zeta$ of the missing data process through the observed covariates $y$ with the same assumption that latent variables $\zeta$ and $\theta$ are correlated.

### *2.2.3 The Generalized Partial Credit Model (GPCM)*

For the observed responses, we consider items with dichotomous and polytomous responses and they will be analyzed in general using the multidimensional generalized partial credit model (GPCM; Muraki, 1992). For persons $i$ ($i = 1, ..., N$) responding to item $k$ ($k = 1, ..., K$) in category $g$ ($g = 0, ..., m_k$). The probability of responding in a category $g$ of item $k$ by person $i$ is given by

$$\psi_{kg}(\theta_i) = p(X_{ikg} = 1|\theta_i, \alpha_k, \beta_k) = \frac{\exp(g\sum_q^Q \alpha_{kq}\theta_{iq} - \beta_{kg})}{1 + \sum_{h=1}^{m_k} \exp(h\sum_q^Q \alpha_{kq}\theta_{iq} - \beta_{kh})} \tag{2.6}$$

where $\alpha_k$ and $\beta_k$ are the item vectors of discrimination and difficulty parameters. where $\alpha_k = \{\alpha_{k1}, ...\alpha_{kq}, ...\alpha_{kQ}\}$ is a Q-dimensional vector of discrimination parameters, $\theta_i = \{\theta_{i1}, ..., \theta_{iq}, ..., \theta_{iQ}\}$ is a Q-dimensional vector of person's parameters and $\beta_{kg}$ is a scalar item parameter for difficulty. We assume $\beta_{k0} = 0$ so that estimates of $\beta_{kg}$ are unique

Model (2.6) will be a specific model depending on the values of some of its parameters. When $m_k = 1$, (2.6) is the multidimensional two-parameter logistic model (2PL; Birnbaum, 1968) and in addition becomes the multidimensional partial credit model (PCM; Masters,1982; Masters & Wright, 1997) when item discrimination $\alpha_k = 1$ and further it is the multidimensional Rasch model for dichotomous items when $m_k = 1$.

## 2.3 MML Estimation

### *2.3.1 Estimation method*

Suppose $\mathbf{x}_i$ is the response pattern of respondent $i$, and $\mathbf{X}$ is the data matrix. Under the MML approach, it is assumed that possibly multidimensional ability parameters $\theta_i$ are independent and identically distributed with density $g(\theta; \lambda)$. Usually, it is assumed the person's ability is normally distributed with population parameters $\lambda$ (which are the mean $\mu$ and variance $\sigma^2$ for the unidimensional case, or the mean vector $\mu$ and the covariance matrix $\mathbf{\Sigma}$ for the multidimensional case). Item parameters $\phi$ consist of discrimination parameters ($\alpha_k$, or $\alpha_q$ for the unidimensional and the multidimensional cases, respectively) and the item difficulties $\beta_k$ whose elements are $(\beta_{k1}, \beta_{k2}, ..., \beta_{kg}, ..., \beta_{km_k})$. MML estimation derives its name from maximizing the log-likelihood that is marginalized with respect to $\theta$, rather than maximizing the joint log-likelihood of all person parameters $\theta$ and item parameters $\phi$. Below we will give a general derivation of MML estimation, and therefore the person parameters $\theta$ are assumed to include the parameters of the missing data indicator $\zeta$, and

likewise $\phi$ includes $\gamma$ and $\delta$. Let $\upsilon$ be a vector of all item and population parameters that is $\upsilon^t = (\phi^t, \lambda^t)$. Then the marginal likelihood of $\upsilon$ is given by

$$L(\upsilon; \mathbf{X}, \mathbf{D}) = \int ... \int \prod_i^N p(\mathbf{x}_i, \mathbf{d}_i | \theta_i, \phi) g(\theta_i, \lambda) d\theta_i$$

$$L(\upsilon; \mathbf{X}, \mathbf{D}) = \prod_i^N \int ... \int p(\mathbf{x}_i, \mathbf{d}_i | \theta_i, \phi) g(\theta_i, \lambda) d\theta_i$$

and hence the marginal log-likelihood of $\upsilon$ is

$$\log L(\upsilon; \mathbf{X}, \mathbf{D}) = \log \prod_i^N \int ... \int p(\mathbf{x}_i, \mathbf{d}_i | \theta_i, \phi) g(\theta_i, \lambda) d\theta_i$$

which is equivalent to the expression

$$\log L(\upsilon; \mathbf{X}, \mathbf{D}) = \sum_i^N \log \int ... \int p(\mathbf{x}_i, \mathbf{d}_i | \theta_i, \phi) g(\theta_i, \lambda) d\theta_i. \qquad (2.7)$$

The reason for maximizing the marginal rather than the joint likelihood is that maximizing the latter does not generally lead to consistent estimates. This is related to the fact that the number of person parameters grows proportional with the number of observations, and, in general, this leads to inconsistency (Neyman & Scott, 1948). Results from simulation studies by Wright and Panchapakesan (1969) and Fischer and Scheiblechner (1970) showed that these inconsistencies can indeed occur in IRT models. Kiefer and Wolfowitz (1956) have shown that MML estimates of structural parameters, say the item and population parameters of an IRT model, are consistent under fairly reasonable regularity conditions, which motivates the general use of MML in IRT models.

Now, the marginal likelihood equations for $\upsilon$ can then be easily derived using Fisher's identity (Efron, 1977; Louis 1982; also see, Glas, 1992, 1998). The first order derivatives with respect to $\upsilon$ can be written as

$$\mathbf{h}(\upsilon) = \frac{\partial}{\partial \upsilon} \log L(\upsilon | \mathbf{X}, \mathbf{D}) = \sum_i^N E(\omega_i(\upsilon) | \mathbf{x}_i, \mathbf{d}_i, \upsilon) \qquad (2.8)$$

where $\omega_i(\upsilon)$ is

$$\begin{aligned}\omega_i(\upsilon) &= \frac{\partial}{\partial \upsilon} \log p(\mathbf{x}_i, \theta_i | \mathbf{d}_i, \upsilon) \\ &= \frac{\partial}{\partial \upsilon} \left[\log p(\mathbf{x}_i | \theta_i, \mathbf{d}_i, \phi) + \log g(\theta_i | \lambda)\right] \end{aligned} \tag{2.9}$$

with

$$p(\mathbf{x}_i | \theta_{i,}, \mathbf{d}_i, \phi) = \prod_k \prod_{g=0}^{m_k} \psi_{kg}(\theta_i)^{d_{ik} x_{ikg}} \tag{2.10}$$

and the expectation is with respect to the posterior distribution $p(\theta_i | \mathbf{x}_i, \mathbf{d}_i, \upsilon)$. The identity in (2.8) is closely related to the EM-algorithm (Dempster, Laird and Rubin, 1977), which is an algorithm for finding the maximum of a likelihood marginalized over unobserved data. The present application fits this framework when the response patterns are viewed as observed data and the ability parameters as unobserved data. Together they are referred to as the complete data. The EM algorithm is applicable in situations where direct inference based on the marginal likelihood is complicated, and the complete data likelihood equations, i.e., equations based on $\omega_i(\upsilon)$ are easily solved. Given some estimate of $\upsilon$ as $\upsilon^*$, the estimate can be improved by solving $\sum_i^N E(\omega_i(\upsilon) | \mathbf{x}_i, \mathbf{d}_i, \upsilon^*) = \mathbf{0}$ with respect to $\upsilon$. Then this new estimate becomes $\upsilon^*$ and the process is iterated until convergence.

Application of this framework to deriving the likelihood equations of the structural parameters of the multidimensional GPCM proceeds as follows. The likelihood equations are obtained upon equating (2.8) to zero, so explicit expressions are needed for (2.9). Given the design vector $\mathbf{d}_i$, the ability parameter $\theta_i$ and the item parameters of the multidimensional GPCM, the probability of response pattern $\mathbf{x}_i$ is given by (2.10). By taking first order derivatives of the logarithm of this expression, the expressions for (2.9) are found as

$$\omega_i(\alpha_{kq}) = d_{ik} \left[\theta_{iq}(x_{ikg} - \psi_{ikg})\right] \tag{2.11}$$

and

$$\omega_i(\beta_{kg}) = d_{ik}(\psi_{ikg} - x_{ikg}) \tag{2.12}$$

where $\psi_{igk} = \psi_{gk}(\theta_i)$, thus the likelihood equations for the item parameters are found upon inserting these expressions into (2.8) and

equate the resulting expressions to zero, hence

$$\sum_{i}^{N} E(\theta_{iq}\psi_{ikg}d_{ik}|\mathbf{x_i}, \mathbf{d}_i, \upsilon) = \sum_{i}^{N} E(d_{ik}\theta_{iq}x_{ikg}|\mathbf{x}_i, \mathbf{d}_i, \upsilon)$$

simplifying further

$$\sum_{i}^{N} E(\theta_{iq}\psi_{ikg}d_{ik}|\mathbf{x}_i, \mathbf{d}_i, \upsilon) = \sum_{i}^{N} d_{ik}x_{ikg}E(\theta_{iq}|\mathbf{x}_i, \mathbf{d}_i, \upsilon) \tag{2.13}$$

and similarly

$$\sum_{i}^{N} E(d_{ik}\psi_{ikg}|\mathbf{x}_i, \mathbf{d}_i, \upsilon) = \sum_{i}^{N} E(d_{ik}x_{ikg}|\mathbf{x}_i, \mathbf{d}_i, \upsilon)$$

then

$$\sum_{i}^{N} E(d_{ik}\psi_{ikg}|\mathbf{x}_i, \mathbf{d}_i, \upsilon) = \sum_{i}^{N} d_{ik}x_{ikg} \tag{2.14}$$

To derive the likelihood equations for the population parameters, the first order derivatives of the logarithm of the density of the ability parameters $g(\theta|\lambda)$, where $\lambda$ is the vector of population parameters which is the mean vector $\mu$ and the covariance matrix $\mathbf{\Sigma}$ are needed. In the present case, $g(\theta|\mu, \mathbf{\Sigma})$ is the well-known expression for the $q$-dimensional multivariate normal distribution with mean vector $\mu$ and the covariance matrix $\mathbf{\Sigma}$, whose probability density is

$$g(\theta_i|\lambda) = g(\theta_i|\mu, \mathbf{\Sigma}) = (2\pi)^{-q/2}\,|\mathbf{\Sigma}|^{-1/2} \exp\left(-1/2(\theta-\mu)^t\mathbf{\Sigma}^{-1}(\theta-\mu)\right)$$

where $|\mathbf{\Sigma}|$ is the determinant of the covariance matrix, so it is easily verified that these derivatives are given by

$$\omega_i(\mu) = 1/2(\mathbf{\Sigma}^{-1}(\theta-\mu)) \tag{2.15}$$

and

$$\omega_i(\mathbf{\Sigma}) = 1/2[(\theta-\mu)(\theta-\mu)^t\mathbf{\Sigma}^{-2} - \mathbf{\Sigma}^{-1}] \tag{2.16}$$

where elements considered in $\mathbf{\Sigma}$ are the diagonals.

The likelihood equations to obtain $\mu$ are again found upon inserting these expressions in (2.8) and equating the resulting expressions to zero, that is

$$\sum_{i}^{N} E(\mathbf{\Sigma}^{-1}(\theta-\mu)|\mathbf{x}_i, \lambda) = \mathbf{0}.$$

and by simplifying the expression by working on the expectations of the stochastic variable $\theta$ and the parameters we solve $\mu$ as

$$\mu = \frac{\sum_i^N E(\theta|\mathbf{x_i}, \lambda)}{N}$$

Similarly for $\mathbf{\Sigma}$, the resulting expression is

$$\sum_i^N E((\theta-\mu)(\theta-\mu)^t\mathbf{\Sigma}^{-2}|\lambda) = \sum_i^N E((\mathbf{\Sigma}^{-1})^t|\lambda)$$
$$\sum_i^N E((\theta-\mu)(\theta-\mu)^t\mathbf{\Sigma}^{-2}|\lambda) = N(\mathbf{\Sigma}^{-1}) \tag{2.17}$$

simplifying leads to

$$\mathbf{\Sigma} = \frac{\sum_i^N E((\theta-\mu)(\theta-\mu)^t|\mathbf{x_i}, \lambda)}{N}.$$

Note that the standard errors are also easily derived in this framework: Mislevy (1986) points out that the information matrix can be approximated as

$$\mathbf{H}(\ \upsilon,\ \upsilon) \approx \sum_i^N E(\ \omega_i(\upsilon) \mid \mathbf{x}_i, \mathbf{d}_i, \upsilon\ )E(\ \omega_i(\upsilon) \mid \mathbf{x}_i, \mathbf{d}_i, \upsilon\ )^t \tag{2.18}$$

and the standard errors are the diagonal elements of the inverse of this matrix.

The basic approach presented so far can be generalized in two ways. First, the assumption that all respondents are drawn from one population can be replaced by the assumption that there are multiple populations of respondents. Usually, it is assumed that each population has a normal ability distribution indexed by a unique mean and variance parameter. Bock and Zimowski (1997) pointed out that this generalization together with the possibility of analyzing incomplete item-administration designs provides a unified approach to such problems as differential item functioning, item parameter drift, non-equivalent groups equating, vertical equating and matrix-sampled educational assessment. Item calibration for CAT also fits within this framework.

### *2.3.2 Observed Covariates*

We will now derive the MML estimation equations for the regression parameters for a model with observed covariates, such as given in (2.5). The population model is now given by

$$g(\theta_i|\mathbf{y}_i,\mathbf{B},\mathbf{\Sigma}) = (2\pi)^{-q/2}\,|\mathbf{\Sigma}|^{-1/2}\exp\left(-1/2(\theta_i-\mathbf{B}^t\mathbf{y}_i)^t\mathbf{\Sigma}^{-1}(\theta_i-\mathbf{B}^t\mathbf{y}_i)\right)$$

where $\mathbf{B}$ is a $p\times q$ matrix of regression parameter coefficients and $\mathbf{\Sigma}$ is a $q\times q$ variance-covariance matrix. Equivalently the general model of the latent variables can be expressed as a linear combination of the observed covariates with parameters regression coefficients and parameter residuals in the matrix form

$$\theta = \mathbf{YB} + \mathbf{E} \tag{2.19}$$

where $\theta$ is the $n\times q$ matrix of latent variables,$\mathbf{Y}$ is a $n\times p$ matrix of observed covariates, and $\mathbf{E}$ is the $n\times q$ matrix of residuals. In general, if we let $p\times q$ matrix $\widehat{\mathbf{B}}$ be the of estimate of $\mathbf{B}$. Then the maximum likelihood estimate of $\mathbf{B}$ is given by

$$\widehat{\mathbf{B}} = (\mathbf{Y}^t\mathbf{Y})^{-1}\mathbf{Y}^t\theta. \tag{2.20}$$

Furthermore, application of Fisher's identity leads to the expression of a vector of the first order partial derivative with respect to $\mathbf{B}$, that is

$$\begin{aligned}\mathbf{h}(\mathbf{B}) = \frac{\partial}{\partial\mathbf{B}}\ln L(\mathbf{B}|\mathbf{X},\mathbf{D}) &= \int\ldots\int(\mathbf{B}-(\mathbf{Y}^t\mathbf{Y})^{-1}\mathbf{Y}^t\theta)p(\theta|\mathbf{X})d\theta\\ &= \mathbf{B}-(\mathbf{Y}^t\mathbf{Y})^{-1}\int\ldots\int\mathbf{Y}^t\theta p(\theta|\mathbf{X})d\theta\\ &= \mathbf{B}-(\mathbf{Y}^t\mathbf{Y})^{-1}\left[\mathbf{Y}^tE(\theta|X)\right].\end{aligned} \tag{2.21}$$

Now, simplifying the expectation of the $\theta$ given the data, we have

$$\mathbf{h}(\mathbf{B}) = \mathbf{B}-(\mathbf{Y}^t\mathbf{Y})^{-1}\sum_i^N\left[\mathbf{y}_i^tE(\theta_i|\mathbf{x}_i)\right]. \tag{2.22}$$

Setting $\mathbf{h}(\mathbf{B})=\mathbf{0}$, we can calculate $\widehat{\mathbf{B}}$ as

$$\widehat{\mathbf{B}}= (\mathbf{Y}^t\mathbf{Y})^{-1}\sum_i^N\left[\mathbf{y}_i^tE(\theta_i|\mathbf{x}_i)\right]. \tag{2.23}$$

We can solve the estimate of the variance-covariance of the residuals $\mathbf{\Sigma}_\varepsilon$ as

$$\widehat{\mathbf{\Sigma}_\varepsilon} = \frac{1}{N}\sum_{i}^{N}\int_{\theta_i}\left(\theta_i - \mathbf{B}^t\mathbf{y}_i\right)\left(\theta_i - \mathbf{B}^t\mathbf{y}_i\right)^t g(\theta|\mathbf{y_i}, \mathbf{B}, \mathbf{\Sigma}_\varepsilon)d\theta. \qquad (2.24)$$

## 2.4 Simulation procedure

A simulation study was undertaken to asses the effect of a missing data process as described in (2.3) and (2.4) on the estimates of item parameters. The simulation study consisted of two parts. The first part extends the study by Holman and Glas (2005) to a situation where the model for the missing data indicators is multidimensional, and studies the effects of including no, part of, or all latent dimensions of this model in the estimation. The second simulation study pertains to the effects of adding observed covariates to the model.

### *2.4.1 Data generation and parameter estimation*

To study the effects of including no, part of, or all latent dimensions of the model for the missing data process in the estimation procedure, latent person parameters were drawn from three-variate normal distribution. The sample size was $N = 500$ persons. The variances of the latent variables was always equal to one. The correlation between the latent trait variables $\theta_i$ and $\zeta_i$, $\rho(\theta, \zeta)$, varied as $0.0, 0.4$ and $0.8$. Also the correlations between the two dimensions of the missing data process $\rho(\zeta_1, \zeta_2)$, varied as $0.0, 0.4$ and $0.8$. The items were either dichotomously and polytomously scored. The test consisted of $K = 10$ items. The values $d_{ik}$ and $x_{ik}$ were drawn from $p(d_{ik}|\ \zeta_i, \gamma_k, \delta_k)$ and $p(x_{ik}|d_{ik}, \theta_{i,}\alpha_k, \beta_k)$, respectively. The data were used to compute MML estimates of the item parameters under various assumptions. Then the values of item parameters estimates over replications $r$ $(r = 1, ..., R,\ R = 100)$, say $\widehat{\phi_r}$ were compared with the values of the parameters used to generate the data using the mean absolute error (MAE) and mean squared error (MSE). There is no index $k$ because all item parameters were equal. The formula to obtained MAE for item parameters is given by

$$MAE(\phi) = \frac{1}{R}\sum_{r=1}^{R}\left|\widehat{\phi_r} - \phi\right| \qquad (2.25)$$

where $R$ denote the number of replications of the simulation procedure and $\widehat{\beta_i}$ is the estimate of the item parameter $\beta_k$. On the other hand to obtained the MSE for $\beta_k$,it is given by

$$MSE(\phi) = \frac{1}{R}\sum_{r=1}^{R}\left(\widehat{\phi_r} - \phi\right)^2. \tag{2.26}$$

For the dichotomous case in the simulation, two conditions were used: in the first, the item parameters for all $k$ were $\alpha_k = 1, \gamma_k = 1, \delta_k = -1$ and $\beta_k = 0$ , these initial entries give us about 25% missing data and, in the second, we considered $\alpha_k = 1, \gamma_k = 1$, $\delta_k = 0$ and $\beta_k = 0$ which results to about 50% missing data. The MAE and MSE results of the item parameters estimates for the combination $K = 10$ and $N = 500$ are given in Table 2.1 and Table 2.2.

For the polytomous case, items with three response categories were used in the simulation. The item parameters for all $k$ were $\alpha_k = 1, \gamma_k = 1, \delta_k = -1$ and $\beta_k = -1, 1$, and $\alpha_k = 1, \gamma_k = 1, \delta_k = 0$ and $\beta_k = -1, 1$. The MAE and MSE results of the item parameters estimates for the combination $K = 10$, $N = 500$ are given in Tables 2.3 and 2.4.

TABLE 2.1. MAE of item parameter estimates under MAR and NON-MAR models(dichotomous Case); Estimation Model: (Observed data: 2PL, missing data: 1PL); Dimension of missing data process=2; N=500; K=10; $\alpha = 1$; $\beta = 0$; $\gamma = 1$; $\rho_1 = \rho(\theta, \zeta)$; $\rho_2 = \rho(\zeta_1, \zeta_2)$.

| | | | | Mean Absolute Error | | | |
|---|---|---|---|---|---|---|---|
| $\delta$ | $\rho_1$ | $\rho_2$ | DMis | $\alpha$ | $\beta$ | $\delta$ | $\gamma$ |
| -1 | .0 | - | - | .168 | .102 | | |
| | .4 | .0 | 0 | .169 | .118 | | |
| | | | 1 | .163 | .113 | .126 | .467 |
| | | | 2 | .163 | .113 | .106 | .162 |
| | | .4 | 0 | .169 | .107 | | |
| | | | 1 | .164 | .102 | .109 | .205 |
| | | | 2 | .165 | .102 | .108 | .149 |
| | | .8 | 0 | .170 | .110 | | |
| | | | 1 | .165 | .104 | .100 | .137 |
| | | | 2 | .165 | .104 | .104 | .140 |
| | .8 | .4 | 0 | .176 | .140 | | |
| | | | 1 | .156 | .105 | .101 | .151 |
| | | | 2 | .160 | .104 | .099 | .135 |
| | | .8 | 0 | .170 | .137 | | |
| | | | 1 | .153 | .103 | .099 | .136 |
| | | | 2 | .156 | .103 | .103 | .137 |
| 0 | .0 | - | - | .225 | .120 | | |
| | .4 | .0 | 0 | .245 | .148 | | |
| | | | 1 | .228 | .133 | .081 | .568 |
| | | | 2 | .223 | .128 | .089 | .154 |
| | | .4 | 0 | .229 | .142 | | |
| | | | 1 | .209 | .124 | .079 | .194 |
| | | | 2 | .209 | .125 | .084 | .147 |
| | | .8 | 0 | .222 | .144 | | |
| | | | 1 | .214 | .121 | .086 | .126 |
| | | | 2 | .214 | .122 | .088 | .126 |
| | .8 | .4 | 0 | .257 | .210 | | |
| | | | 1 | .187 | .128 | .078 | .158 |
| | | | 2 | .186 | .129 | .083 | .136 |
| | | .8 | 0 | .245 | .220 | | |
| | | | 1 | .192 | .133 | .083 | .121 |
| | | | 2 | .194 | .133 | .083 | .121 |

TABLE 2.2. MSE of item parameter estimates under MAR and NON-MAR model (dichotomous Case); Estimation Model: (Observed data: 2PL, missing data: 1PL); Dimension of missing data process=2; N=500; K=10; $\alpha = 1$; $\beta = 0$; $\gamma = 1$; $\rho_1 = \rho(\theta, \zeta)$; $\rho_2 = \rho(\zeta_1, \zeta_2)$.

| | | | | Mean Squared Error | | | |
|---|---|---|---|---|---|---|---|
| $\delta$ | $\rho_1$ | $\rho_2$ | DMis | $\alpha$ | $\beta$ | $\delta$ | $\gamma$ |
| -1 | .0 | - | - | .046 | .016 | | |
| | .4 | .0 | 0 | .046 | .021 | | |
| | | | 1 | .043 | .020 | .023 | .312 |
| | | | 2 | .043 | .020 | .018 | .043 |
| | | .4 | 0 | .047 | .018 | | |
| | | | 1 | .044 | .016 | .019 | .065 |
| | | | 2 | .044 | .016 | .019 | .039 |
| | | .8 | 0 | .047 | .019 | | |
| | | | 1 | .044 | .017 | .016 | .031 |
| | | | 2 | .044 | .017 | .017 | .032 |
| | .8 | .4 | 0 | .051 | .029 | | |
| | | | 1 | .039 | .017 | .016 | .035 |
| | | | 2 | .042 | .017 | .016 | .030 |
| | | .8 | 0 | .047 | .028 | | |
| | | | 1 | .038 | .017 | .015 | .029 |
| | | | 2 | .040 | .017 | .017 | .030 |
| 0 | .0 | - | - | .089 | .023 | | |
| | .4 | .0 | 0 | .110 | .034 | | |
| | | | 1 | .088 | .028 | .010 | .423 |
| | | | 2 | .085 | .026 | .013 | .040 |
| | | .4 | 0 | .088 | .031 | | |
| | | | 1 | .072 | .024 | .010 | .062 |
| | | | 2 | .072 | .024 | .012 | .036 |
| | | .8 | 0 | .087 | .032 | | |
| | | | 1 | .077 | .023 | .012 | .025 |
| | | | 2 | .076 | .023 | .013 | .027 |
| | .8 | .4 | 0 | .023 | .062 | | |
| | | | 1 | .056 | .026 | .010 | .038 |
| | | | 2 | .056 | .026 | .011 | .029 |
| | | .8 | 0 | .104 | .067 | | |
| | | | 1 | .062 | .028 | .011 | .023 |
| | | | 2 | .064 | .028 | .012 | .024 |

TABLE 2.3. MAE of item parameter estimates under MAR and NON-MAR model (Polytomous Case); Estimation Model: (Observed data: PCM, missing data: 1PL); Dimension of missing data process=2; N=500; K=10; $\alpha = 1$; $\beta = -1, 1$; $\gamma = 1$; $\rho_1 = \rho(\theta, \zeta)$; $\rho_2 = \rho(\zeta_1, \zeta_2)$.

| | | | | Mean Absolute Error | | | | |
|---|---|---|---|---|---|---|---|---|
| $\delta$ | $\rho_1$ | $\rho_2$ | DMis | $\alpha$ | $\beta 1$ | $\beta 2$ | $\delta$ | $\gamma$ |
| -1 | .0 | - | - | .137 | .135 | .194 | | |
| | .4 | .0 | 0 | .142 | .129 | .194 | | |
| | | | 1 | .139 | .126 | .192 | .127 | .470 |
| | | | 2 | .138 | .125 | .193 | .103 | .167 |
| | | .4 | 0 | .138 | .139 | .198 | | |
| | | | 1 | .136 | .129 | .194 | .109 | .192 |
| | | | 2 | .135 | .129 | .194 | .107 | .162 |
| | | .8 | 0 | .136 | .137 | .206 | | |
| | | | 1 | .133 | .126 | .193 | .098 | .138 |
| | | | 2 | .132 | .127 | .192 | .100 | .139 |
| | .8 | .4 | 0 | .152 | .153 | .247 | | |
| | | | 1 | .140 | .129 | .200 | .100 | .154 |
| | | | 2 | .138 | .126 | .200 | .103 | .138 |
| | | .8 | 0 | .138 | .150 | .241 | | |
| | | | 1 | .129 | .125 | .196 | .098 | .130 |
| | | | 2 | .128 | .125 | .197 | .102 | .130 |
| 0 | .0 | - | - | .187 | .156 | .239 | | |
| | .4 | .0 | 0 | .182 | .173 | .259 | | |
| | | | 1 | .175 | .148 | .250 | .080 | .548 |
| | | | 2 | .174 | .145 | .242 | .088 | .155 |
| | | .4 | 0 | .189 | .173 | .257 | | |
| | | | 1 | .182 | .150 | .241 | .078 | .182 |
| | | | 2 | .182 | .150 | .243 | .084 | .143 |
| | | .8 | 0 | .188 | .182 | .274 | | |
| | | | 1 | .183 | .151 | .246 | .087 | .131 |
| | | | 2 | .183 | .151 | .246 | .090 | .129 |
| | .8 | .4 | 0 | .197 | .228 | .367 | | |
| | | | 1 | .165 | .148 | .245 | .081 | .152 |
| | | | 2 | .167 | .143 | .247 | .086 | .137 |
| | | .8 | 0 | .195 | .241 | .411 | | |
| | | | 1 | .170 | .154 | .250 | .088 | .120 |
| | | | 2 | .171 | .153 | .249 | .090 | .123 |

TABLE 2.4. MSE of item parameter estimates under MAR and NON-MAR model (Polytomous Case); Estimation Model: (Observed data: PCM, missing data: 1PL); Dimension of missing data process=2; N=500; K=10; $\alpha = 1$; $\beta = -1, 1$; $\gamma = 1$; $\rho_1 = \rho(\theta, \zeta)$; $\rho_2 = \rho(\zeta_1, \zeta_2)$.

| | | | | Mean Squared Error | | | | |
|---|---|---|---|---|---|---|---|---|
| $\delta$ | $\rho_1$ | $\rho_2$ | DMis | $\alpha$ | $\beta 1$ | $\beta 2$ | $\delta$ | $\gamma$ |
| -1 | .0 | - | - | .031 | .029 | .062 | | |
| | .4 | .0 | 0 | .033 | .027 | .058 | | |
| | | | 1 | .031 | .025 | .059 | .023 | .322 |
| | | | 2 | .031 | .024 | .061 | .016 | .046 |
| | | .4 | 0 | .031 | .032 | .061 | | |
| | | | 1 | .030 | .027 | .060 | .018 | .058 |
| | | | 2 | .030 | .027 | .060 | .019 | .044 |
| | | .8 | 0 | .030 | .031 | .065 | | |
| | | | 1 | .029 | .025 | .059 | .015 | .030 |
| | | | 2 | .029 | .025 | .059 | .016 | .032 |
| | .8 | .4 | 0 | .037 | .037 | .090 | | |
| | | | 1 | .029 | .026 | .064 | .015 | .036 |
| | | | 2 | .030 | .024 | .063 | .017 | .030 |
| | | .8 | 0 | .031 | .037 | .087 | | |
| | | | 1 | .026 | .025 | .061 | .016 | .027 |
| | | | 2 | .026 | .025 | .061 | .017 | .026 |
| 0 | .0 | - | - | .062 | .039 | .091 | | |
| | .4 | .0 | 0 | .057 | .047 | .101 | | |
| | | | 1 | .053 | .035 | .100 | .010 | .418 |
| | | | 2 | .052 | .033 | .095 | .012 | .039 |
| | | .4 | 0 | .062 | .049 | .100 | | |
| | | | 1 | .057 | .036 | .097 | .010 | .054 |
| | | | 2 | .057 | .036 | .098 | .011 | .035 |
| | | .8 | 0 | .060 | .055 | .115 | | |
| | | | 1 | .057 | .038 | .098 | .012 | .028 |
| | | | 2 | .056 | .038 | .098 | .012 | .027 |
| | .8 | .4 | 0 | .064 | .079 | .187 | | |
| | | | 1 | .041 | .035 | .092 | .010 | .034 |
| | | | 2 | .043 | .033 | .095 | .012 | .030 |
| | | .8 | 0 | .064 | .088 | .225 | | |
| | | | 1 | .046 | .037 | .101 | .012 | .023 |
| | | | 2 | .047 | .036 | .101 | .013 | .024 |

All four tables have the same format. In all tables, $\delta$ refers to the difficulty parameter for the missing data process used in generating data. Consider Table 2.1. The first row pertains to a base-line condition where $\rho(\theta, \zeta) = 0.0$. So ignorability holds, and there are 25% missing data. The values of the MAE($\alpha$) and MAE($\beta$) given in the two columns labeled $\alpha$ and $\beta$; they are the mean absolute errors over the 100 replications, and they serve as a baseline. The next three rows pertain to data generated using $\rho(\theta, \zeta) = 0.4$ and $\rho(\zeta_1, \zeta_2) = 0.0$. These data were analyzed using no, one and two dimensions for the missing data indicator. The column DMis refers to the number of dimension of the latent variable for the missing data process. The columns denoted by $\alpha$, $\beta$, $\delta$ and $\gamma$ are the estimated values of the mean absolute errors of the item parameters for the observed data $\alpha$ (discrimination), $\beta$ (difficulty) and the missing data process $\delta$ (difficulty), $\gamma$ (discrimination). For polytomous case, reported in the Table 2.3 there are two columns for the mean absolute error of the location parameters referred as $\beta_1$ and $\beta_2$. The analogous mean squared errors are given in Table 2.2 and Table 2.4.

The simulations (please refer to Table 2.1 until Table 2.4) showed that both MAE and MSE values of the item parameters in the parameter estimates were inflated when the model for missing data process was excluded in the parameter estimation. The effect increased as correlation between the latent variables for both observed data and the missing data process increased. For instance if we consider Table 2.1, when $\delta = 0$, (that is, when there are 50% missing data) the baseline, which refers to $MAR$ data, shows that MAE($\alpha$) = 0.225 and MAE($\beta$) = 0.120. When $\rho(\theta, \zeta) = 0.4$ and $\rho(\zeta_1, \zeta_2) = 0.0$, and the missing data is ignored (DMis = 0), the MAE for $\alpha$ and $\beta$ have values 0.245 and 0.148 respectively. So the first conclusion is that ignoring the missing data process leads to inflated estimation errors.

When the model for the missing data process was included in the analysis, that is, when the $NONMAR$ model was used, the MAE values dropped to 0.228 for $\alpha$ and 0.133 for $\beta$ when DMis=1 and MAE($\alpha$) = 0.223 and MAE($\beta$) = 0.128, when DMis=2. In general, a decrease in the values of the MAE and the MSE of the item parameters was observed and this decrease was positively related to the number of dimensions included. Similar results are also observed for the values of MAE and MSE of the item parameters $\delta$ and $\gamma$ for

missing data process. So the second conclusion is that invoking the missing data process leads to a reduction of estimation errors, even if it is not completely invoked.

The third conclusion that can be drawn from the tables is that when the missing data process is completely modeled, the estimation errors can even fall below the errors of the baseline. For instance, in Table 2.1 we see that for $\delta = 0.0$, the MAE($\alpha$) = 0.225 for the baseline and MAE($\alpha$) = 0.194 for $\rho(\theta, \zeta) = \rho(\zeta_1, \zeta_2) = 0.8$ and DMis = 2. Obviously, invoking a model for the missing data indicator results in the exploitation of collateral information.

The fourth conclusion pertains to a main effect of the extent to which MAR is violated. Inspection of the tables shows that if we ignore the missing data process (DMis = 0), the magnitude of the estimation error for $\rho(\theta, \zeta) = 0.8$ is greater than the magnitude for $\rho(\theta, \zeta) = 0.4$. For instance, in Table 2.1 we see that conditionally on $\rho(\zeta_1, \zeta_2) = 0.4$, the MAEs for $\alpha$ are .229 and .257, respectively. Finally, if we consider all results, there is no clear effect of $\rho(\zeta_1, \zeta_2)$

### *2.4.2 Data generation and parameter estimation with observed covariates*

The simulation procedure used was analogous to the simulation procedure in the previous section, but with added feature of including observed covariates. To achieve comparability with the previous section, the regression coefficients were chosen as follows. Let $\Sigma_\theta$ be the covariance matrix of both the latent abilities for the observed responses and the missing data indicator. As in the previous section, there was one dimension for the observed responses and there were two dimensions for the missing data process. Only the case $\rho(\zeta_1, \zeta_2) = 0.8$ was considered here. Further, either $\rho(\theta, \zeta) = 0.4$ or $\rho(\theta, \zeta) = 0.8$. Let $\Sigma_\varepsilon$ be the diagonal matrix of the variances of the error terms. These variances were all equal to 0.15. The regression coefficients $\mathbf{B}$ were chosen such that

$$\Sigma_\theta = \mathbf{B}\mathbf{B}^t + \Sigma_\varepsilon.$$

Note that the matrix $\mathbf{B}$ is now the Cholesky-decomposition of the matrix $\Sigma_\theta - \Sigma_\varepsilon$, so the upper off-diagonal elements are equal to zero. The latent variables were ordered in such a way that the regression model for the latent variable for the observations only depended

on the first covariate, and the two latent variables for the missing data indicator depended on the first two and all three covariates, respectively.

As before, the sample size was $N = 500$. Again the test length was $K = 10$ and the item parameters were also as used above. One hundred replications were made for every combination of $\delta$, $\rho(\theta, \zeta)$ and DMis, where DMis is again the number of dimensions included for the missing data process.

The results are given in the Tables 2.5, 2.6, 2.7 and 2.8. The format of the tables is analogous to the previous four tables, except for an added column *ncov*, which refers to the number of covariates that were included in the parameter estimation. Note that also the baseline model where $\rho(\theta, \zeta) = 0.0$ (the MAR model) includes a covariate. This was done to enable the comparison with the NONMAR models.

TABLE 2.5. MAE of item parameter estimates under MAR and NONMAR model (dichotomous Case); Estimation Model: (Observed data: 2PL, missing data: 1PL); variance=0.15; N=500; K=10; $\alpha = 1$; $\beta = 0$; $\gamma = 1$; $\rho_1 = \rho(\theta, \zeta)$; $\rho_2 = \rho(\zeta_1, \zeta_2)$.

| | | | | | Mean Absolute Error | | | |
|---|---|---|---|---|---|---|---|---|
| $\delta$ | $\rho_1$ | $\rho_2$ | DMis | ncov | $\alpha$ | $\beta$ | $\delta$ | $\gamma$ |
| -1 | .0 | - | - | 1 | .121 | .095 | | |
| | .4 | .8 | 0 | 1 | .157 | .117 | | |
| | | | 1 | 2 | .131 | .097 | .102 | .150 |
| | | | 2 | 3 | .120 | .097 | .095 | .104 |
| | .8 | .8 | 0 | 1 | .188 | .142 | | |
| | | | 1 | 2 | .159 | .110 | .092 | .127 |
| | | | 2 | 3 | .126 | .101 | .089 | .100 |
| 0 | .0 | - | - | 1 | .145 | .117 | | |
| | .4 | .8 | 0 | 1 | .170 | .164 | | |
| | | | 1 | 2 | .150 | .116 | .083 | .134 |
| | | | 2 | 3 | .140 | .114 | .078 | .094 |
| | .8 | .8 | 0 | 1 | .313 | .228 | | |
| | | | 1 | 2 | .204 | .134 | .092 | .125 |
| | | | 2 | 3 | .155 | .126 | .084 | .097 |

TABLE 2.6. MSE of item parameter estimates under MAR and NONMAR model (dichotomous Case); Estimation Model: (Observed data: 2PL, missing data: 1PL); variance=0.15; N=500; K=10; $\alpha = 1$; $\beta = 0$; $\gamma = 1$; $\rho_1 = \rho(\theta, \zeta)$; $\rho_2 = \rho(\zeta_1, \zeta_2)$.

| | | | | | Mean Squared Error | | | |
|---|---|---|---|---|---|---|---|---|
| $\delta$ | $\rho_1$ | $\rho_2$ | DMis | NCOV | $\alpha$ | $\beta$ | $\delta$ | $\gamma$ |
| -1 | .0 | - | - | 1 | .023 | .014 | | |
| | .4 | .8 | 0 | 1 | .042 | .020 | | |
| | | | 1 | 2 | .028 | .015 | .017 | .036 |
| | | | 2 | 3 | .022 | .015 | .015 | .016 |
| | .8 | .8 | 0 | 1 | .059 | .031 | | |
| | | | 1 | 2 | .039 | .019 | .013 | .024 |
| | | | 2 | 3 | .024 | .016 | .012 | .015 |
| 0 | .0 | - | - | 1 | .034 | .022 | | |
| | .4 | .8 | 0 | 1 | .051 | .040 | | |
| | | | 1 | 2 | .038 | .022 | .011 | .029 |
| | | | 2 | 3 | .031 | .021 | .010 | .014 |
| | .8 | .8 | 0 | 1 | .190 | .070 | | |
| | | | 1 | 2 | .075 | .028 | .013 | .025 |
| | | | 2 | 3 | .037 | .025 | .011 | .015 |

TABLE 2.7. MAE of item parameter estimates under MAR and NONMAR model (Polytomous Case); Estimation Model: (Observed data: PCM, missing data: 1PL); variance=0.15; N=500; K=10; $\alpha = 1$; $\beta = -1, 1$; $\gamma = 1$; $\rho_1 = \rho(\theta, \zeta)$; $\rho_2 = \rho(\zeta_1, \zeta_2)$.

| | | | | | Mean Absolute Error | | | | |
|---|---|---|---|---|---|---|---|---|---|
| $\delta$ | $\rho_1$ | $\rho_2$ | DMis | ncov | $\alpha$ | $\beta 1$ | $\beta 2$ | $\delta$ | $\gamma$ |
| -1 | .0 | - | - | 1 | .109 | .118 | .175 | | |
| | .4 | .8 | 0 | 1 | .139 | .133 | .212 | | |
| | | | 1 | 2 | .115 | .120 | .179 | .099 | .149 |
| | | | 2 | 3 | .108 | .119 | .177 | .093 | .103 |
| | .8 | .8 | 0 | 1 | .143 | .159 | .254 | | |
| | | | 1 | 2 | .130 | .125 | .198 | .103 | .132 |
| | | | 2 | 3 | .107 | .119 | .180 | .096 | .104 |
| 0 | .0 | - | - | 1 | .126 | .146 | .217 | | |
| | .4 | .8 | 0 | 1 | .166 | .180 | .309 | | |
| | | | 1 | 2 | .138 | .143 | .211 | .090 | .143 |
| | | | 2 | 3 | .128 | .140 | .211 | .084 | .101 |
| | .8 | .8 | 0 | 1 | .191 | .241 | .417 | | |
| | | | 1 | 2 | .159 | .148 | .238 | .089 | .126 |
| | | | 2 | 3 | .127 | .140 | .219 | .084 | .097 |

TABLE 2.8. MSE of item parameter estimates under MAR and NONMAR model (Polytomous Case); Estimation Model: (Observed data: PCM, missing data: 1PL); variance=0.15 ; N=500; K=10; $\alpha = 1$; $\beta = -1, 1$; $\gamma = 1$; $\rho_1 = \rho(\theta, \zeta)$ ; $\rho_2 = \rho(\zeta_1, \zeta_2)$.

| | | | | | Mean squared Error | | | | |
|---|---|---|---|---|---|---|---|---|---|
| $\delta$ | $\rho_1$ | $\rho_2$ | DMis | ncov | $\alpha$ | $\beta 1$ | $\beta 2$ | $\delta$ | $\gamma$ |
| -1 | .0 | - | - | 1 | .019 | .022 | .049 | | |
| | .4 | .8 | 0 | 1 | .032 | .028 | .069 | | |
| | | | 1 | 2 | .021 | .023 | .050 | .016 | .036 |
| | | | 2 | 3 | .018 | .022 | .049 | .014 | .016 |
| | .8 | .8 | 0 | 1 | .033 | .039 | .093 | | |
| | | | 1 | 2 | .026 | .025 | .060 | .017 | .027 |
| | | | 2 | 3 | .018 | .022 | .051 | .014 | .017 |
| 0 | .0 | - | - | 1 | .026 | .034 | .074 | | |
| | .4 | .8 | 0 | 1 | .045 | .052 | .135 | | |
| | | | 1 | 2 | .031 | .033 | .069 | .013 | .034 |
| | | | 2 | 3 | .026 | .031 | .069 | .011 | .015 |
| | .8 | .8 | 0 | 1 | .063 | .089 | .227 | | |
| | | | 1 | 2 | .041 | .034 | .093 | .012 | .025 |
| | | | 2 | 3 | .024 | .031 | .076 | .011 | .015 |

Referring to Table 2.5, when we have $\delta = 0$, i.e., 50% missing data. The baseline showed entries for MAE($\alpha$) = 0.145 and MAE($\beta$) = 0.117 (as compared to the first simulation in Table 2.1, MAE($\alpha$) = 0.225 and MAE($\beta$) = 0.120). This increase in precision is due to the inclusion of a covariate. When we increased the correlation to $\rho(\theta, \zeta) = 0.4$ and $\rho(\zeta_1, \zeta_2) = 0.8$, results showed that when the missing data process was ignored and only the covariate for $\theta$ was included in the estimation, the values MAE($\alpha$) = 0.170 and MAE($\beta$) = 0.164 were obtained. When one dimension for the missing data process using the $NONMAR$ model which include two covariates were considered i.e., $ncov = 2$, results showed MAE($\alpha$) = 0.150 and MAE($\beta$) = 0.116. Further, when two dimensions for the missing data process in the $NONMAR$ model were considered i.e., when three covariates were included in the model for the missing data, results obtained were MAE($\alpha$) = 0.140 and MAE($\beta$) = 0.114

It can be seen that increasing the correlation of the latent variables $\theta$ and $\zeta$ that is increasing the violation of ignorability, resulted in a more bias in the parameter estimates when the covariates are ignored. Including them reduced the bias to a value close to the baseline.

# 3

# Modeling Nonignorable Missing Data in Speeded Tests

ABSTRACT: If a test is administered under a limited-time condition, items at the end of the test are often not endorsed. In most instances, the pattern of missing responses depends on the ability that is measured and, therefore, the missing data are not ignorable in statistical inference. In the present paper, the data are modeled using a combination of two item response theory (IRT) models: one IRT model for the observed response data and one IRT model for the missing data indicator. The missing data indicator is modeled using the sequential model by Tutz (1990, also see, Verhelst, Glas & de Vries, 1997). The two IRT models are connected by invoking the assumption that their latent person parameters have a joint multivariate normal distribution. The model parameters are estimated using marginal maximum likelihood. Simulation studies showed that treating the missing data as ignorable leads to considerable bias in the parameter estimates. Further, it was found that including an IRT model for the missing data removes this bias in the parameter estimates. The impact of the method in practical situations is illustrated with data from the calibration of a time-limit test for measuring intelligence.

KEYWORDS: ignorability, item response theory, marginal maximum likelihood, nonignorable missing data, sequential model, step model

## 3.1 Introduction

Missing data can be organized into two categories: ignorable and nonignorable missing data. If the missing data are missing at random (MAR) and the parameter of interest and the parameters of the missing data process are distinct, the missing data are ignorable.

That is, with these assumptions, inferences based on the likelihood function and likelihood ratios that ignore the missing data process are valid and consistent (Rubin, 1976; Little & Rubin, 1987; Heitjan, 1994).

When the missing data are nonignorable, the likelihood function and likelihood ratios that ignored the missing data process give rise to biased item parameter estimates (Holman and Glas, 2005). An appropriate method to deal with these problems is to model the missing data process (Heckman, 1979). The idea is to identify and model the explanatory variables in the missing data mechanism or process that caused the missing data. Basing inferences concurrently on this model and the relevant model for the observed data, reduces bias caused by ignoring nonignorable missing data (see for instance O'Muircheartaigh & Moustaki, 1999); Moustaki & O'Muircheartaigh, 2000; Bartholomew & Knott, 1999; Moustaki & Knott, 2000; Holman & Glas, 2005).

In this chapter, we are dealing with item nonresponses in tests and examinations where responses are missing consecutively on items at the end of the test, that is, the respondent has not reached the end of the test because of a time limit. It must be expected that the number of items endorsed is correlated with the respondent's ability level and therefore, the missingness is nonignorable. This form of missingness is closely related to missingness caused by skipping of items by respondents with a low ability. Holman & Glas (2005) show that ignoring this missing data process can lead to bias in the estimates of the item parameters. Bradlow and Thomas (1998) also mentioned that ignoring this type of missing data process could produce bias in the parameter estimates.

In this chapter, it is shown that the missing data indicator of a test with a time limit can be modeled by the sequential model by Tutz (1990), also known as the steps model (Verhelst, Glas, & De Vries, 1997). The observed responses will be modeled by the 2PL model, but this choice is not essential. The step model for the missing data indicator could be combined with any parametric IRT model.

This chapter is made up of six sections and is organized as follows. After this section, a general notation is presented for IRT models for the missing data process and the model for observed data will be discussed. Then a presentation about the estimation procedure using the marginal maximum likelihood method follows. In the next

section the results of a number of simulation studies will be presented. An application of the method using an intelligence test will be undertaken in the fifth section. Finally, the last section gives a discussions of the results, and some conclusions and recommendations for further research.

## 3.2 A General IRT Model

### *3.2.1 General IRT model for missing data*

Let $\mathbf{X}$ be a two-dimensional data matrix with elements $x_{ik}$, where the persons are indexed $i = 1, ..., N$ and items $k = 1, ..., K$. When the combination of $i$ and $k$ is observed, the entry $x_{ik}$ is the observation, otherwise it is equal to some arbitrary constant. We define a design matrix $\mathbf{D}$ of the same dimension as $X$ with elements

$$d_{ik} = \begin{cases} 0 & \text{if } x_{ik} \text{ is missing} \\ 1 & \text{if } x_{ik} \text{ is observed.} \end{cases}$$

To model the responses missing as a result of the speededness of the test, we will focus on the unobserved responses at the end of the response pattern. So the focus will be on a string of missing data indicators $d_{ik} = 0$, for $k = k', ..., K$. Intermediate missing responses will be considered ignorable missing data.

The table below presents a $N \times 2K$ data matrix , where $N$ is the total number of respondents and $K$ is the total numbers of items. The matrix contains the observed data $\mathbf{X}$ (with missing data indicated by 9 as a dummy ) and the missing data indicator $\mathbf{D}$.

| | Observed data **X** | | | | | | Missing data indicator **D** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Persons | 1 | 2 | 3 | $k$ | . | $K$ | 1 | 2 | 3 | $k$ | . | $K$ |
| 1 | 0 | 1 | 9 | 9 | 9 | 9 | 1 | 1 | 0 | - | - | - |
| 2 | 1 | 1 | 9 | 9 | 9 | 9 | 1 | 1 | 0 | - | - | - |
| 3 | 1 | 0 | 1 | 9 | 9 | 9 | 1 | 1 | 1 | 0 | - | - |
| . | . | . | . | . | . | . | . | . | . | . | . | . |
| $i$ | 1 | 1 | 0 | 1 | 9 | 9 | 1 | 1 | 1 | 1 | 0 | - |
| . | . | . | . | . | . | . | . | . | . | . | . | . |
| | 1 | 0 | 1 | 0 | 1 | 9 | 1 | 1 | 1 | 1 | 1 | 0 |
| $N$ | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |

The item nonresponse occurred due to time limit condition and interacts with the level ability of the respondent. In a case of dichotomously scored items, respondent $i$ answering to item $k$ can get a correct 1 or incorrect 0 response. Then he stumbled on an item which has 0 entry in **D** and 9 in **X**,and the succeeding items are skipped as well. To model the missing data process of this case, we use the steps model (Verhelst, Glas, & De Vries;1997) given by

$$p(d_{i1} = d_{i2} = ... = d_{ik-1} = 1 \ \& \ d_{ik} = 0) = \left[\prod_{h=1}^{k} p_k\left(\zeta_i\right)\right] \left[1 - p_{k+1}(\zeta_i)\right] \quad \text{if } 0 \leq k < K \tag{3.1}$$

and

$$p(d_{i1} = d_{i2} = ... = d_{ik-1} = d_{ik} = 1) = \prod_{k=1}^{K} p_k\left(\zeta_i\right) \quad \text{if } k = K, \tag{3.2}$$

where we assumed that

$$p_k\left(\zeta_i\right) = \frac{\exp(\gamma_k \zeta_i - \delta_k)}{1 + \exp(\gamma_k \zeta_i - \delta_k)}. \tag{3.3}$$

So $p_k\left(\zeta_i\right)$ is equivalent to the 2PL model for dichotomously scored items. The model entails that the respondent makes item-steps until the first wrong response, and then stops taking item-steps. Usually the data show too little variation to estimate the slope parameter $\gamma_k$, so usually we assume that $\gamma_k = 1$. Note that $\zeta_i$ refers to the latent variable for the missing data process. Further, we impose a restriction on the difficulty of the item -steps:

$$\delta_k = \tau + (k - K)\eta,$$

where $\tau$ is the baseline (overall) level and $\eta$ models a monotone change in the probability of an observation as a function of the position of the item in the test. The reason for this restriction on $\delta_k$ is that the first item-steps are usually taken by all respondents, so the difficulty of these steps cannot be estimated. Further, the restriction supports a monotonously decreasing probability of observing a response.

### *3.2.2 Combined IRT models for Observed data and missing data*

The models that we will use for the comparison are analogous to the $MAR$ and $NONMAR$ models described in Holman and Glas (2005) and in the previous chapter (refer to equation (2.2) and (2.3)). The likelihood of the $MAR$ model is given by

$$\prod_{i,k} p(x_{ik}|d_{ik}, \theta_{i,}\alpha_k, \beta_k)p(d_{ik}|\zeta_i, \gamma_k, \delta_k)g(\zeta_i)g(\theta_i) \tag{3.4}$$

where $p(x_{ik}|d_{ik}, \theta_{i,}\alpha_k, \beta_k)$ is the measurement model, $\theta_{i,}$ is the latent person ability parameter, and $\alpha_k, \beta_k$ are item parameters of the observed data. Further, $p(d_{ik}|\zeta_i, \gamma_k, \delta_k)$ is the model for the missing data indicator and $\gamma_k, \delta_k$ are item parameters of the missing data process. Finally, $g(\zeta_i)$ and $g(\theta_i)$ are the densities of the latent parameters. We assume these densities to be standard normal. In (3.4), the latent variables $\theta$ for the observed data and $\zeta$ for the missing data process are not correlated and hence we can ignore the model for the missing data i.e. for maximum likelihood estimation we can ignore $p(d_{ik}|\zeta_i, \gamma_k, \delta_k)g(\zeta_i)$. On the other hand, the $NONMAR$ model also described in Chapter 2 in this thesis, is the model where the missing data process is included in the estimation. In that case, the latent variables for both the observed data and the missing data process, $\theta$ and $\zeta$, respectively, are correlated by with a correlation parameter $\mathbf{\Sigma}$. The likelihood of the $NONMAR$ model is written as

$$\prod_{i,k} p(x_{ik}|d_{ik}, \theta_{i,}\alpha_k, \beta_k)p(d_{ik}|\zeta_i, \gamma_k, \delta_k)g(\zeta_i, \theta_i|\mathbf{\Sigma})), \tag{3.5}$$

where $g(\cdot)$ is the density of $\zeta_i$ and $\theta_i$ which is assumed to follow a multivariate normal distribution with mean vector 0 and variance-covariance $\mathbf{\Sigma}$. Expressions (3.5) will be used in the procedure to make

inferences when all latent variables for observed data and missing data process are considered and then compared to the results on values of item parameters estimates when the (3.4) model was used.

### *3.2.3 The Generalized Partial Credit Model (GPCM)*

In general, the observed responses will be modeled by a multidimensional version of the generalized partial credit model (Muraki, 1992). In the unidimensional case, the person's ability or proficiency is represented by a scalar parameter. However, in many cases it is a priori clear that multiple abilities are involved in producing the observed responses or the dimensionality of the ability structure might not be clear at all. In these cases, multidimensional ability parameters are needed to describe the ability or proficiency level of a person. Béguin & Glas (2001) state that multidimensional IRT models can serve confirmatory and exploratory purposes.

For persons $i$ $(i = 1, ..., N)$ responding to item $k$ $(k = 1, ..., K)$ the probability of responding in a category $g$ $(g = 0, ..., m_k)$ is given by

$$\psi_{kg}(\theta_i) = p(X_{ikg} = 1|\theta_i, \alpha_k, \beta_k) = \frac{\exp(g\sum_q^Q \alpha_{kq}\theta_{iq} - \beta_{kg})}{1 + \sum_{h=1}^{m_k} \exp(h\sum_q^Q \alpha_{kq}\theta_{iq} - \beta_{kh})} \tag{3.6}$$

where $\alpha_k = \{\alpha_{k1}, ...\alpha_{kq}, ...\alpha_{kQ}\}$ is a Q-dimensional vector of discrimination parameters or factor loadings, $\theta_i = \{\theta_{i1}, ..., \theta_{iq}, ..., \theta_{iQ}\}$ is a Q-dimensional vector of person's parameters and $\beta_{kg}$ is a scalar location parameter.

Model (3.6) will be a specific model depending on the values of some of its parameters. When $m_k = 1$, (3.6) is the multidimensional two-parameter logistic model (2PL; Birnbaum, 1968) which is the one we use in the simulation studies reported in this chapter and, further, (3.6) becomes the multidimensional partial credit model (PCM; Masters, 1982; Masters & Wright, 1997) when $\alpha_k = 1$ and additionally, the multidimensional Rasch model for dichotomous items when $m_k = 1$ and $\alpha_k = 1$.

Note that the model for the missing data indicator (3.3) is a special case of the GPCM given by (3.6). Therefore, both models can be combined into one concurrent model, for instance a model of the form of (3.5), by assuming a $Q$-dimensional model where the model for the item responses only loads on the first $Q-1$ dimensions, while the model for the missing data indicator only loads on the $Q$-th

dimension. The ensemble of the latent parameters of a respondent then has a $Q$-variate normal distribution with a mean equal to zero and a covariance matrix $\mathbf{\Sigma}$.

## 3.3 MML Estimation

Suppose $\mathbf{x}_i$ is the response pattern of respondent $i$, and let $\mathbf{X}$ be the data matrix. Under MML approach, it is assumed that possibly multidimensional ability parameters $\theta_i$ are independent and identically distributed with density $g(\theta;\lambda)$. Usually, it is assumed that person's ability is normally distributed with population parameters $\lambda$ (which are the mean $\mu$ and the variance $\sigma^2$ for the unidimensional case, or the mean vector $\mu$ and the covariance matrix $\mathbf{\Sigma}$ for the multidimensional case). Item parameters $\phi$ consist of discrimination parameters ($\alpha_k$, or $\alpha_k$ for the unidimensional and the multidimensional cases, respectively) and the item difficulties $\beta_k$ whose elements are $(\beta_{k1}, \beta_{k2}, ..., \beta_{kg}, ..., \beta_{km_k})$. Given the remark in the previous section that the models for the item responses and the missing data indicator can be brought together in one concurrent model, MML estimation will be described without explicitly distinguishing between item parameters and person parameters associated with the observations or the indicators.

MML estimation derives its name from maximizing the log-likelihood that is marginalized with respect to $\theta$, rather than maximizing the joint log-likelihood of all person parameters $\theta$ and item parameters $\phi$. Let $\upsilon$ be a vector of all item and population parameters that is $\upsilon^t = (\phi^t, \lambda^t)$. Then the marginal likelihood of $\upsilon$ is given by

$$L(\upsilon;\mathbf{X},\mathbf{D}) = \int ... \int \prod_i^N p(\mathbf{x}_i,\mathbf{d}_i|\theta_i,\phi) g(\theta_i;\lambda) d\theta_i$$

that is

$$L(\upsilon;\mathbf{X},\mathbf{D}) = \prod_i^N \int ... \int p(\mathbf{x}_i,\mathbf{d}_i|\theta_i,\phi) g(\theta_i;\lambda) d\theta_i$$

and hence the marginal log-likelihood of $\upsilon$ is

$$\log L(\upsilon;\mathbf{X},\mathbf{D}) = \log \prod_i^N \int ... \int p(\mathbf{x}_i,\mathbf{d}_i|\theta_i,\phi) g(\theta_i;\lambda) d\theta_i$$

which is equivalent to the expression

$$\log L(\upsilon; \mathbf{X}, \mathbf{D}) = \sum_{i}^{N} \log \int ... \int p(\mathbf{x}_i, \mathbf{d}_i|\theta_i, \phi) g(\theta_i; \lambda) d\theta_i. \qquad (3.7)$$

We maximized the marginal likelihood since it gives us consistent estimates as compared to the ones obtained using the joint likelihood which can be inconsistent. Neyman & Scott (1948) stated that if the number of person parameters grows proportional with the number of observations, then in general this leads to inconsistency when using joint likelihood. Simulation studies of Wright and Panchapakesan (1969) and Fischer and Scheiblechner (1970) showed that these inconsistencies can indeed occur in IRT models. Kiefer and Wolfowitz (1956) have shown that marginal maximum likelihood estimates of structural parameters, say the item and population parameters of an IRT model, are consistent under fairly reasonable regularity conditions, which motivates the general use of MML in IRT models.

To derive MML equations, we will introduce the vector of derivatives

$$\begin{aligned} \omega_i(\upsilon) &= \frac{\partial}{\partial \upsilon} \log p(\mathbf{x}_i, \mathbf{d}_i, \theta_i|\upsilon) \qquad (3.8) \\ &= \frac{\partial}{\partial \upsilon} \left[\log p(\mathbf{x}_i, \mathbf{d}_i|\theta_i, \phi) + \log g(\theta_i|\lambda)\right]. \end{aligned}$$

Using Fisher's identity (Efron, 1977; Louis 1982; also see, Glas, 1992, 1998), then the marginal likelihood equations for $\upsilon$ can then be easily derived. The first order derivatives with respect to $\upsilon$ is written as

$$\mathbf{h}(\upsilon) = \frac{\partial}{\partial \upsilon} \log L(\upsilon|\mathbf{X}, \mathbf{D}) = \sum_{i}^{N} E(\omega_i(\upsilon)|\mathbf{x}_i, \mathbf{d}_i, \upsilon) \qquad (3.9)$$

where $\omega_i(\upsilon)$ is the expression in (3.8) and the expectation is with respect to the posterior distribution $p(\theta_i|\mathbf{x}_i, \mathbf{d}_i, \upsilon)$. The identity in (3.9) is closely related to the EM-algorithm (Dempster, Laird and Rubin, 1977), which is a very useful algorithm for finding the maximum of a likelihood marginalized over unobserved data. This framework fits the present application when the response patterns are viewed as observed data and the ability parameters as unobserved data. Together they are referred to as the complete data. When direct inference based on the marginal likelihood is complicated, the

EM algorithm is applicable in this situations. The complete data likelihood equations, i.e., equations based on $\omega_i(\upsilon)$ are easily found. Given some estimate of $\upsilon$ as $\upsilon^*$, the estimate can be improved by solving $\sum_i^N E(\omega_i(\upsilon)|\mathbf{x}_i, \mathbf{d}_i, \upsilon^*) = \mathbf{0}$ with respect to $\upsilon$. Then this new estimate becomes $\upsilon^*$ and the process is iterated until convergence.

Applications of this framework in deriving the likelihood equations of the structural parameters of the multidimensional GPCM proceeds as follows. We will only consider finding the item parameter estimates for the item responses, because the item parameter estimates for the missing data indicators is completely analogous. The complete likelihood is given by

$$p(\mathbf{x}_i|\theta_{i,}, \mathbf{d}_i, \phi) = \prod_k \prod_{g=0}^{m_k} \psi_{kg}(\theta_i)^{d_{ik}x_{ikg}}. \tag{3.10}$$

The likelihood equations are obtained upon equating (3.9) to zero, so explicit expressions are needed for (3.8). Given the design vector $\mathbf{d}_i$, the ability parameter $\theta_i$ and the item parameters of the multidimensional GPCM, the probability of response pattern $\mathbf{x}_i$ is given by (3.10). By taking first order derivatives of the logarithm of this expression, the expressions for (3.8) are found as

$$\omega_i(\alpha_{kq}) = d_{ik}\left[\theta_{iq}(x_{ikg} - \psi_{ikg})\right] \tag{3.11}$$

and

$$\omega_i(\beta_{kg}) = d_{ik}(\psi_{ikg} - x_{ikg}), \tag{3.12}$$

where $\psi_{igk}=\psi_{gk}(\theta_i)$, thus the likelihood equations for the item parameters are found upon inserting these expressions into (3.9) and equate the resulting expressions to zero, hence

$$\sum_i^N E(\theta_{iq}\psi_{ikg}d_{ik}|\mathbf{x_i}, \mathbf{d}_i, \upsilon) = \sum_i^N E(d_{ik}\theta_{iq}x_{ikg}|\mathbf{x}_i, \mathbf{d}_i, \upsilon)$$

simplifying further

$$\sum_i^N E(\theta_{iq}\psi_{ikg}d_{ik}|\mathbf{x}_i, \mathbf{d}_i, \upsilon) = \sum_i^N d_{ik}x_{ikg}E(\theta_{iq}|\mathbf{x}_i, \mathbf{d}_i, \upsilon) \tag{3.13}$$

and similarly

$$\sum_i^N E(d_{ik}\psi_{ikg}|\mathbf{x}_i, \mathbf{d}_i, \upsilon) = \sum_i^N E(d_{ik}x_{ikg}|\mathbf{x}_i, \mathbf{d}_i, \upsilon)$$

then

$$\sum_{i}^{N} d_{ik} E(\psi_{ikg}|\mathbf{x}_i, \mathbf{d}_i, \upsilon) = \sum_{i}^{N} d_{ik} x_{ikg} \tag{3.14}$$

To derive the likelihood equations for the population parameters, the first order derivatives of the logarithm of the density of the ability parameters $g(\theta;\lambda)$, where $\lambda$ is the vector of population parameters which is the mean vector $\mu$ and the covariance matrix $\mathbf{\Sigma}$ are needed. In the present case, $g(\theta;\mu,\mathbf{\Sigma})$ is the well-known expression for the $Q$-dimensional multivariate normal distribution with mean vector $\mu$ and the covariance matrix $\mathbf{\Sigma}$, whose probability density is

$$g(\theta_i;\lambda) = g(\theta_i|\mu,\mathbf{\Sigma}) = (2\pi)^{-q/2} \left|\mathbf{\Sigma}\right|^{-1/2} \exp\left(-1/2(\theta-\mu)^t \mathbf{\Sigma}^{-1}(\theta-\mu)\right)$$

where $|\mathbf{\Sigma}|$ is the determinant of the covariance matrix, so it is easily verified that these derivatives are given by

$$\omega_i(\mu) = 1/2(\mathbf{\Sigma}^{-1}(\theta-\mu)) \tag{3.15}$$

and

$$\omega_i(\mathbf{\Sigma}) = 1/2[(\theta-\mu)(\theta-\mu)^t \mathbf{\Sigma}^{-2} - (\mathbf{\Sigma}^{-1})^t] \tag{3.16}$$

where elements considered in $\mathbf{\Sigma}$ are the diagonals.

The likelihood equations to obtain $\mu$ are again found upon inserting these expressions in (3.9) and equating the resulting expressions to zero, that is

$$\sum_{i}^{N} E(\mathbf{\Sigma}^{-1}(\theta-\mu)|\mathbf{x}_i,\lambda) = \mathbf{0}$$

and by simplifying the expression by working on the expectations of the stochastic variable $\theta$ and the parameters we solve $\mu$ as

$$\mu = \frac{\sum_i^N E(\theta|\mathbf{x_i},\lambda)}{N}$$

Similarly for $\mathbf{\Sigma}$, the resulting expression is

$$\sum_{i}^{N} E((\theta-\mu)(\theta-\mu)^t \mathbf{\Sigma}^{-2}|\lambda) = \sum_{i}^{N} E((\mathbf{\Sigma}^{-1})^t|\lambda)$$

$$\sum_{i}^{N} E((\theta-\mu)(\theta-\mu)^t \mathbf{\Sigma}^{-2}|\lambda) = N(\mathbf{\Sigma}^{-1}) \tag{3.17}$$

and simplifying leads to

$$\mathbf{\Sigma} = \frac{\sum_i^N E((\theta-\mu)(\theta-\mu)^t|\mathbf{x_i},\lambda)}{N}$$

Note that the standard errors are also easily derived in this framework: Mislevy (1986) pointed out that the information matrix can be approximated as

$$\mathbf{H}(\ \upsilon,\ \upsilon) \approx \sum_i^N E(\ \omega_i(\upsilon) \mid \mathbf{x}_i, \mathbf{d}_i, \upsilon\ ) E(\ \omega_i(\upsilon) \mid \mathbf{x}_i, \mathbf{d}_i, \upsilon\ )^t \qquad (3.18)$$

and the standard errors are the diagonal elements of the inverse of this matrix.

The basic approach presented so far can be generalized in two ways. First, the assumption that all respondents are drawn from one population can be replaced by the assumption that there are multiple populations of respondents. Usually, it is assumed that each population has a normal ability distribution indexed by a unique mean and covariance matrix. This generalization together with the possibility of analyzing incomplete item-administration designs provides a unified approach to such problems as differential item functioning, item parameter drift, non-equivalent groups equating, vertical equating and matrix-sampled educational assessment as pointed out by Bock and Zimowski (1997). Further, item calibration for CAT also fits within this framework.

## 3.4 Simulation Studies

Simulation studies were conducted to asses the effect in the bias of the item parameter estimates when a model for missing data is ignored or included in a model for estimation as described in (3.4) and (3.5). We divided the simulation study into two parts. The first part consists of the data generation using the Rasch model (RM or $1PL$) and the second part was the estimation of the parameters. Two models were used in the estimation of item parameters. The RM model was used for the estimation of item difficulty parameter $\widetilde{\beta}_{MAR}$ when the model for missing data process was ignored (MAR model). The RM version of the sequential (steps) model for the missing data model was used for the model of the missing data when this model

was included in the estimation that was concurrent with the estimation of the RM model for the observed data. The item difficulty $\delta$ of the conceptual items for the missing data indicator has $\tau$ (overall level) and $\eta$ (increment) as components. Their main purposed was described in the previous section.

For a sample size $N = 500$ persons, latent trait values ($\theta_i$, $\zeta_i$) were drawn from a bivariate normal distribution with means 0, and a covariance matrix $\mathbf{\Sigma}$ with diagonal elements equal to one and correlation $\rho$. This correlation between the latent variables for the observed data and the missing data process where chosen to vary from 0.0, 0.2, 0.4, 0.6 and 0.8. The test was made of $K = 10$ items and the items were dichotomously scored. The values $d_{ik}$ and $x_{ik}$ were drawn from $p(d_{ik}|\ \zeta_i, \theta_i, \delta_k)$ and $p(x_{ik}|d_{ik}, \theta_i, \alpha_k, \beta_k)$ respectively. The generated data were used to compute estimates of the item parameters $\widetilde{\beta}_{MAR}$ when the $MAR$ model was used and item parameter estimates $\widehat{\beta}_{obs}, \widehat{\delta}_{obs}$, $\widehat{\tau}_{obs}$ and $\widehat{\rho}$ when $NONMAR$ model was used, respectively.

The values of $\widetilde{\beta}_{MAR}, \widehat{\beta}_{obs}, \widehat{\delta}_{obs}$, $\widehat{\tau}_{obs}$ and $\widehat{\rho}$ were compared with the values of the parameters used to generate the data (true values) using the mean absolute error (MAE) and mean squared error (MSE). For the formulas to obtained MAE and MSE for the model parameters refer to the equations (2.25) and (2.26) in Chapter 2.

One hundred replications were made for the combination of $K = 10$ and $N = 500$ and $\rho = 0.0$, 0.2, 0.4, 0.6 and 0.8. The same replications for the combination $n = 1000$ and same $k$ and $\rho$ was also done. The difficulty parameters for the observed data is $\beta_k = 0$ while difficulty parameters for the conceptual items were $\delta_k$ =-8, -7, -6, -5, -4, -3, -2, -1, 0, and 1, respectively. These values were chosen such that the item parameters will go from easy to difficult, that is, the probability of observing an item response decreases. So most respondents can respond the first items until they run out of time and then they omit the succeeding items. This is the situation of missingness we are dealing with, where missingness can not be ignored since the response mechanism depends on the ability of the respondents. The result of the simulations are given on Table 3.1 and Table 3.2 for $N = 500$ and $N = 1000$.

TABLE 3.1. MAE of item parameter estimates under MAR and NONMAR model (dichotomous Case); Estimation Model: (Observed data: 2PL; missing data: Steps model); Dimension of missing data process: 1; N=500, 1000; K=10; $\alpha = 1$; $\beta = 0$ ; $\gamma = 1$; $\rho = \rho(\theta, \zeta)$.

| | | Mean Absolute Error(MAE) | | | | |
|---|---|---|---|---|---|---|
| N | $\rho$ | $\widehat{\beta}_{obs}$ | $\eta$ | $\tau$ | $\rho$ | $\widetilde{\beta}_{MAR}$ |
| 500 | .0 | .107 | .121 | .288 | .094 | .107 |
| | .2 | .112 | .116 | .294 | .090 | .117 |
| | .4 | .112 | .122 | .299 | .088 | .121 |
| | .6 | .114 | .104 | .253 | .129 | .126 |
| | .8 | .110 | .070 | .182 | .138 | .139 |
| 1000 | .0 | .083 | .077 | .192 | .061 | .083 |
| | .2 | .077 | .082 | .206 | .067 | .081 |
| | .4 | .079 | .078 | .193 | .075 | .091 |
| | .6 | .078 | .065 | .157 | .075 | .103 |
| | .8 | .079 | .049 | .128 | .087 | .124 |

TABLE 3.2. MSE of item parameter estimates under MAR and NONMAR model (dichotomous Case); Estimation Model: (Observed data: 2PL; missing data: Steps model); Dimension of missing data process: 1; N=500, 1000; K=10; $\alpha = 1$; $\beta = 0$; $\gamma = 1$; $\rho = \rho(\theta, \zeta)$.

| | | Mean Squared Error(MSE) | | | | |
|---|---|---|---|---|---|---|
| N | $\rho$ | $\widehat{\beta}_{obs}$ | $\eta$ | $\tau$ | $\rho$ | $\widetilde{\beta}_{MAR}$ |
| 500 | .0 | .020 | .031 | .170 | .015 | .020 |
| | .2 | .022 | .023 | .137 | .011 | .024 |
| | .4 | .022 | .024 | .147 | .014 | .028 |
| | .6 | .024 | .023 | .134 | .016 | .030 |
| | .8 | .024 | .010 | .057 | .009 | .044 |
| 1000 | .0 | .012 | .010 | .059 | .005 | .012 |
| | .2 | .010 | .010 | .060 | .006 | .012 |
| | .4 | .011 | .010 | .059 | .006 | .016 |
| | .6 | .011 | .008 | .050 | .007 | .022 |
| | .8 | .011 | .004 | .030 | .004 | .032 |

We start the discussion of our results by introducing the notations in the tables. Notation $\rho(\theta, \zeta)$ refers to the correlation of the latent variables of the observed data $\theta$ and the missing data process $\zeta$. The MAE($\widehat{\beta}_{obs}$) and MSE($\widehat{\beta}_{obs}$) refers to the mean absolute error and mean squared error, respectively, of the estimates of the difficulty parameter $\widehat{\beta}_{obs}$ when the $NONMAR$ model was used. MAE($\widetilde{\beta}_{MAR}$) and MSE($\widetilde{\beta}_{MAR}$) refers to the mean absolute error and mean squared error, respectively, of the estimates of the difficulty parameter $\widetilde{\beta}_{MAR}$ that ignored the model for the missing data ($MAR$ model). The other notations for the MAE's and MSE's of $\eta, \tau$ and $\rho$ refers to the increment of the 'conceptual' items, the difficulty parameter of the last 'conceptual' item and the correlation between $\theta$ and $\zeta$, respectively.

The first row of each table are baselines, that is when there is no correlation between the two latent variables, so when ignorability holds. The results showed that when the correlation increases, the MAE and the MSE of the estimates under the assumption of MAR increased considerably. For instance, if we look specifically at Table 3.1, where $\rho$ increases from 0.0 to 0.8 with intervals of 0.2, the MAE( $\widetilde{\beta}_{MAR}$) had values of 0.107, 0.117, 0.121, 0.126 and 0.139. These values of the MAE (or the bias) for the $\widetilde{\beta}_{MAR}$ were inflated as expected since the missing data process was excluded. It was also true for MSE of $\widetilde{\beta}_{MAR}$. These results were analogous to the results of the simulations of Holman & Glas (2005) and the results reported in the previous chapter in this thesis.

The MAE and MSE values for $\widehat{\beta}_{obs}$ only showed random fluctuation. Looking again in the Table 3.1, MAE($\widehat{\beta}_{obs}$) had values of 0.107, 0.112, 0.112, 0.114 and 0.110. On the other hand, MAE's of $\widehat{\eta}_{obs}$ showed 0.121, 0.116, 0.122, 0.104, 0.070 and $\widehat{\tau}_{obs}$ showed 0.192, 0.206, 0.193, 0.157, 0.128. The errors of these parameters estimates showed a decreasing trend. Since the marginal distribution of $\mathbf{D}$ did not change as a function of the correlation, this trend cannot be explained as a result of having more observations in $\mathbf{D}$ or more information in the responses in $\mathbf{D}$. A possible explanation for such a trend is that an increase in the correlation between $\theta$ and $\zeta$ results in more collateral information on $\zeta$ through $\theta$, and therefore more information on $\tau$ and $\eta$. This collateral informations resulted on a decrease in the standard error of the item parameter estimates for the missing data indicators.

The MAE values for estimated correlation $\rho$ were 0.094, 0.090, 0.088, 0.129 and 0.138, which is an increasing trend. We have no clear explanation of this phenomenon at the moment of this writing.

## 3.5 Real Data Application

To get an idea of the impact of the approach in a real data situation, data from a calibration sample of an intelligence test for children in primary education was analyzed (van Dijk & Tellegen, 2004). The data set was made up 3145 children responding to of 30 items of a speeded form of the test. The percentage of missing data was equal to 27%. The first 5 items were responded to by all children, the last five items were endorsed by 1004, 855, 786, 622 and 508 children, respectively. The data ware analyzed using both the MAR (ignorable) and NONMAR (nonignorable) models. The estimated correlation between the latent parameters of the observed data and missing data is 0.429, signifying that the missing data process cannot be ignored in the estimation. For both approaches, the values of the items parameters estimates were compared. Results of the parameters estimates are given in Table 3.3. The notation *diff* means the difference between the estimates of the parameters. $\widehat{\alpha}$ and $\widehat{\beta}$ were the estimates of the item discrimination and difficulty respectively of the model that includes the model for the missing data while $\tilde{\alpha}$ and $\tilde{\beta}$ were the estimates of the item discrimination and difficulty respectively of the model that ignored the missing data. The differences between the estimates obtained using the two methods is plotted in Figure 3.1. It can be seen that there is no trend on the difference between the discrimination parameters, but the differences in the item difficulty parameters clearly increase after the 20th item.

TABLE 3.3. Item parameter estimates under MAR and NONMAR model Real data (speeded test),N=3145 examinees K=30 items

| item | $\widehat{\alpha}$ | $\tilde{\alpha}$ | *diff* | $\widehat{\beta}$ | $\tilde{\beta}$ | *diff* |
|---|---|---|---|---|---|---|
| 1 | 0.876 | 0.847 | 0.029 | -4.303 | -4.277 | -0.026 |
| 2 | 1.254 | 1.281 | -0.027 | -3.529 | -3.550 | 0.021 |
| 3 | 1.208 | 1.210 | -0.002 | -3.132 | -3.130 | -0.002 |
| 4 | 0.777 | 0.773 | 0.004 | -1.692 | -1.690 | -0.002 |
| 5 | 1.154 | 1.140 | 0.014 | -0.687 | -0.684 | -0.003 |
| 6 | 0.340 | 0.351 | -0.011 | -1.398 | -1.400 | 0.002 |
| 7 | 1.002 | 0.986 | 0.016 | 0.1930 | 0.1920 | 0.001 |
| 8 | 1.403 | 1.400 | 0.003 | 0.2680 | 0.2660 | 0.002 |
| 9 | 0.701 | 0.701 | 0.000 | -0.377 | -0.378 | 0.001 |
| 10 | 0.298 | 0.292 | 0.006 | 0.477 | 0.4760 | 0.001 |
| 11 | 1.583 | 1.593 | -0.010 | 0.197 | 0.1890 | 0.008 |
| 12 | 1.563 | 1.538 | 0.025 | 0.300 | 0.2890 | 0.011 |
| 13 | 0.342 | 0.347 | -0.005 | -0.078 | -0.081 | 0.003 |
| 14 | 0.811 | 0.819 | -0.008 | -0.479 | -0.490 | 0.011 |
| 15 | 0.893 | 0.885 | 0.008 | 0.907 | 0.8930 | 0.014 |
| 16 | 0.468 | 0.464 | 0.004 | 0.562 | 0.5520 | 0.010 |
| 17 | 0.402 | 0.394 | 0.008 | 0.997 | 0.9850 | 0.012 |
| 18 | 0.446 | 0.422 | 0.024 | -0.957 | -0.969 | 0.012 |
| 19 | 0.530 | 0.529 | 0.001 | 0.858 | 0.8380 | 0.020 |
| 20 | 0.681 | 0.666 | 0.015 | 1.473 | 1.4370 | 0.036 |
| 21 | 0.696 | 0.685 | 0.011 | 1.279 | 1.2380 | 0.041 |
| 22 | 0.920 | 0.908 | 0.012 | -0.052 | -0.111 | 0.059 |
| 23 | 0.689 | 0.689 | 0.000 | 1.396 | 1.3490 | 0.047 |
| 24 | 0.658 | 0.649 | 0.009 | 1.135 | 1.0800 | 0.055 |
| 25 | 1.071 | 1.052 | 0.019 | 1.971 | 1.8710 | 0.100 |
| 26 | 1.209 | 1.192 | 0.017 | 2.591 | 2.4690 | 0.122 |
| 27 | 0.485 | 0.473 | 0.012 | 0.961 | 0.9060 | 0.055 |
| 28 | 0.276 | 0.265 | 0.011 | 2.083 | 2.0480 | 0.035 |
| 29 | 0.698 | 0.689 | 0.009 | 2.514 | 2.4260 | 0.088 |
| 30 | 0.467 | 0.457 | 0.010 | 1.916 | 1.8500 | 0.066 |

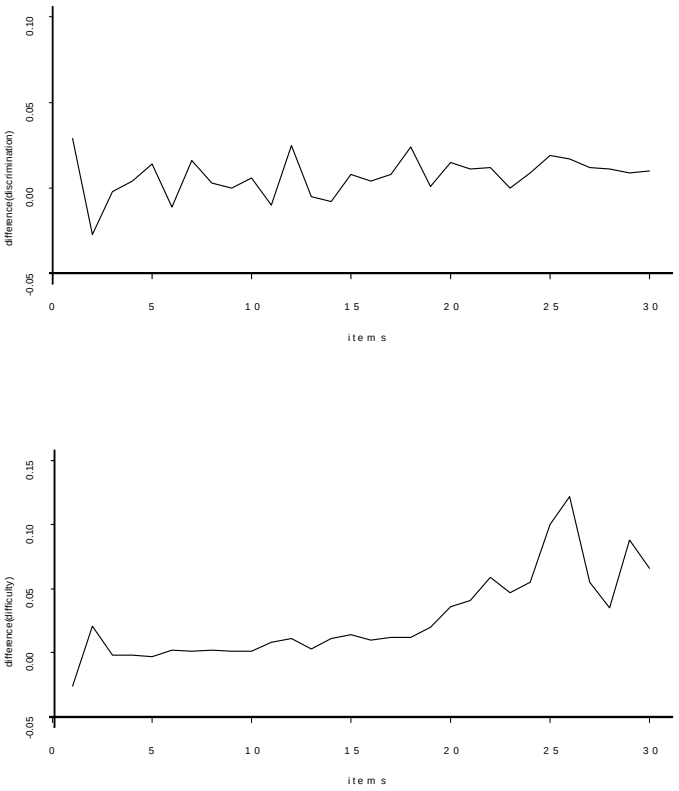



FIGURE 3.1. Plot of the differences of item discrimination and difficulty estimates between MAR and NONMAR models.

The impact of the difference between the MAR and NONMAR models will be studied further by computing the global reliability of the test. Usually, this is done by classical test theory. However, if missing data are present, it is more convenient to compute the global reliability via IRT. In an IRT framework, an index of reliability is based on the identity

$$Var(\theta) = E(Var(\theta|x)) + Var(E(\theta|x))$$

(Bechger, Maris, Verstralen & Béguin, 2003). This identity entails that the total variance of the ability parameters is a sum of two components. The first component, $E(Var(\theta|x))$, relates to the uncertainty about the ability parameter. The posterior variance of ability, $Var(\theta|x)$, gives an indication of the uncertainty with respect to the ability parameter, once we have observed the response pattern $x$. By considering its expectation over the distribution of $x$, we obtain an estimate of the average uncertainty over the respondents' ability parameters. The second term, $Var(E(\theta|x))$, is related to the systematic measurement component. The expectation serves as an estimate of ability, and by considering the variance of these expectations over the distribution of $x$, we get an indication of the extent to which the respondents can be distinguished on the basis of their observed responses. Therefore, a reliability index taking values between zero and one can be computed as the ratio of the systematic variance and the total variance, that is

$$\rho = \frac{Var(E(\theta|x))}{Var(\theta)}. \tag{3.19}$$

In the present application, we can compute $Var(E(\theta|x))$ in two ways: under the MAR assumption where we only condition of the observed responses, and under the NONMAR assumption, where we condition on both the observed responses $x$ and the missing data indicator $d$. In the latter case, we integrate over both latent variables involved, so the expectation in the numerator of (3.19) is computed as

$$\begin{aligned} E(\theta|x,d) &= \int\int \theta \; p(\theta,\zeta|x,d)d\theta d\zeta \\ &= \int\int \theta \; \frac{p(x|d,\theta)p(d|\zeta)g(\theta,\zeta|\Sigma)}{p(x,d)} d\theta d\zeta. \end{aligned}$$

Under the MAR assumption the global reliability was computed as $\rho = 0.658$, under the NONMAR assumption it was computed as $\rho = 0.738$. So in the present case, taking the missing data process into account leads to a substantial increase in the estimate of global reliability.

## 3.6 Discussion

The results of the simulation study showed that when an IRT model for the missing data process was included in the estimation together with an IRT model for the observed data, even how much we increased the correlation between latent variables $\theta$ and $\zeta$ that is, we want to make the missing data mechanism more nonignorable, the bias in the item parameters remained constant and lower compared to the case when the model for the missing data was ignored in the estimation. We conclude that the bias in the IRT parameter estimates is reduce when an IRT model for the missing data process is included in the estimation.

The method as applied in the real speeded test data indicated that it is possible to model the missing data with an IRT model. The results showed that the difference in the item parameters, especially the difficulty parameters (refer to Figure 3.1), gets bigger. This is expected since the respondents were under time limit conditions and the items were getting difficult to endorse. So they skipped items more in the end where item nonresponse were incurred. It was shown that the estimate of the global reliability was larger when the missing data process was taken into account.

For further research, it is of great interest to investigate the effect in the bias of the model parameters estimates when observed covariates are included in the model for the missing data given that the data came from a speeded test. From the results of the previous chapter we can expect that inclusion of the observed covariates in the model for the missing data will support the reduction of the bias in the parameters estimates. It is also further recommended to investigate the effect in the bias of the model parameter estimates when more complex IRT models for the observed scores are used. Further, the concept of using the step model to model speededness needs not be confined to a likelihood based framework. It can also be applied to the complex IRT models that are usually estimated

in a Bayesian framework. Examples are models with multiple raters, multiple item types, missing data (Patz & Junker, 1999a,b), models for testlet structures (Bradlow, Wainer & Wang, 1999, Wainer, Bradlow & Du, 2000), and models with a multi-level structure on the ability parameters (Fox & Glas, 2001, 2002, 2003). Implementation of NONMAR models in a Bayesian framework will be the topic of the next two chapters.

# 4

# Detecting Nonignorable Missing Data using the Splitter Item Technique

ABSTRACT: Researchers are often confronted with missing data. Direct statistical inference is appropriate if the missing data are ignorable. In a framework of item response theory, two methods based on the splitter item technique are proposed for deciding whether the missing data are ignorable or nonignorable. In the first method, the observed item response data are split according to the values of the splitter item. Then, the estimated marginal distributions of the item parameters corresponding to both data sets are compared for detecting differences. In the second method, an IRT model for the observed data is extended with group specific item parameters. These extra parameters provide information regarding item parameter differences across groups. They are estimated using MCMC and they do not interfere with the estimation of the other model parameters. In a simulation study concerning item-selection designs, both methods are illustrated and compared using probit IRT models.

KEYWORDS: Ignorability, Item response theory, Markov chain Monte Carlo, Missing data, Splitter item technique.

## 4.1 Introduction

When data are collected using questionnaire or proficiency items (usually in a sample survey), it is possible that there will be missing observations. For making meaningful inferences it is necessary to find out if it is appropriate to ignore the process that causes the missing data. The missing data process or response mechanism is nonignorable when it depends on a respondent's unobserved response and ignorable when the probability of a nonresponse is independent of the respondent's unobserved response. Bayesian (likelihood) inferences based on the observed data are equivalent to the inferences based

on the complete posterior (likelihood) reflecting both the observed data and the response mechanism when the response mechanism is ignorable.

Most of the literature on missing categorical data assumes an ignorable response mechanism. However, handling nonignorable nonresponse is getting more attention. Fay (1986), Baker and Laird (1988), and Green and Park (2003) proposed a class of log-linear models for categorical responses subject to nonignorable nonresponse. In a simulation study, Park and Brown (1994, 1997) showed that it is important to decide whether the underlying response mechanism is ignorable or nonignorable. Lord (1983) was one of the first to develop a mathematical model for omitting behavior when the usual item response theory (IRT) models for dichotomously scored multiple choice items cannot handle appropriately omitted responses. O'Muircheartaigh and Moustaki (1999), and Moustaki and Knott (2000) proposed so-called symmetric pattern models for handling item nonresponse in attitude scales. A set of questions is used to measure some underlying latent attitude or ability but the observed item responses contain missing values. They developed a nonignorable nonresponse model based on a latent basic response propensity that describes the tendency of respondents to respond. The probability of an item response depends on the response propensity value. An individual's response to an item does not depend on its propensity value but only on the value of the individual's latent attitude. Bradlow and Zaslavsky (1999) proposed an IRT model for ordinal customer satisfaction data. The item nonresponse, that might be due to either lack of a strong opinion or indifference about the question, was modeled by a logistic regression model. Bradlow and Thomas (1998) showed in a simulation study that common IRT models cannot be used for likelihood or Bayesian inference when the missing data mechanism cannot be ignored. In this particular case, assuming an ignorable response mechanism leads to bias in parameter estimates. Holman and Glas (2005) proposed several IRT models for modeling nonignorable nonresponse.

A relevant issue when analyzing item response data with missing data concerns the process that causes the missing data. When the response mechanism is ignorable, a statistical analysis based on the observed data always leads to correct inference of the data. When the response mechanism is nonignorable, one can eliminate bias only by

constructing a model that correctly represents the response mechanism (Little, 1982). However, such models are highly sensitive to misspecification error and they substantially complicate the statistical inference. Therefore, it is recommendable that first the necessity of such a complex model, that is, a model for the observed data extended with a missing-data model, is verified. On the other hand, most of the literature on missing data for categorical problems assumes that the process that caused the missing data can be ignored. In these cases, the assumptions for ignorability should be checked.

In the present paper, two methods will be proposed to verify whether the missing data mechanism can be ignored or not in case of item nonresponse. It will be assumed that the probability of the observed pattern of missing data may be depending on possible values of the missing data and/or the parameters of the data and the parameters of the missing data may not be distinct. No other variables relate to the item score missingness. Both cases lead to a nonignorable response mechanism (Rubin, 1976). In the first method, the splitter item technique (Molenaar 1983; Van den Wollenberg, 1979) is used for splitting the data in two groups depending whether the response item was observed or missing. Then, the marginal posterior distributions of the item parameters corresponding to both groups are compared. In the second method, an IRT model for the observed data is extended such that item parameters may fluctuate across groups. The extra parameters in this more general model are called Bayesian modification indices (Fox and Glas, 2005) and provide information about the relevance of the model extension. In this particular case, they are used to test whether the response mechanism is nonignorable. The parameters of the IRT model for the observed data are estimated using MCMC (Gelfand and Smith, 1990). The BMI values are sampled given the sampled values of the IRT parameters. However, these extra draws do not influence the Markov chain and the chain remains restricted to the manifold of the posterior of the IRT model. It will be shown that the estimated marginal posterior distribution of the BMI values are closely related to their true marginal posterior distribution. As a result, BMI values are sampled as by-products of the MCMC procedure for estimating the parameters of the IRT model for the observed data. The MCMC estimation procedure can be time-consuming and it is, therefore,

preferable to compute certain fit statistics during the estimation of the model parameters.

In the next section, a general notation is given for IRT models for the observed data and models for the missing data process. Models for the missing data process are introduced to illustrate the aspect of distinctness. Then, the splitter item method will be described and details will be given of both methods for testing whether the missing data process can be safely ignored. Next, both methods will be applied in three experiments concerning item selection with artificial data. Finally, the last section contains a discussion and suggestion for further research.

## 4.2 Model and Notation

### *4.2.1 IRT model for the observed data*

The categorical outcome, $y_{ik}$, represents the item response of person $i$ ($i = 1, \ldots, N$) on item $k$ ($k = 1, \ldots, K$). These item responses may be dichotomous or polytomous. Let $\theta_i$ denote the latent abilities or attitudes of the respondents responding to the $K$ items. They are collected in the latent vector $\boldsymbol{\theta}$. For dichotomous item responses a two-parameter IRT model is used for specifying the relation between the examinee level on a latent variable and the probability of a particular item response. That is

$$P\big(y_{ik} = 1 \mid \theta_i, a_k, b_k\big) = \Phi\big(a_k\theta_i - b_k\big), \tag{4.1}$$

where $a_k$ is the item discrimination parameter, and $b_k$ is the item difficulty parameter. The item parameters will also be denoted by $\boldsymbol{\xi}_k$, with $\boldsymbol{\xi}_k = (a_k, b_k)$. The function $\Phi$ is the cumulative standard normal distribution. For polytomous item responses, the probability that an individual obtains a grade $c$ ($c = 1, \ldots, C$) on item $k$ is defined by a graded response model (GRM)

$$P\big(y_{ik} = c \mid \theta_i, a_k, \boldsymbol{\kappa}_k\big) = \Phi\big(a_k\theta_i - \kappa_{kc-1}\big) - \Phi\big(a_k\theta_i - \kappa_{kc}\big) \tag{4.2}$$

where the boundaries between the response categories are represented by an ordered vector of thresholds $\boldsymbol{\kappa}$ such that $\kappa_{kr} > \kappa_{ks}$ whenever $r > s$, with $\kappa_{k0} = -\infty$ and $\kappa_{kC} = \infty$. In this case let $\boldsymbol{\xi}_k = (a_k, \boldsymbol{\kappa}_k)$. Consequently, there are a total of $C - 1$ threshold parameters and one discrimination parameter for each item.

### *4.2.2 A latent variable model for the missing data process*

The data matrix of the observed data is partitioned into two parts, the observed part $\mathbf{y}_{obs}$ and the missing part $\mathbf{y}_{mis}$. The pattern of the missing data is given by a matrix $\mathbf{d}$, of the same dimension as $\mathbf{y}$ and equals one when an item is observed and zero otherwise. Although the proposed techniques can be applied to any missing data process, it is assumed that the binary responses (response, nonresponse) are indicators of an underlying latent variable $\boldsymbol{\zeta}$, which represents the tendency to respond (see, for example, Holman & Glas, 2005; Moustaki & Knott, 2000; O'Muircheartaigh & Moustaki, 1999). The actual response $y_{ik}$ itself depends on the individual's attitude level but the probability of a response depends on the individual's response propensity. The nonresponse or missing observations may include unit non-response, where a respondent does not respond to any of the items, and item non-response where the respondent does respond to some but not all of the items. Attention will be focused on item nonresponse although unit nonresponse is a more serious problem.

The occurrence of missing data is viewed as a random phenomenon. That is, the occurrence of missing data, in terms of item nonresponse of persons responding to an item, is governed by a random process that caused the missingness. Let $\boldsymbol{\zeta}, \boldsymbol{\phi}$ denote the parameters of the missing-data process, where $\boldsymbol{\zeta}$ are person parameters and $\boldsymbol{\phi}$ item parameters. Then, $p(\mathbf{d} \mid \boldsymbol{\zeta}, \boldsymbol{\phi})$ represents the latent variable model for the missing data mechanism. This latent variable model may exists of one or more factors, and can be defined as a confirmatory factor model or an item response theory model. That is, it will be assumed that the pattern of missing data are represented as a function of one or more latent variables. In the present paper, attention is focused on a nonignorable response model by allowing the attitude parameter $\boldsymbol{\theta}$ to affect the probability of responding. So, $p(\mathbf{d} \mid \boldsymbol{\theta}, \boldsymbol{\zeta}, \boldsymbol{\phi})$ is a nonignorable response model. An individual response depends on both the individual's ability or attitude and propensity to respond. For example, examinees with high math abilities may have a higher probability of responding to a math item than examinees with low math abilities. Further, the probability of a missing response depends on an attitude as well as a personality trait (Holman & Glas, 2005), or when measuring customer satisfaction, nonresponse is related to the latent opinion, since a nonresponse indicates a lack of knowledge or interest (Bradlow & Zaslavsky, 1999).

## 4.3 Detecting a Nonignorable Missing Data Mechanism

The complete data-likelihood of $(\mathbf{y}_{obs}, \mathbf{d})$, given the model parameters can be factorized as

$$p\big(\mathbf{y}_{obs}, \mathbf{d} \mid \boldsymbol{\theta}, \boldsymbol{\zeta}, \boldsymbol{\xi}, \boldsymbol{\phi}\big) = p\big(\mathbf{y}_{obs} \mid \mathbf{d}, \boldsymbol{\theta}, \boldsymbol{\xi}\big) p\big(\mathbf{d} \mid \boldsymbol{\theta}, \boldsymbol{\zeta}, \boldsymbol{\phi}\big), \tag{4.3}$$

where it is assumed that the missing data are missing at random. Inferences for $(\boldsymbol{\xi}, \boldsymbol{\phi})$ are based on this joint distribution combined with the priors for the model parameters. It is a priori assumed that the attitudes or abilities underlying the observed responses are independent of the propensity to respond. Therefore, the prior for each latent variable is a standard normal distribution. This way the mixture of models, the model for the observed responses and the missing data mechanism, is identified. Both parameters $\boldsymbol{\xi}$ and $\boldsymbol{\phi}$ have proper noninformative priors. It follows that the marginal posterior distribution of the item parameters $\boldsymbol{\xi}$ can be specified as,

$$p\big(\boldsymbol{\xi} \mid \mathbf{y}_{obs}, \mathbf{d}\big) \propto \int\int\int p\big(\mathbf{y}_{obs} \mid \mathbf{d}, \boldsymbol{\theta}, \boldsymbol{\xi}\big) p\big(\mathbf{d} \mid \boldsymbol{\theta}, \boldsymbol{\zeta}, \boldsymbol{\phi}\big) \times p(\boldsymbol{\theta}) p(\boldsymbol{\zeta}) p(\boldsymbol{\xi}) p(\boldsymbol{\phi})\, d\boldsymbol{\phi}\, d\boldsymbol{\zeta}\, d\boldsymbol{\theta} \tag{4.4}$$

When the $\boldsymbol{\theta}$ values do not interfere with the probability of responding, inferences about $\boldsymbol{\xi}$, ignoring the process that causes the missing data, is appropriate. In this particular case, the missing data mechanism is ignorable, also assuming that the missing data are missing at random, and equation (4.4) simplifies to

$$p\big(\boldsymbol{\xi} \mid \mathbf{y}_{obs}, \mathbf{d}\big) \propto \int p\big(\mathbf{y}_{obs} \mid \mathbf{d}, \boldsymbol{\theta}, \boldsymbol{\xi}\big) p(\boldsymbol{\theta}) p(\boldsymbol{\xi}) d\boldsymbol{\theta}. \tag{4.5}$$

As a result, inferences based on the distribution (4.5) are equivalent to inferences based on the full distribution, see the right-hand side of Equation (4.4).

### *4.3.1 The splitter item technique*

In a rich-data situation the data can be split in two samples according to the scores of one item, the splitter item. This method of splitting the data is quite common and can be used for general problems

like model assessment and selection. In this particular case, it is of interest to test whether the manner of splitting affects the statistical inference. That is, the splitter item technique is used to test whether the process that causes the missing data can be ignored.

Two samples are obtained when the observed item response data are divided according to the scores on a particular item $k$. In Figure 4.1 the splitting of the data $(\mathbf{y}_{obs}, \mathbf{d})$ is given in a diagram. The missing data indicator of item $k$ splits the data in two samples. The first sample, denoted as the observed group; the observed item responses of individual $i$ except those to item $k$, $\mathbf{y}_{i,obs}^{(-k)}$ with $d_{ik} = 1$, $i = 1, \ldots, n$. The second sample, denoted as the missing group: the observed item responses of individual $i$ except those to item $k$, $\mathbf{y}_{i,mis}^{(-k)}$ with $d_{ik} = 0$, $i = 1, \ldots, n$.

The occurrence of missing data patterns is modeled by a latent variable model. As a result, this splitting of the data in two groups depends on the values for $\boldsymbol{\zeta}$, $\boldsymbol{\theta}$ and some model parameters $\boldsymbol{\phi}$. After splitting the data, the marginal posterior distributions of the item parameters for the observed data are $p_{mis}\big(\boldsymbol{\xi} \mid \mathbf{y}_{obs}, \mathbf{d}^{(-k)}, d_k = 0\big)$ and $p_{obs}\big(\boldsymbol{\xi} \mid \mathbf{y}_{obs}, \mathbf{d}^{(-k)}, d_k = 1\big)$ for the missing and observed group, respectively. It follows that the statistical inferences derived from these posterior distributions are different when $\boldsymbol{\theta}$ affects the probability of responding to an item and accordingly influences the way the data are divided.

For example, assume that students were permitted to choose a subset of items of varying difficulty and that the better students choose the easier items, since they can better decide which items are easier (Bradlow & Thomas, 1998). In this case, $\boldsymbol{\theta}$ represent the individuals' math abilities, and the data are divided in a high level and low level group. The weaker students may select the harder items and make them appear even more difficult. So, the estimates of the corresponding difficulty parameter are biased. The better students select the more easier items and make them appear even more easier. Analogous, biased estimates of the difficulty parameter are obtained.

In summary, the splitter item technique is used to detect a nonignorable missing data mechanism by comparing the marginal posterior distributions of the item parameters given both samples. Results can be compared using summary statistics for the sampled values from the marginal posterior distributions, such as the posterior mean or median.

### *4.3.2 BMI based on the splitter item technique*

BMI were introduced by Fox and Glas (2005) for detecting model violations of the 2PNO model, differential item functioning and violations of the assumptions of local independence. They extended the 2PNO model with extra parameters such that the assumption to be tested is violated. An indication of a model violation is found when the estimated extra parameters are significantly different from zero. The marginal distribution of the extra parameters is unknown but samples from a so-called Bayesian modification (BM) distribution, that is a good approximation of the true marginal posterior distribution, can be obtained from an extra sampling step in an MCMC algorithm for sampling the parameters of the 2PNO model. BMI are useful when the number of model violations are large, or when estimating the parameters of the more general models is difficult and/or time-consuming.

The relation between the categorical outcomes and the underlying latent variables, equation (4.1) and (4.2), can also be explained in terms of a random variable $z_{ik}$ with mean $a_k\theta_i - b_k$ or $a_k\theta_i$ for binary or polytomous outcomes, respectively, and variance 1 (see, Albert, 1992; Johnson & Albert, 1999). The binary response $y_{ik}$ is the indicator of $z_{ik}$ being positive, and the polytomous response can be viewed as an indicator of $z_{ik}$ falling into one of the line segment associated with response categories. It follows that,

$$\begin{aligned} z_{ik} &= a_k\theta_i - b_k + e_{ik} \text{ such that } \begin{cases} y_{ik} = 0 \leftrightarrow z_{ik} \leq 0 \\ y_{ik} = 1 \leftrightarrow z_{ik} > 0 \end{cases} \\ z_{ik} &= a_k\theta_i + e_{ik} \text{ such that } y_{ik} = c \leftrightarrow \kappa_{kc-1} < z_{ik} < \kappa_{kc}, \end{aligned} \tag{4.6}$$

where $-\infty = \kappa_{k0} < \kappa_{k1} < \ldots < \kappa_{kC} = \infty$, and $z_{ik}$ normally distributed. The resulting model is the ordinal probit model for dichotomous or polytomous data, respectively. This ordinal probit model will be considered as the null-model.

The data are divided, according to the values of splitter item, in an observed ($j = 1$) and a missing group ($j = 0$). In case of nonignorable nonresponses, the item parameter estimates given the item responses of the observed group will differ from the item parameter estimates given the item responses of the missing group. This item bias can be modeled by extending the null model, equation (4.6), with fixed group effects that represent the item bias due to nonignorable nonresponses since the grouping of the data is based upon

the values (observed/missing) of the splitter item. Extending the two-parameter model for dichotomous data leads to

$$z_{ijk} = a_k\theta_{ij} - b_k + \big(\lambda_{1jk}\omega_{ij} + \lambda_{2jk}\big) + e_{ijk}, \tag{4.7}$$

where $j = 0, 1$, and $\omega_{ij}$ is an explanatory variable which might be $\theta$ or an observed covariate either within the test (test score) or outside the test. The magnitude of the extra parameters, $\boldsymbol{\lambda}_{jk} = (\lambda_{1jk}, \lambda_{2jk})$, depends on the extent to which the difference $z_{ijk}$ and $a_k\theta_{ij} - b_k$ is properly modeled. It follows that the response mechanism is not ignorable when the estimated group effects are significantly different from zero. Note that item bias in the discrimination parameter is modeled when one of the explanatory variables is a function of $\boldsymbol{\theta}$.

This fixed effects model in (4.7) can be written as a linear regression model using an indicator variable $\mathbf{x}_2$. The $i$th case of $\mathbf{x}_2$ equals one when the $i$th case of the splitter item is observed and zero otherwise or vice versa. In this case, the fixed effects model is identified by fixing one of the two group effects to zero, and the subscript $j$ of parameter $\boldsymbol{\lambda}$ can be dropped. The multiple regression model then is as follows:

$$\mathbf{z}_k = a_k\boldsymbol{\theta} - b_k + \mathbf{x}_2\big(\lambda_{1k}\boldsymbol{\omega} + \lambda_{2k}\big) + \mathbf{e}_k, \tag{4.8}$$

where the $\mathbf{z}_k$ and $\boldsymbol{\theta}$ are the augmented responses and latent attitudes or abilities of all respondents, respectively. In the same way the ordinal probit model, equation (4.6), can be extended to handle item bias. It follows that,

$$\mathbf{z}_k = a_k\boldsymbol{\theta} + \mathbf{x}_2\big(\lambda_{1k}\boldsymbol{\omega} + \lambda_{2k}\big) + \mathbf{e}_k, \tag{4.9}$$

where $\mathbf{e}_k$ are independent standard normally distributed. As for the two-parameter model, item bias in the discrimination parameter is modeled when one of the explanatory variables is a function of $\boldsymbol{\theta}$. Item bias in the threshold parameters is modeled by $\lambda_{2k}$ since it allows thresholds to vary across groups. In the simplest case,

$$\begin{aligned} P\big(z_{ijk} \le \kappa_{kc} \mid \theta_{ij}, a_k, \boldsymbol{\kappa}_k, \lambda_2\big) &= \Phi\big(\kappa_{kc} - (a_k\theta_{ij} + \lambda_{2k})\big) \\ &= \Phi\big((\kappa_{kc} - \lambda_{2k}) - a_k\theta_{ij}\big). \end{aligned} \tag{4.10}$$

As a result, in group $j$ the original thresholds for item $k$, $\boldsymbol{\kappa}_k$, are simultaneously shifted yielding the thresholds $\boldsymbol{\kappa}_k + \lambda_{2k}$. Thus, the effective thresholds vary across groups when the missing data mechanism cannot be ignored. The thresholds for item $k$, $\boldsymbol{\kappa}_k$, represent the average across groups.

## 4.4 Bayesian Estimation

Bayesian inference typically requires the computation of the posterior distribution for a collection of random variables (parameters or unknown observables). Therefore, numerous simulation-based methods have been developed and implemented within the Bayesian paradigm, e.g. importance sampling (Chen, Shao, & Ibrahim, 2000; Ripley, 1987), and Markov Chains Monte Carlo (MCMC) algorithms (see for e.g., Robert & Casella 1999; Gelfand & Smith, 1990; Gelman, Carlin, Stern & Rubin, 2004). In specific, MCMC procedures for sampling the parameters of logistic and probit IRT models were formulated by, among others, Albert (1992), Fox and Glas (2001, 2003), Hendrawan (2004), Johnson and Albert (1999), Maris and Maris (2002), and Patz and Junker (1999a, 1999b). A wide range of MCMC algorithms were developed for other latent variable models (e.g. Casella & Robert 1999; Congdon, 2002). More specific, Bradlow and Zaslavsky (1999) developed different MCMC schemes for sampling the parameters of a latent variable model for a missing data mechanism.

When employing the splitter item technique, and modeling both observed data sets, all parameters are sampled using MCMC. This way, values are sampled from $p_{mis}\big(\boldsymbol{\xi} \mid \mathbf{y}_{obs}, \mathbf{d}^{(-k)}, d_k = 0\big)$ and $p_{obs}\big(\boldsymbol{\xi} \mid \mathbf{y}_{obs}, \mathbf{d}^{(-k)}, d_k = 1\big)$ by sampling from their full conditionals. These group specific sampling steps are easily derived from the general procedure for sampling item parameter values. For example, in case of a normal ogive model for binary item responses with splitter item $K$. Let $t$ denote the iteration number of the Markov chain.

**Algorithm 1**

*Sample augmented data* $\mathbf{z}$*, for* $i = 1, \ldots, n_j, k = 1, \ldots, K-1$ *and* $j = 0, 1$*:*

$$z_{ijk}^{(t)} \mid \mathbf{y}_{obs}, \mathbf{d}^{(-k)}, \theta_i^{(t-1)}, \boldsymbol{\xi}_{jk}^{(t-1)} \sim \begin{cases} \mathcal{N}\big(a_{jk}\theta_i - b_{jk}, 1\big), \\ \quad \textit{if } y_{ijk} \textit{ is missing} \\ \mathcal{N}\big(a_{jk}\theta_i - b_{jk}, 1\big) I(z_{ijk} > 0), \\ \quad \textit{if } y_{ijk} = 1 \\ \mathcal{N}\big(a_{jk}\theta_i - b_{jk}, 1\big) I(z_{ijk} < 0), \\ \quad \textit{if } y_{ijk} = 0 \end{cases}$$

*Sample latent parameters* $\boldsymbol{\theta}$*, for* $i = 1, \ldots, n_j$*, and* $j = 0, 1$*:*

$$\theta_{ij}^{(t)} \mid \mathbf{z}_j^{(t)}, \boldsymbol{\xi}_j^{(t-1)} \sim \mathcal{N}\big((\mathbf{a}_j^t \mathbf{a}_j)^{-1} \mathbf{a}_j^t (\mathbf{z}_{ij} + \mathbf{b}_j), (\mathbf{a}_j^t \mathbf{a}_j)^{-1}\big) p(\theta_{ij})$$

*where* $\mathbf{a}_j = (a_{j1}, \ldots, a_{jK-1})$.

*Sample item parameters* $\boldsymbol{\xi}_{jk}$*, for* $k = 1, \ldots, K-1$ *and* $j = 0, 1$*:*

$$\boldsymbol{\xi}_{jk}^{(t)} \mid \mathbf{z}_{jk}^{(t)}, \boldsymbol{\theta}_j^{(t)} \sim \mathcal{N}\Big(\hat{\boldsymbol{\xi}}_{jk}, \big(\mathbf{H}_j^t \mathbf{H}_j\big)^{-1}\Big) p(\boldsymbol{\xi}_{jk})$$

*where* $\mathbf{H}_j = (\boldsymbol{\theta}_j, \mathbf{1}_{n_j})$ *and* $\hat{\boldsymbol{\xi}}_{jk} = \big(\mathbf{H}_j^t \mathbf{H}_j\big)^{-1} \mathbf{H}_j^t \mathbf{z}_{jk}$.

In summary, values from the marginal distribution of the group specific item parameter estimates are easily obtained using MCMC when modeling the observed data. Then, summaries of these marginal posterior distributions can be used to decide whether the missing data process can be safely ignored.

### *4.4.1 Sampling BMI parameters*

The BMI values are sampled as an extra step in an MCMC algorithm for estimating the null-model parameters. This way, sampled values of the BM distribution are obtained when estimating the parameters of the null-model. The extra step in the MCMC algorithm consists of sampling values of $\boldsymbol{\lambda}$ given sampled values of the IRT null-model parameters. These extra draws do not influence the chain, and the Markov chain remains restricted to the manifold of the posterior corresponding to the null-model. It will be shown that the resulting estimate of the marginal posterior of $\boldsymbol{\lambda}$ is a good approximation of the true marginal posterior distribution.

A general model that includes the models in equation (4.8) and (4.9) for the observed data, with the extra BMI parameters $\boldsymbol{\lambda}$ for modeling the item bias can be written as,

$$\mathbf{z}_k = \mathbf{x}_1 \boldsymbol{\gamma}_k + \mathbf{x}_2 \boldsymbol{\lambda}_k + \mathbf{e}_k, \tag{4.11}$$

where $\mathbf{x}_1$ is an $n \times r$ and $\mathbf{x}_2$ an $n \times 2$ matrix with rank $r$ and 2, respectively, and $\mathbf{e}_k$ are normally distributed with variance $\sigma^2$. Note that $\mathbf{x}_1 = \big(\boldsymbol{\theta}, -\mathbf{1}\big)$ with $r = 2$ and $\boldsymbol{\gamma}_k = \big(a_k, b_k\big)$ for binomial observed data and $\mathbf{x}_1 = \boldsymbol{\theta}$ with $r = 1$ and $\boldsymbol{\gamma}_k = a_k$ for ordinal polytomous data. The full conditional distribution of the BMI parameters can

be specified explicitly. Therefore, define the least squares estimate of $\boldsymbol{\gamma}_k$ and $\boldsymbol{\lambda}_k$ as

$$\begin{pmatrix} \hat{\gamma}_k \\ \hat{\boldsymbol{\lambda}}_k \end{pmatrix} = \begin{pmatrix} \mathbf{x}_1^t\mathbf{x}_1 & \mathbf{x}_1^t\mathbf{x}_2 \\ \mathbf{x}_2^t\mathbf{x}_1 & \mathbf{x}_2^t\mathbf{x}_2 \end{pmatrix}^{-1} \mathbf{x}^t\mathbf{z}_k = \begin{pmatrix} \mathbf{v}_{11} & \mathbf{v}_{12} \\ \mathbf{v}_{21} & \mathbf{v}_{22} \end{pmatrix} \mathbf{x}^t\mathbf{z}_k = \mathbf{v}\mathbf{x}^t\mathbf{z}_k,$$

with $\mathbf{v} = (\mathbf{x}^t\mathbf{x})^{-1}$ and $\mathbf{x} = (\mathbf{x}_1, \mathbf{x}_2)$. It follows from linear regression theory (see, e.g., Box & Tiao, 1973, p. 116-118) that the true marginal distribution of $\boldsymbol{\lambda}_k$ given $\mathbf{z}_k$ according to the full model is a bivariate t-distribution $t_2\big[\hat{\boldsymbol{\lambda}}_k, s^2\mathbf{v}_{22}, n-(r+2)\big]$, where $r+2$ equals the dimension of $\big(\boldsymbol{\gamma}_k, \boldsymbol{\lambda}_k\big)$ and $s^2$ is an estimate of the residual variance of the model in equation (4.11) using a noninformative reference prior for $\boldsymbol{\lambda}_k$ and $\boldsymbol{\gamma}_k$.

Let $\mathbf{z}$ and $\boldsymbol{\theta}$ be given. Then, the null-model is given by

$$\mathbf{z}_k = \mathbf{x}_1\boldsymbol{\gamma}_k + \mathbf{e}_k, \tag{4.12}$$

where $\mathbf{e}_k$ are normally distributed with variance $\sigma_0^2$. An MCMC algorithm is extended by sampling BMI values of $\boldsymbol{\lambda}$. Let $t$ denote the iteration number of the Markov chain.

**Algorithm 2**

*sample* $\boldsymbol{\gamma}_k^{(t)} \mid \mathbf{z}_k, \sigma_0^{2^{(t-1)}}$

*sample* $\sigma_0^{2^{(t)}} \mid \mathbf{z}_k, \boldsymbol{\gamma}_k^{(t)}$

*sample* $\boldsymbol{\lambda}_k^{(t)} \mid \mathbf{z}_k, \boldsymbol{\gamma}_k^{(t)}, \sigma_0^{2^{(t)}}$

Notice that the sampled values of $\boldsymbol{\lambda}_k$ do not interfere with the sampling of the other parameters. Further, the grouping index $j$ only applies for sampling BMI values. It is assumed that the elements of $\boldsymbol{\lambda}$, $\boldsymbol{\gamma}_k$, and $\log(\sigma_0^2)$ are uniformly and independently distributed. Then, the full conditional distribution of $\boldsymbol{\gamma}_k$ equals

$$\boldsymbol{\gamma}_k \mid \mathbf{z}_k, \mathbf{x}_1, \sigma_0^2 \sim \mathcal{N}\big(\hat{\gamma}_{k,\mathbf{x_1}}, \sigma_0^2\mathbf{v}_{11}\big) \tag{4.13}$$

where $\hat{\gamma}_{k,\mathbf{x_1}}$ is the least squares estimate of $\boldsymbol{\gamma}_k$ given $\mathbf{x_1}$ and $\mathbf{z}$. Further, the full conditional of $\sigma_0^2$ equals

$$\sigma_0^{2^{(t)}} \mid \mathbf{z}_k, \boldsymbol{\gamma}_k \sim \mathcal{IG}\Big(\frac{n}{2}, \sum_i \Big(z_{ik} - \mathbf{x}_1\boldsymbol{\gamma}_k^{(t)}\Big)^2/2\Big). \tag{4.14}$$

Accordingly, as an extra MCMC step values of the BMI parameters are sampled from the full conditional distribution,

$$\boldsymbol{\lambda}_k \mid \mathbf{z}_k, \mathbf{x}, \boldsymbol{\gamma}_k, \sigma_0^2 \sim \mathcal{N}\Big(\hat{\boldsymbol{\lambda}}_k + \mathbf{v}_{12}^t \mathbf{v}_{11}^{-1}\big(\boldsymbol{\gamma}_k - \hat{\boldsymbol{\gamma}}_{k,\mathbf{x}_1}\big), \sigma_0^2\big(\mathbf{v}_{22} - \mathbf{v}_{12}^t \mathbf{v}_{11}^{-1}\mathbf{v}_{12}\big)\Big). \tag{4.15}$$

The Bayesian Modification (BM) distribution is obtained by integrating the conditional distribution of $\boldsymbol{\lambda}_k$ with respect to the null model parameters using MCMC. That is, the MCMC algorithm is used for obtaining sampled values from the Bayesian Modification (BM) distribution. Let $\tilde{p}(.)$ denote the BM distribution, and $\big\{\big(\boldsymbol{\gamma}_k^{(m)}, \sigma_0^{2(m)}\big), m = 1, \ldots, M\big\}$ an MCMC sample from the joint posterior distribution $p\big(\boldsymbol{\gamma}_k, \sigma_0^2 \mid \mathbf{z}\big)$. It follows that

$$\begin{aligned}\tilde{p}\big(\boldsymbol{\lambda}_k \mid \mathbf{z}_k, \mathbf{x}\big) &= \int_0^\infty \int_\Omega \tilde{p}\big(\boldsymbol{\lambda}_k \mid \mathbf{z}_k, \mathbf{x}, \boldsymbol{\gamma}_k, \sigma_0^2\big) p\big(\boldsymbol{\gamma}_k, \sigma_0^2 \mid \mathbf{z}_k, \mathbf{x}_1\big) d\boldsymbol{\gamma}_k d\sigma_0^2 \\ &= \lim_{M\to\infty} 1/M \sum_{m=1}^{M} \tilde{p}\Big(\boldsymbol{\lambda}_k \mid \mathbf{z}_k, \mathbf{x}, \boldsymbol{\gamma}_k^{(m)}, \sigma_0^{2(m)}\Big),\end{aligned} \tag{4.16}$$

where $\Omega = \{\boldsymbol{\gamma}_k \in \mathbb{R}^r\}$. In the Appendix it is shown that the marginal BM distribution in equation (4.16) is the bivariate t-distribution $t_2\big[\hat{\boldsymbol{\lambda}}_k, s_0^2 \mathbf{v}_{22}, n - r\big]$, using a noninformative reference prior for $\boldsymbol{\lambda}_k$ and $\boldsymbol{\gamma}_k$. Here, $s_0^2$ is an estimate of the residual variance of the null-model in equation (4.12). As a result, the marginal BM distribution approximates the true marginal posterior distribution very good. It is not expected that the residual variance, $s_0^2$, differs much from the larger residual variance $s^2$ since the measurement null-model contains all relevant (latent) variables.

## 4.5 Simulated Examples

Bradlow and Thomas (1998) analyzed simulated response data from an examination that allowed students to choose a subset of items. In this choice-based examination, a subset of items was presented in pairs of items and the examinees choose to respond to one of them. No responses are given to those items that are not selected. The choice mechanism can only be ignored when the examinees randomly select items. Recently, the fairness of item-selection has been further investigated by Allen, Holland, and Thayer (2005).

The two experiments by Bradlow and Thomas (1998) are used to demonstrate the splitter item technique for detecting nonignorable missing data. In both experiments, 5000 abilities and 20 difficulty parameter values were generated from a standard normal distribution. These parameter values were used to generate item response data according the Rasch model. The last ten items were considered as paired items. In a third experiment polytomous IRT data were generated according the ordinal probit IRT model. A response mechanism simulated that respondents with a negative strong opinion are more likely to give a nonresponse. This corresponds with well-known cases where respondents refuse to give a socially undesirable answer.

In all three experiments below, Gibbs sampling algorithms were used for estimating the IRT models for binary and polytomous data. For each model, 20,000 iterations were used for estimating the model parameters with a burn-in period of 1,000 iterations. Convergence of the Markov chains was easily established using plots of sampled values and using several convergence diagnostics (Gelman et. al., 2004). For further details regarding the sampling procedure refer to Albert (1992) and Johnson and Albert (1999). All IRT models were identified by fixing the scale of the posterior distribution of the latent variable with mean zero and variance one.

### *4.5.1 Experiment 1*

In the first experiment, the examinees with positive abilities chose the easier items within pairs with probability $p_1 = .95$ and the harder item with probability $p_2 = .05$. These examinees can often decide which of the two items is easier. The examinees with negative abilities chose one of the items randomly. So, the distribution of the response mechanism depends on the ability parameters $\boldsymbol{\theta}$ underlying the observed responses and the difficulty parameters. As a result, the missing data are missing at random but the parameters of the missing data process, $\boldsymbol{\phi}$, are not a priori distinct from $\boldsymbol{\theta}$. Inferences about $\boldsymbol{\theta}$ cannot be based on the posterior distribution $p(\boldsymbol{\theta} \mid \mathbf{y}_{obs})$ ignoring the missing data mechanism.

The BMI parameters $\boldsymbol{\lambda}$ were defined according to equation (4.8) with indicator variable $\mathbf{x}_2$ equal to one if the corresponding value for the splitter item was missing and zero otherwise. These BMI parameters represent item bias for the so-called missing group, the set of observed item response data with nonresponses for the splitter

item. According to missing data mechanism it was expected that the item difficulties varied over groups since the distribution of abilities varied across groups.

In Table 4.1 are the posterior means and standard deviations given of the difficulty parameters for different subsets of item response data. The posterior means of $p\big(\mathbf{b} \mid \mathbf{y}_{obs}\big)$ correspond to the estimated item difficulties given all observed item response data. It can been seen that for each paired item the difficult item is overestimated and the easy item is underestimated. In most cases the better students choose the easier item and make them appear even more easier. This is in contrast to the harder items. These items were selected by the weaker students and they make them appear even more difficult. The observed item response data were grouped according to the values of item 20. This splitter item was paired with item 19, item 19 was the easier item. As a result, most of the better students chose to make item 19 and they are denoted as the missing group. Most of the weaker students chose to make the more difficult item 20, denoted as the observed group. It can be seen that the true item difficulties are highly overestimated by the posterior means of $p_{obs}\big(\mathbf{b} \mid \mathbf{y}_{obs}, \mathbf{d}_{20} = \mathbf{1}\big)$ since the item responses of the observed group correspond to the weaker students. Subsequently, the posterior means of $p_{mis}\big(\mathbf{b} \mid \mathbf{y}_{obs}, \mathbf{d}_{20} = \mathbf{0}\big)$ are lower than the true item difficulties. The difficulty parameter of item 19 could not be estimated since the students of the observed group did not respond to item 19. It can be concluded that the pattern of missing data is affected by the abilities of the students. That is, the examinee's propensity to respond correlates with their ability and this results in nonignorable missing data.

The BMI values are sampled under the null-model for the missing group. The posterior means are all negative indicating that the students in the missing group make the items appear more easier. The estimated BMI values cannot capture the difference between the estimates given all data and the estimates given only the item responses of the missing group. This follows from the fact that the other model parameters are estimated under the null model. However, the estimated BMI values are for most items significant given the 95% highest posterior density intervals (HPD), that is, the value zero was not contained in the HPD region. The grouping of the data according to splitter item 20 resulted in various significant fixed group effects indicating that the way of grouping the data (observed/missing) af-

fects the results. The set of responses to the last ten items contains missing values that caused a reduction in size of the estimated BMI values.

The choice of the splitter item may affect the results. The last ten items can be considered as potential splitter items. All BMI values are sampled in one MCMC algorithm for estimating the null model for each possible splitter item. This procedure requires only extra sampling steps. In Figure 4.2, the posterior distributions of the BMI's are given for splitter item 12, 17, and 20. These items are all easy items in the pairs of items. It can be seen that the results are not depending on the splitter item since the estimated BMI values and their distributions are comparable.

### *4.5.2 Experiment 2*

In the second simulation, each examinee first responded to both items of a pair, and, contingent on the responses, then submitted only one response and left the other response missing. This was done according to the following rule. For each of the paired items, each examinee chose randomly one of the items when both responses were either correct or incorrect. If one of the responses within a pair was correct and the other one incorrect, then the examinee chose the correct one with probability $p_1 = .75$ and the incorrect one with probability $1 - p_1 = .25$. The response mechanism cannot be ignored since the missing data are not missing at random. That is, the distribution of the missing data mechanism depends on the missing values.

In the second and third column of Table 4.2, the true and estimated item difficulties are given using all item response data. As expected, the item difficulties of the paired items are underestimated. This follows from the fact that students are tended to select items when they knew the correct answer. The last item was used as a splitter item and the data were grouped in an observed and a missing group. However, if examinees responded to item 20 they did not respond to item 19, and the other way around. As a result, the splitter item technique does not provide any additional information since the responses to item 19 of the missing group correspond with all observed responses to item 19, and the observed group did not respond to item 19. The patterns of missing data corresponding to a pair of items are only depending on the values of the missing item responses to this pair of items. Therefore, it was not expected to detect any

differences between the estimated item difficulties corresponding to the grouped data.

In Table 4.2 it can been seen that the estimated posterior means based on the item responses of the observed group (fifth column) are slightly higher than the estimated posterior means based on the item responses of the missing group (seventh column). Because item 20 is much easier than item 19, the observed group consists of weaker students. They knew the correct answer to item 20 but not to item 19. However, this effect is in most cases very small and not significant given the posterior standard deviations. This is supported by the estimated BMI's, defined as in experiment 1. These estimated BMI values were around zero and not significant.

### *4.5.3 Experiment 3*

In collecting data via surveys it is often assumed that the respondents are willing to cooperate and respond honestly to the survey questions. In case of sensitive topics, respondents may be tended to provide more socially desirable answers or provide nonresponses. In the same way, lack of a strong opinion or indifference can also lead to nonresponses (see, e.g., Baker and laird, 1988; Bradlow and Zaslavsky, 1999). De Leeuw, Hox, and Huisman (2003), and Sijtsma and van der Ark (2003) discuss several types of missing item scores. Item response data were generated according to the ordinal probit model with three response categories and discrimination parameters set to one. The attitude parameters were generated from a standard normal distribution. The ordered threshold parameters were generated from an uniform distribution restricted to the interval $[-.75, -.50] \cup [.50, .75]$. For the last ten items out of twenty items, nonresponses were generated. Respondents scoring in the lowest category had a probability of 40% of a nonresponse and others 20%. As a result, the missing data are not missing at random since the distribution of the missing data mechanism depends on the missing values. Respondents with low attitude values provided responses in the lowest category, and, subsequently, they had a higher propensity to give a nonresponse than respondents with high attitude values. The willingness to give a (negative, mild, positive) response was correlated with the respondents' attitude being measured, that is, the attitude parameter was correlated with the parameter of the missing data mechanism.

The last item was used as a splitter item, and the data were split in two groups, a missing and an observed group. The item parameters were estimated using MCMC given all observed data and the group specific item response data. In Figure 4.3 are the threshold estimates given corresponding to the full and partitioned data set. It can be seen that the true threshold values are underestimated when using all observed data. About 25% of the item response data is missing and about 44% of the missing item responses were scores in the lowest category causing an underestimation of the true threshold values. It appears as if respondents score relatively often in a second or third category. However, respondents, who were inclined to score in the first category, more often refused to give an answer. It follows that the estimated threshold parameters given the item responses of the missing group are higher than the estimates given all observed data. The missing group contain respondents with lower attitudes and they are more inclined to score in the first category. Most of the corresponding 95% HPD regions of the estimated threshold parameters given the item response data of the missing group do not contain the estimated threshold values given all observed item response data. The partitioning of the data based on the values of the splitter item (observed/missing) resulted in different item parameter estimates and, subsequently, the missing data are nonignorable.

Again, BMI values are sampled under the null-model for the missing group. In Figure 4.4 are the marginal posterior distributions given of all BMI parameters using the last item as the splitter item. The estimated BMI values captured the difference between the estimates given all data and the estimates given only the item responses of the missing group. That is, more than 50% of the estimated BMI values are significant given 95% highest posterior density intervals (HPD). Note that the estimated posterior means are negative since this corresponds with a shift upwards in threshold values, see Equation (4.10).

In all three experiments, the mechanism for generating missing item responses was known to produce nonignorable missing data. The proposed method detected nonignorable missing data in two experiments. In all three experiments, it was investigated that the splitter item technique did not detect significant differences in item parameter estimates across groups in case of ignorable missing item

response data. In this case, the data were grouped completely at random, and significant differences were also not to be expected.

## 4.6 Discussion

The splitter item technique can be used to test whether the response mechanism leads to ignorable or nonignorable missing data. In this proposed procedure, it is tested if the item parameter estimates differ across the subsets of item response data. Differences in item parameter estimates across subsets indicate nonignorable missing data since the splitting of the data was done according to the values of the splitter item (observed/missing). Two methods were proposed for detecting differences in estimates across groups. In the first method, all parameters are estimated given the subsets of item response data. Then, summary statistics of the estimated marginal posterior distributions of the item parameters can be used for detecting differences. In the second method, parameters of an IRT model for binary or ordinal responses are estimated given all observed data using MCMC and, as an additional sampling step, BMI values are sampled. These BMI values provide information regarding any fluctuations in item parameter values across subsets of item response data. In the Appendix, it is shown that the Bayesian Modification distribution is a good approximation of the true marginal posterior distribution of the item parameters. As a result, the BMI values can be obtained as a by-product of the MCMC algorithm for estimating the parameters of an IRT model.

Further study is focused on the generalization of the BMI approach to more complex models. One of the main advantages of estimating IRT models using a fully Bayesian approach is that traditional frequentist approaches break down because of the infeasible numerical evaluation of the multiple integrals involved in solving the estimation equations. The splitter item technique in combination with BMI becomes particularly interesting when estimating complex IRT models, like testlet response models (Bradlow, Wainer and Wang, 1999), models with multidimensional latent abilities (Béguin and Glas, 2001), and multilevel IRT models (Fox, 2004; Fox and Glas, 2001, 2003) and it is in the realm of these models that more research needs to be done.

## 4.7 Appendix

The marginal BM distribution in equation (4.16) of the BMI parameter can be obtained by integration using a noninformative reference prior for $\boldsymbol{\lambda}_k$ and $\boldsymbol{\gamma}_k$.

$$\begin{aligned}\tilde{p}\big(\boldsymbol{\lambda}_k \mid \mathbf{z}_k, \mathbf{x}\big) &= \int_0^\infty \int_\Omega \tilde{p}\big(\boldsymbol{\lambda}_k \mid \mathbf{z}_k, \mathbf{x}, \boldsymbol{\gamma}_k, \sigma_0^2\big) p\big(\boldsymbol{\gamma}_k, \sigma_0^2 \mid \mathbf{z}_k, \mathbf{x}_1\big) d\boldsymbol{\gamma}_k d\sigma_0^2 \\ &\propto \int_0^\infty \int_\Omega \sigma_0^{-(n+r+1)} \exp\Bigg(\frac{-1}{2\sigma_0^2}\Bigg(\Bigg[\big(\boldsymbol{\lambda}_k - \hat{\boldsymbol{\lambda}_k}\big) - \mathbf{v}_{12}^t \mathbf{v}_{11}^{-1} \\ &\quad \big(\boldsymbol{\gamma}_k - \hat{\boldsymbol{\gamma}}_{k,\mathbf{x}_1}\big)\Bigg]^t \mathbf{w}_{22} \Bigg[\big(\boldsymbol{\lambda}_k - \hat{\boldsymbol{\lambda}_k}\big) - \mathbf{v}_{12}^t \mathbf{v}_{11}^{-1} \big(\boldsymbol{\gamma}_k - \hat{\boldsymbol{\gamma}}_{k,\mathbf{x}_1}\big)\Bigg] \\ &\quad + (n-2)s_0^2 + \big(\boldsymbol{\gamma}_k - \hat{\boldsymbol{\gamma}}_{k,\mathbf{x}_1}\big)^t \mathbf{v}_{11}^{-1} \big(\boldsymbol{\gamma}_k - \hat{\boldsymbol{\gamma}}_{k,\mathbf{x}_1}\big)\Bigg)\Bigg) d\boldsymbol{\gamma}_k d\sigma_0^2\end{aligned}$$

where $\Omega = \{\boldsymbol{\gamma}_k \in \mathbb{R}^2\}$ and $\mathbf{w}_{22} = \big(\mathbf{v}_{22} - \mathbf{v}_{12}^t \mathbf{v}_{11}^{-1} \mathbf{v}_{12}\big)^{-1}$. Terms can be gathered together by defining $\boldsymbol{\Lambda}_k = (\boldsymbol{\gamma}_k, \boldsymbol{\lambda}_k)$ and using a specific form of the inverse of a partitioned full rank symmetric matrix, that is,

$$\mathbf{v}^{-1} = \begin{bmatrix} \mathbf{v}_{11}^{-1} + \mathbf{v}_{11}^{-1} \mathbf{v}_{12} \mathbf{w}_{22} \mathbf{v}_{12}^t \mathbf{v}_{11}^{-1} & -\mathbf{v}_{11}^{-1} \mathbf{v}_{12} \mathbf{w}_{22} \\ -\mathbf{w}_{22} \mathbf{v}_{12}^t \mathbf{v}_{11}^{-1} & \mathbf{w}_{22} \end{bmatrix}. \tag{4.17}$$

It follows that

$$\begin{aligned}\tilde{p}(\boldsymbol{\Lambda}_k \mid \mathbf{z}_k) &\propto \int_0^\infty \int_\Omega \sigma_0^{-(n+r+1)} \exp\Bigg(\frac{-1}{2\sigma_0^2}\Bigg((n-2)s_0^2 + \big(\boldsymbol{\Lambda}_k - \\ &\qquad \hat{\boldsymbol{\Lambda}_k}\big)^t \mathbf{v}^{-1} \big(\boldsymbol{\Lambda}_k - \hat{\boldsymbol{\Lambda}_k}\big)\Bigg) d\boldsymbol{\gamma}_k d\sigma_0^2 \\ &\propto \int_\Omega \Big[\nu\, s_0^2 + \big(\boldsymbol{\Lambda}_k - \hat{\boldsymbol{\Lambda}_k}\big)^t \mathbf{v}^{-1} \big(\boldsymbol{\Lambda}_k - \hat{\boldsymbol{\Lambda}_k}\big)\Big]^{-(\nu+r+2)/2} d\boldsymbol{\gamma}_k, \end{aligned} \tag{4.18}$$

where $\nu = n - r$. This last step in equation (4.18) follows from the integral formula

$$\int_0^\infty x^{-(p+1)} \exp\Big(\frac{-a}{x^2}\Big) dx = \frac{1}{2} a^{-p/2} \Gamma(p/2), \tag{4.19}$$

with $a, p$ greater than zero and $\Gamma(.)$ the Gamma function. Finally, the right hand side of equation (4.18) can be recognized as the multivariate t-distribution. As a result, the marginal distribution of $\boldsymbol{\lambda}_k$, a

subset of $\mathbf{\Lambda}_k$, has a multivariate t-distribution (Box and Tiao, 1973)

$$\boldsymbol{\lambda}_k \mid \mathbf{z}_k \sim t_2 \left[ \hat{\boldsymbol{\lambda}_k}, s_0^2 \mathbf{v}_{22}, \nu \right]. \tag{4.20}$$

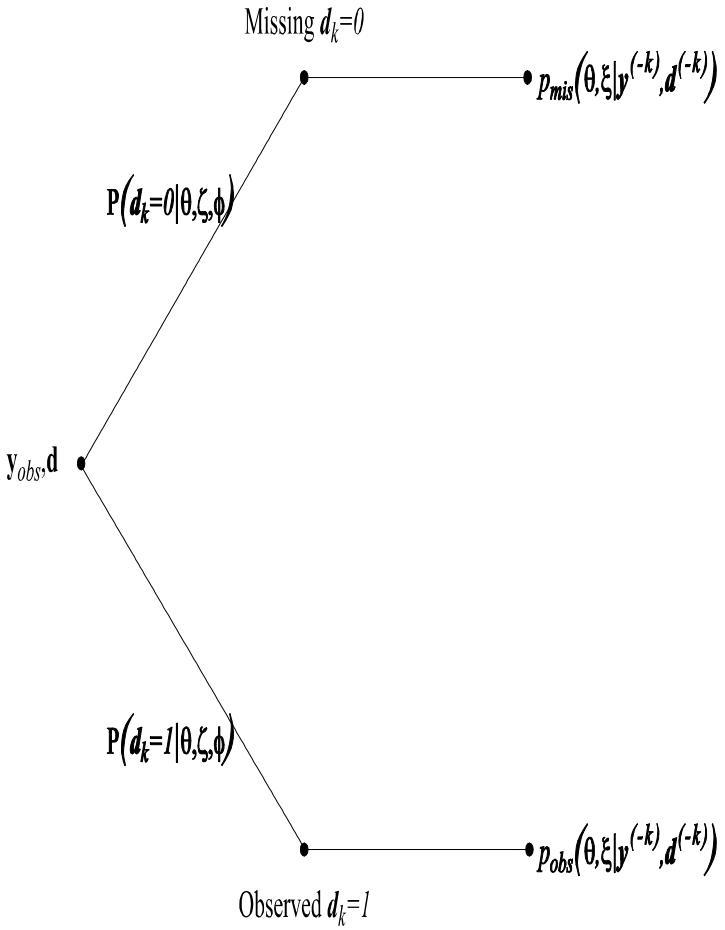



FIGURE 4.1. Splitting the observed item response data.

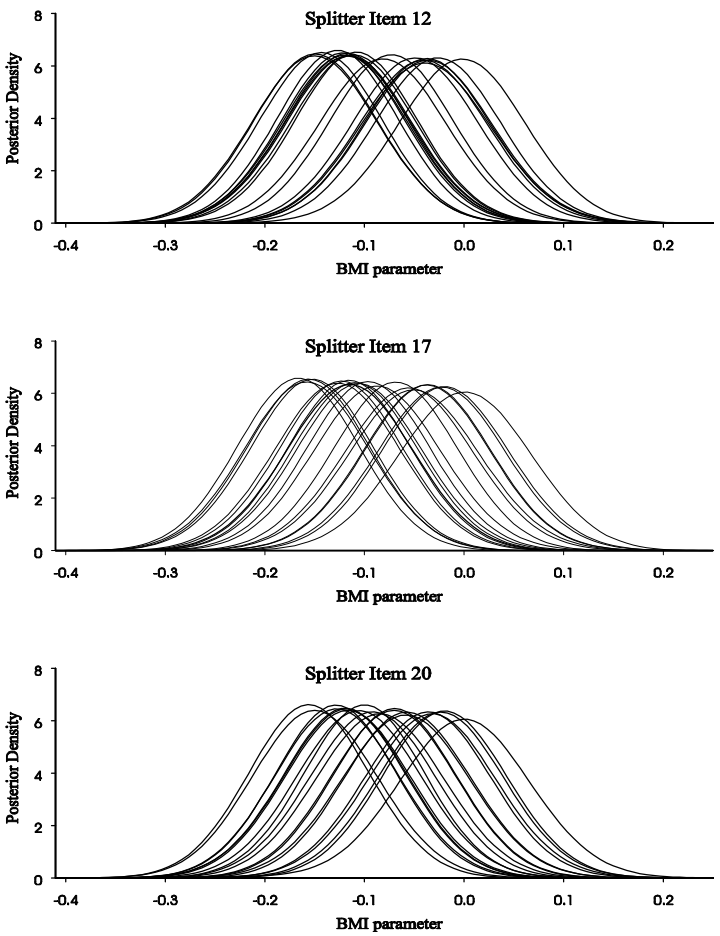



FIGURE 4.2. Posterior distributions of BMI parameters representing differences in item difficulties, corresponding to splitter items 12, 17, and 20.

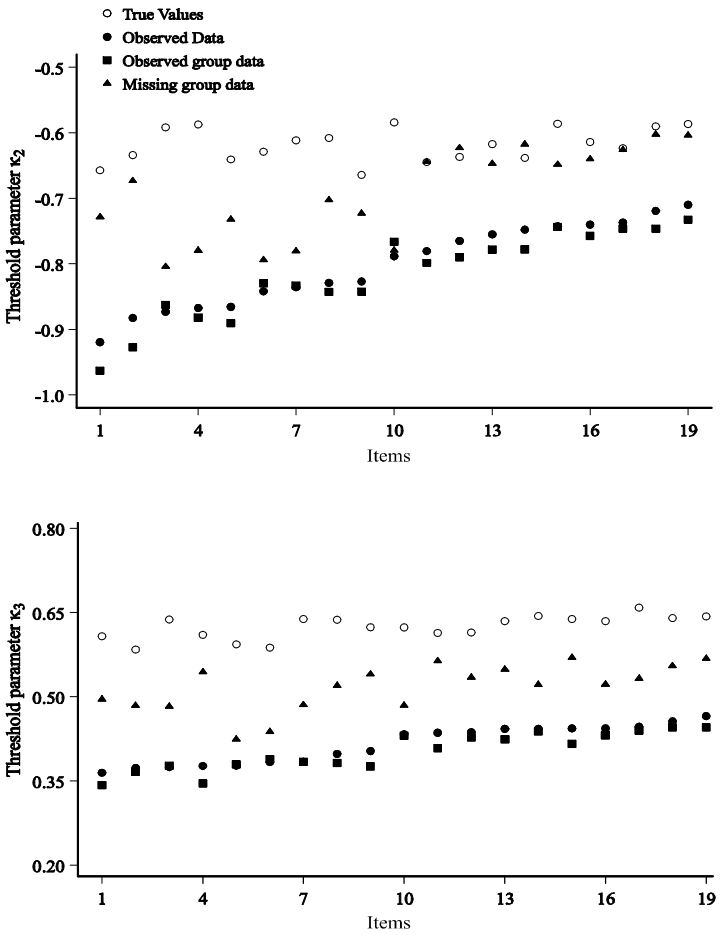



FIGURE 4.3. Threshold parameter estimates given all observed data, and the item responses of the missing and observed group where item 20 served as a splitter item.

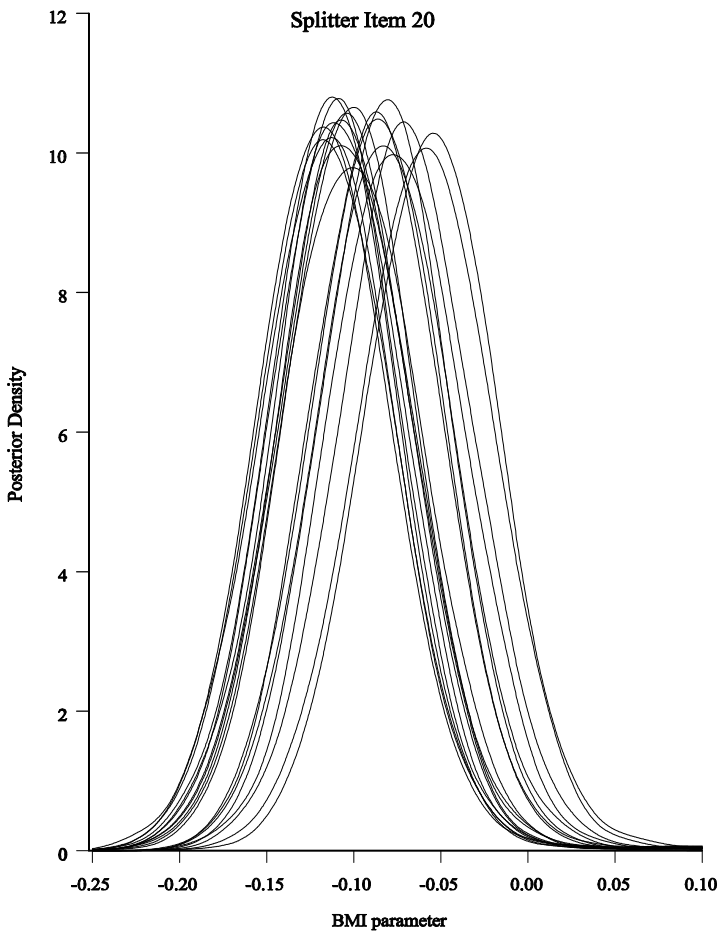



FIGURE 4.4. Posterior distributions of BMI parameters representing differences in item thresholds corresponding to splitter items 20.

TABLE 4.1. Experiment 1: The response mechanism depends upon the examinee ability and item difficulties.

| | | | | Splitter Item 20 | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $p(\mathbf{b} \mid \mathbf{y}_{obs})$ | | $p_{obs}(\mathbf{b} \mid \mathbf{y}_{obs})$ | | $p_{mis}(\mathbf{b} \mid \mathbf{y}_{obs})$ | | $\tilde{p}_{mis}(\boldsymbol{\lambda} \mid \mathbf{y}_{obs})$ | |
| Item | $\mathbf{b}$ | Mean | sd | Mean | sd | Mean | sd | $\text{BMI}_{mis}$ | HPD |
| 1 | .90 | .91 | .03 | 1.55 | .07 | .64 | .03 | −.12 | [−.19, −.05] |
| 2 | .89 | .91 | .03 | 1.69 | .08 | .62 | .03 | −.12 | [−.19, −.05] |
| 3 | .51 | .48 | .02 | 1.16 | .06 | .19 | .03 | −.16 | [−.23, −.09] |
| 4 | 1.17 | 1.13 | .03 | 1.70 | .08 | .88 | .03 | −.09 | [−.16, −.02] |
| 5 | 1.50 | 1.48 | .04 | 2.45 | .18 | 1.18 | .04 | −.07 | [−.14, −.01] |
| 6 | −.66 | −.63 | .02 | .00 | .04 | −.90 | .03 | −.10 | [−.17, −.04] |
| 7 | 1.10 | 1.11 | .03 | 1.81 | .09 | .81 | .03 | −.13 | [−.19, −.05] |
| 8 | −1.41 | −1.39 | .04 | −.74 | .04 | −1.71 | .06 | −.07 | [−.15, −.01] |
| 9 | −.63 | −.64 | .03 | .05 | .04 | −.95 | .04 | −.13 | [−.19, −.05] |
| 10 | 1.06 | 1.02 | .03 | 1.75 | .09 | .72 | .03 | −.15 | [−.22, −.07] |
| 11 | −1.10 | −1.18 | .04 | −.52 | .06 | −1.44 | .05 | −.06 | [−.14, .01] |
| 12 | .29 | .44 | .05 | .97 | .07 | .16 | .07 | −.04 | [−.11, .04] |
| 13 | .12 | .12 | .03 | .78 | .07 | −.15 | .03 | −.10 | [−.17, −.03] |
| 14 | .44 | .63 | .05 | 1.14 | .07 | .37 | .07 | −.03 | [−.11, .05] |
| 15 | .24 | .17 | .03 | .95 | .08 | −.12 | .03 | −.12 | [−.19, −.05] |
| 16 | .29 | .45 | .04 | .95 | .07 | .24 | .07 | −.02 | [−.10, .05] |
| 17 | .00 | .13 | .05 | .65 | .06 | −.21 | .08 | −.02 | [−.10, .06] |
| 18 | −1.09 | −1.09 | .03 | −.37 | .06 | −1.38 | .05 | −.08 | [−.16, −.01] |
| 19 | −.15 | −.18 | .03 | − | − | −.41 | .03 | −.06 | [−.14, .02] |
| 20 | 1.00 | 1.14 | .05 | − | − | − | − | − | − |

TABLE 4.2. Experiment 2:The response mechanism depends upon the examinees unobserved item responses

| | | | | Splitter Item 20 | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $p(\mathbf{b} \mid \mathbf{y}_{obs})$ | | $p_{obs}(\mathbf{b} \mid \mathbf{y}_{obs})$ | | $p_{mis}(\mathbf{b} \mid \mathbf{y}_{obs})$ | | $\tilde{p}_{mis}(\boldsymbol{\lambda} \mid \mathbf{y}_{obs})$ | |
| Item | $\mathbf{b}$ | Mean | sd | Mean | sd | Mean | sd | $\text{BMI}_{mis}$ | HPD |
| 1 | −.25 | −.27 | .02 | −.23 | .03 | −.32 | .03 | .02 | [−.05, .08] |
| 2 | −.35 | −.38 | .02 | −.36 | .03 | −.41 | .03 | .00 | [−.06, .07] |
| 3 | .85 | .84 | .03 | .85 | .04 | .83 | .04 | −.03 | [−.10, .04] |
| 4 | −.20 | −.25 | .02 | −.24 | .03 | −.25 | .03 | −.01 | [−.07, .06] |
| 5 | .90 | .88 | .03 | .92 | .03 | .82 | .04 | .00 | [−.07, .07] |
| 6 | −.55 | −.60 | .02 | −.59 | .03 | −.62 | .03 | −.01 | [−.08, .06] |
| 7 | .35 | .30 | .02 | .31 | .03 | .28 | .03 | −.01 | [−.08, .06] |
| 8 | −.50 | −.55 | .02 | −.52 | .03 | −.59 | .03 | −.02 | [−.09, .05] |
| 9 | −.05 | −.08 | .02 | −.03 | .03 | −.14 | .03 | .04 | [−.03, .11] |
| 10 | .95 | .92 | .03 | .96 | .04 | .89 | .04 | .03 | [−.04, .10] |
| 11 | .30 | .06 | .03 | .05 | .04 | −.03 | .04 | .00 | [−.07, .07] |
| 12 | .75 | .48 | .03 | .56 | .04 | .38 | .05 | .02 | [−.05, .09] |
| 13 | −.45 | −.73 | .03 | −.67 | .04 | −.83 | .05 | .03 | [−.04, .10] |
| 14 | .70 | .32 | .03 | .36 | .05 | .26 | .05 | −.00 | [−.08, .07] |
| 15 | .25 | −.13 | .04 | −.10 | .04 | −.18 | .05 | .01 | [−.06, .09] |
| 16 | −.75 | −1.03 | .04 | −.97 | .05 | −1.10 | .06 | −.00 | [−.08, .06] |
| 17 | −.65 | −1.06 | .04 | −.99 | .05 | −1.13 | .07 | −.00 | [−.08, .07] |
| 18 | −1.00 | −1.32 | .05 | −1.31 | .06 | −1.33 | .07 | −.00 | [−.08, .07] |
| 19 | .10 | −0.28 | .03 | − | − | −.28 | .03 | .00 | [−.06, .07] |
| 20 | −.85 | −1.10 | .04 | − | − | − | − | .00 | [−.08, .08] |

# 5
# Fixed effect IRT Model

ABSTRACT: A fixed effect item response theory (IRT) model is developed for modeling group specific item parameters. Two applications are presented. The first application is that the proposed model can be used to detect whether a response mechanism is ignorable using the splitter item technique. The second application is the detection of differential item functioning. In the latter application, the fixed effect item parameters can model item parameter differences between groups. Simulation studies are presented to show the feasibility and performance of the method on both applications.

KEYWORDS: analysis of variance, differential item functioning, fixed effect, item response theory model, MCMC.

## 5.1 Introduction

Interest is often focused on the possibility that educational and psychological measures are biased against a particular group of respondents. So-called external bias occurs when test scores have different correlations with non-test variables for two or more groups of examinees. Another form of bias occurs when correlations among item responses differs across two or more groups. This measurement bias leads to noninvariant measurement scales (e.g., the measurement scale is not invariant across groups). This form of item bias is denoted as differential item functioning (DIF). DIF is often modeled using IRT. In the framework of IRT, an item displays DIF when any of the item parameters differs across groups. Statistics for detection of DIF based on IRT models are summarized in Muraki, Mislevy, and Bock (1987), and Thissen, Steinberg, and Wainer (1988, 1993), and references therein. The detection of DIF is complicated due to the fact that group differences in the distribution of the latent vari-

able cause differences in response probabilities that as such are not signs of DIF. In other words, differences in the ability distribution between groups do not constitute DIF. Items are biased or noninvariant when respondents at the same level of the latent variable have different response distributions on the item.

Another common problem in educational and psychological measurement is the occurrence of nonignorable missing data. Rubin (1987) identified a number of situations in which statistical inferences based on the observed data and ignoring the distribution of the missing data indicators become biased. Roughly speaking, this bias does not occur if the distribution of the missing data indicator does not depend on the missing data. If the missing data cannot be ignored, a concurrent probability model must be defined for the observed and missing data, and inferences are made averaging over the missing data. Examples of such models were proposed by O'Muircheartaigh and Moustaki (1999, also see, Moustaki & O'Muircheartaigh, 2000; Moustaki & Knott, 2000; Bernaards & Sijtsma, 1999, 2000; Conaway, 1992; Park & Brown, 1994; Holman & Glas, 2005). Below it will be shown that a splitter item technique (Molenaar 1983; Van den Wollenberg, 1979) can be used for testing ignorability. In the splitter item technique, the sample of respondents are splitting up in two groups depending whether the response on the splitter item was observed or missing. Differences in item parameter estimates obtained in the two groups may then indicate nonignorable missing data.

In general, a unidimensional IRT model is appropriate for data in which a single common factor, say a latent variable, underlies the item responses. The person's response pattern on a particular set of items provides the basis for estimating the level on the latent variable level. IRT models involve an assumption about the distribution of the item response given the latent variable. Besides on the latent ability variable, the item response function also depends on item parameters which are distinct from the ability variable. In a fixed effect IRT model, group specific item parameters are added to the response function to model group specific fixed effects such as DIF, or differences in response behavior between subgroups formed using a splitter item that might indicate a violation of the ignorability assumption.

Though the approach that will be sketched below is quite general, the two-parameter logistic and normal ogive models will be used

as an example. Estimation will be developed in a Bayesian framework. The development of powerful sampling-based estimation techniques have stimulated the application of Bayesian methods. Markov chain Monte Carlo (MCMC) methods, such as Gibbs sampling and Metropolis-Hastings (M-H), can be used to simultaneously estimate all model parameters. An MCMC implementation will be introduced for the sampling of all model parameters that combines various advantages of different MCMC schemes for sampling IRT parameters.

In the next section, a general notation is given for fixed effect IRT models. Then, it will be shown how the model can be used to detect nonignorable missing data when using the splitter item technique. Next, it will be shown how the model can be used to explore DIF. Both applications are illustrated using artificial data. The last section contains a discussion and suggestion for further research.

## 5.2 A Fixed Effects IRT Model

The two-parameter normal ogive (2PNO) and the two-parameter logistic (2PL) models can be used to describe the relationship between a set of binary response items and a latent variable. Let a response of a person $i$ to an item labeled $k$ be coded by a $y_{ik}$. The probability of a correct response of a person $i$ on an item $k$ is defined as

$$P\big(y_{ik}=1 \mid \theta_i, a_k, b_k\big) = \begin{cases} \big[1+\exp(-D(a_k\theta_i - b_k))\big]^{-1}, \\ \text{for the 2PL} \\ \Phi\big(a_k\theta_i - b_k\big), \\ \text{for the 2PNO}, \end{cases} \tag{5.1}$$

where $a_k$ is the item discrimination parameter, and $b_k$ is the item difficulty parameter in both models. The item parameters will also be denoted by $\xi_k$, with $\xi_k = (a_k, b_k)^t$. Function $\Phi$ is the cumulative standard normal distribution, and the factor $D$, usually taken to be 1.7, is a scaling factor introduced to scale the parameters of the logistic function as close as possible to the parameters of the normal ogive function.

Let $\lambda_{kj}$ express the difference between a group $j$ specific difficulty parameter, indexed $k$, and a fixed difficulty parameter $b_k$ across groups indexed $j = 1, \ldots, J$. So, group specific difficulty parameters

$b_{kj}$ can be expressed as

$$b_{kj} = b_k + \lambda_{kj}, \tag{5.2}$$

where the difference $\lambda_{kj}$ is called a $j$th factor level effect or the $j$th treatment effect in ANOVA terms with the usual constraint that $\sum_j \lambda_{jk} = 0$ for $k = 1, \ldots, K$.

In a regression approach equivalent to an one-way ANOVA a design matrix $\mathbf{x}$ defines the grouping structure. Indicator variables are needed that take on values 0,1, or $-1$. It follows that:

$$a_k\boldsymbol{\theta} - (b_k + \mathbf{x}\boldsymbol{\lambda}_k) = \begin{pmatrix} \theta_{11} & -1 \\ \theta_{21} & -1 \\ \vdots & \vdots \\ \theta_{n_1 1} & -1 \\ \theta_{12} & -1 \\ \vdots & \vdots \\ \theta_{n_2,2} & -1 \\ \vdots & \vdots \\ \theta_{1J} & -1 \\ \vdots & \vdots \\ \theta_{1n_J} & -1 \end{pmatrix} \begin{pmatrix} a_k \\ b_k \end{pmatrix} -$$

$$\begin{pmatrix} 1 & 0 & 0 & \cdots & 0 \\ 1 & 0 & 0 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 1 & 0 & 0 & \cdots & 0 \\ 0 & 1 & 0 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 1 & 0 & \cdots & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ -1 & -1 & -1 & \cdots & -1 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ -1 & -1 & -1 & \cdots & -1 \end{pmatrix} \begin{pmatrix} \lambda_{k1} \\ \lambda_{k2} \\ \lambda_{k3} \\ \vdots \\ \lambda_{kJ-1} \end{pmatrix},$$

such that $\lambda_{kJ} = -\lambda_{k1} - \lambda_{k2} - \cdots - \lambda_{kJ-1}$. The indicator variable $\mathbf{x}$ denotes the specific group-membership. As a result, in the fixed effects IRT model the probability of a correct response of a person $i$

on an item $k$, is defined as

$$P\big(y_{ik}=1 \mid \theta_i, a_k, b_k, \boldsymbol{\lambda}_k\big) = \begin{cases} \big[1+\exp(-D(a_k\theta_i-(b_k+\mathbf{x}_i^t\boldsymbol{\lambda}_k)))\big]^{-1}, \\ \text{for the 2PL} \\ \Phi\big(a_k\theta_i-(b_k+\mathbf{x}_i^t\boldsymbol{\lambda}_k)\big), \\ \text{for the 2PNO.} \end{cases} \tag{5.3}$$

In the present paper, attention is focused on differences in difficulty parameters. However, the fixed effects IRT model is easily extended to model differences in discrimination parameters across groups.

In the fixed effects IRT model, interest is focused in the individual group means of item parameters $\lambda_{kj}$, Equation (5.2), and they are of interest in themselves. The interest is not focused on the variance in item parameters across groups. In that case, the $\lambda_{kj}$ are to be considered as random effects, and they are specified as independently distributed observations with a distribution. Subsequently, main interest is focused on this distribution. In the fixed effects approach it is a priori assumed that the $\lambda_{kj}$ bear no strong relationship to one another. In the cases where might be more realistic to assume that the $\lambda_{kj}$ are thought of as coming from a distribution, numerical problems occur when estimating variance components given a small number of groups. In this situation a fixed effects analysis can be very useful and avoids the complex statistical modeling of a mixture distribution and specification of hyperprior distributions. Fixed effects analyses from the Bayesian viewpoint have been tackled by, among others, Jeffreys (1961) and Lindley (1965). A random effects approach in IRT modeling has been considered by Janssen, Tuerlinckx, Meulders, and De Boeck (2000). In that approach, item parameters are considered as independent observations from a group specific population distribution, that is, the items in the test are seen as a random sample from this distribution. Subsequently, interest is focused on this item population distribution.

## 5.3 Testing for Non-Ignorable Missing Data

Holman and Glas (2005) propose an IRT model for taking non-ignorable missing data into account. In this model, the observed responses and the missing data indicators are modeled using distinct

IRT models, and the two latent variables associated with these two IRT models have a two-variate normal distribution. If the covariance between the two latent variables is non-zero, ignorability is violated. In that case, if the parameters of the IRT model for the observed responses are estimated ignoring the missingness, they prove to be biased (Holman & Glas, 2005). To assess this violation of ignorability, the data can be divided into two samples using a splitter item, say item $k$. The first group consists of respondents who have an observed response on this item, the second group consists of respondents who have a missing value on this item. Accordingly, the first sample will be denoted as the observed group; the observed item responses of individual $i$ except those to item $k$, $\mathbf{y}_{i,obs}^{(-k)}$ with $d_{ik} = 1$, $i = 1, \ldots, n$. The second sample will be denoted as the missing group: the observed item responses of individual $i$ except those to item $k$, $\mathbf{y}_{i,mis}^{(-k)}$ with $d_{ik} = 0$, $i = 1, \ldots, n$. In fact, the observed data is grouped in two sets. This can also be accomplished by specifying the indicator variable $\mathbf{x}$ in such a way that it represents the grouping structure defined by the splitter item. In that case, the fixed effects parameter $\boldsymbol{\lambda}$ represents item parameter differences between the observed and missing data set. Interest is focused on the marginal posterior distribution of $\boldsymbol{\lambda}$, $p\big(\boldsymbol{\lambda} \mid \mathbf{y}\big)$. When the missing data are nonignorable, the item parameters differ across groups, and the estimated $\boldsymbol{\lambda}$ values are different from zero. So, the splitter item technique is used to detect a nonignorable missing data mechanism by testing whether the fixed effects parameters are significantly different from zero.

## 5.4 Modeling Differential Item Functioning

The value of the ICC at a specific value for the latent variable corresponds to the conditional probability of a correct response given the level of the latent variable. When an ICC differ across groups then it is said that this item function differently and exhibit DIF. So, respondents across groups with the same level of the latent variable have different probabilities of scoring this item correct.

Several techniques for detection of DIF items based on IRT models have been proposed (see, .e.g., Glas, 2001; Glas & Verhelst, 1995; Hambleton & Rogers, 1989; Kelderman, 1989). In most cases, attention is focused on differences in response probabilities between

groups conditional on the level of the latent variable. Thissen, Steinberg, and Wainer (1993), and Glas (1998, 2001) considered DIF as a special case of IRT model misfit. They both used statistical tests in an IRT framework to explore DIF. In a frequentist framework, Glas (1998, 2001) modeled DIF in a common IRT model using multiple background or categorical dummy variables, where these variables model DIF. In this approach, the parameters of the IRT model are estimated and Lagrange Multiplier (LM) tests for DIF, based on the model extension using background variables, are performed for each item. In the present Bayesian approach, all parameters of the fixed effects IRT model are simultaneously estimated. Subsequently, the Bayes factor can be used to identify DIF items.

As an example, consider items that may function differently across groups, say, gender and nations. To model differences in ICC's across gender ($s = 1, 2$) and nations ($r = 1, \ldots, R$) define a fixed effects (probit) IRT model as:

$$P\big(y_{iksr} = 1 \mid \theta_i, a_k, b_k, \lambda_{k1s}, \lambda_{k2r}\big) = \Phi\big(a_k\theta_i - (b_k + \lambda_{k1s} + \lambda_{k2r})\big), \tag{5.4}$$

where $\lambda_{1s}$ is the main effect of being female (s = 2), and $\lambda_{2r}$ is the main effect of being grouped in nation $r$, with $\lambda_{11} = 0$ and $\lambda_{21} = 0$ taken as a baseline related to a so-called focal group. Subsequently, let indicator variable $\mathbf{x}$ represent this grouping structure, and let the fixed effects IRT model with two grouping variables be given by (5.3). Note that interaction effects between gender and nations are easily incorporated.

## 5.5 Estimating Model Parameters

Direct posterior inference is not possible since the joint posterior distribution is very complex. However, samples from this distribution can be obtained using MCMC methods. Then, inferences concerning the model parameters can be made using the sampled values. Below, M-H and Gibbs sampling algorithms are used for sampling parameter values for the item parameters, fixed effects parameters, and the ability parameters from their posterior distributions. Using the method of data augmentation, realizations from a complicated posterior density can be obtained by augmenting the variables of interest by one or more additional variables such that sampling from

the full conditional distributions is easy. Albert (1992) constructed an MCMC chain using the auxiliary variable method for estimating the two-parameter normal ogive model. Generating realizations from the full conditionals is complicated but with the introduction of this augmented variable the full conditionals are tractable and easy to simulate from. Maris and Maris (2002) developed an auxiliary variable method for logistic IRT models that handles different prior distributions in a flexible way. The augmented data are defined in such a way that each full conditional becomes an indicator function with bounds specified by the other parameter values. As a result, the sampling of the parameters is easy. However, the sampled values are highly correlated due to this incorporated dependency structure. As a result, the samples cannot be drawn freely from the target distribution but are restricted to a subspace specified by the other parameter values.

In the present paper, a combination of both methods for simultaneously estimating the parameters of a fixed effects two-parameter IRT model is outlined. In this approach, it is easy to handle different kinds of prior information, the convergence is fast, and the samples are not highly correlated. Fox and Hendrawan (2005) proposed this method for the MCMC estimation of two-parameter IRT models.

Let $\mathcal{L}(0,1)$ and $\mathcal{N}(0,1)$ denote the standard logistic and standard normal distribution function, respectively. Further, define augmented data $\mathbf{z}$,

$$z_{ik} \mid y_{ik}, \theta_i, a_k, b_k, \boldsymbol{\lambda}_k \sim \begin{cases} \mathcal{L}(0,1), \\ \text{for the 2PL} \\ \mathcal{N}(0,1), \\ \text{for the 2PNO}, \end{cases} \tag{5.5}$$

where $y_{ik}$ is the indicator that assumes a value one if $z_{ik} > D((b_k + \mathbf{x}_i^t \boldsymbol{\lambda}_k) - a_k\theta_i)$ and zero otherwise ($D = 1.7$ for the 2PL and $D = 1$ for the 2PNO model). Note that the augmentation step defines a probit or logit analysis. The full conditional distribution of the model parameters are each tractable and easy to simulate from given the augmented data.

- Full conditional distribution of $\boldsymbol{\theta}$. The prior for $\boldsymbol{\theta}$ is a normal distribution with mean parameter $\mu$ and variance parameter $\sigma$. It follows that

$$p(\theta_i \mid \mathbf{y}, \mathbf{z}, \mathbf{a}, \mathbf{b}, \boldsymbol{\lambda}, \mu, \sigma) \propto \prod_k I(z_{ik} \geq D((b_k + \mathbf{x}_i^t \boldsymbol{\lambda}_k) - a_k \theta_i))^{y_{ik}}$$
$$I(z_{ik} < D((b_k + \mathbf{x}_i^t \boldsymbol{\lambda}_k) - a_k \theta_i))^{1-y_{ik}} p(\theta_i \mid \mu, \sigma)$$
$$= I\Bigg( \max_{k|y_{ik}=1} \frac{(b_k + \mathbf{x}_i^t \boldsymbol{\lambda}_k) - z_{ik}/D}{a_k} < \theta_i <$$
$$\min_{k|y_{ik}=0} \frac{(b_k + \mathbf{x}_i^t \boldsymbol{\lambda}_k) - z_{ik}/D}{a_k} \Bigg) p(\theta_i \mid \mu, \sigma),$$

where $I(\cdot)$ is an indicator function assuming a value one if the condition in the argument is fulfilled and is equal to zero otherwise.

- Full conditional distribution of $\mathbf{a}$,$\mathbf{b}$,$\boldsymbol{\lambda}$. The fixed effects parameters, $\boldsymbol{\lambda}$, are taken to be a priori exchangeable. That is, $\lambda_{kj}$, $j = 1, \ldots, J$ are assumed independent and normally distributed with mean zero and variance $\sigma_\lambda$, with a large value for $\sigma_\lambda$ to specify a diffuse proper prior and to specify independence among the fixed effects parameters. Independent proper noninformative priors for the discrimination and difficulty parameters are specified, that is,

$$p(a_k, b_k) = p(a_k) p(b_k) \propto I(a_k \in \mathcal{A}) I(b_k \in \mathcal{B}),$$

where $\mathcal{A}$ and $\mathcal{B}$ are a sufficiently large bounded intervals in $\mathbb{R}^+$ and $\mathbb{R}$, respectively. As a result,

$$p(\mathbf{a}, \mathbf{b}, \boldsymbol{\lambda}) = p(a_k) p(b_k) \prod_j p(\lambda_{kj}) \propto \prod_{k,j} p(\lambda_{kj}) I(a_k \in \mathcal{A}) I(b_k \in \mathcal{B}).$$

Define augmented data $\mathbf{z}_k^*$,

$$\begin{aligned} \mathbf{z}_k^* &= D(a_k \boldsymbol{\theta} - (b_k + \mathbf{x} \boldsymbol{\lambda}_k)) + \boldsymbol{\epsilon}_k \\ \mathbf{z}_k^* &= \mathbf{H} \boldsymbol{\Xi}_k + \boldsymbol{\epsilon}_k \end{aligned} \tag{5.6}$$

where $\mathbf{H} = D(\boldsymbol{\theta}, -\mathbf{1}, -\mathbf{x})$, $\boldsymbol{\Xi}_k = (a_k, b_k, \boldsymbol{\lambda}_k)^t$, and $\boldsymbol{\epsilon}_k$ equals the augmented data $\mathbf{z}_k$ and they are standard normal or standard logistic distributed. The full conditional distribution can be specified as follows

$$\boldsymbol{\Xi}_k \mid \mathbf{z}_k^*, \boldsymbol{\theta} \sim \mathcal{N}\Big( \hat{\boldsymbol{\Xi}}_k, c\big(\mathbf{H}^t \mathbf{H}\big)^{-1} \Big) p(\boldsymbol{\Xi}_k), \tag{5.7}$$

where

$$\hat{\boldsymbol{\Xi}}_k = \left(\mathbf{H}^t\mathbf{H}\right)^{-1}\mathbf{H}^t\mathbf{z}_k^*,$$

and $c = 1$ or $c = \pi^2/3$ in case of 2PNO or 2PL augmented data, respectively. Note that the standard logistic cumulative distribution resembles the normal cumulative distribution with mean zero and variance $\pi^2/3$. A M-H probability can be used to correct any deficiencies in the approximation, since the tail of the logistic distribution is somewhat longer. However, almost every value is accepted since both distributions are quite comparable. In fact, a very good proposal distribution is specified in equation (5.7) for the fixed effects 2PL model.

## 5.6 Bayesian Inference

Summary statistics, such as the posterior mean or median, are used to report the results. A Bayesian confidence interval can provide information about the 'most likely' parameter values. In general, a $100(1-\alpha)\%$ credible set, $C_{\boldsymbol{\lambda}}(\mathbf{y})$, for $\boldsymbol{\lambda}$ is any set of values with

$$1-\alpha \leq P\big(C_{\boldsymbol{\lambda}}(\mathbf{y}) \mid \mathbf{y}\big) = \int_{C_{\boldsymbol{\lambda}}(\mathbf{y})} p\big(\boldsymbol{\lambda} \mid \mathbf{y}\big)d\boldsymbol{\lambda}. \tag{5.8}$$

It will be assumed that the (marginal) posterior density function is unimodal. The null-hypothesis $\boldsymbol{\lambda} = \boldsymbol{\lambda}_0$ is of particular interest, however, it is not realistic to have a precise null-hypothesis. This is better represented as

$$H_0 : |\boldsymbol{\lambda} - \boldsymbol{\lambda}_0| \leq \epsilon \text{ versus } H_1 : |\boldsymbol{\lambda} - \boldsymbol{\lambda}_0| > \epsilon, \tag{5.9}$$

where $\epsilon$ is "small". The point null hypothesis will be seen as an approximation for the small interval null as in Equation (5.9). In general, a Bayesian confidence region can be determined and conclusions are directly drawn from this region. That is, $C_{\boldsymbol{\lambda}}(\mathbf{y})$ provides information about the location of $\boldsymbol{\lambda}$, its distance to $\boldsymbol{\lambda}_0$, and if this distance makes a practical difference. Berger and Delampady (1987) argued that Bayesian credible intervals are often inappropriate when testing $\boldsymbol{\lambda} = \boldsymbol{\lambda}_0$ with a specific value $\boldsymbol{\lambda}_0$. They stated that the likelihood of a special point $\boldsymbol{\lambda}_0$, say, outside a confidence region $C_{\boldsymbol{\lambda}}(\mathbf{y})$ is often not too much smaller than the average likelihood in $C_{\boldsymbol{\lambda}}(\mathbf{y})$.

As a result, there is no strong evidence for rejecting $\boldsymbol{\lambda}_0$. Besides reporting a credible region, the Bayes factor can be used to test the null-hypothesis. Note that the computation of the Bayes factor against $H_0$ is easily constructed from the MCMC output for estimating the fixed effects IRT model parameters. Let $M_0$ denote the model with $\boldsymbol{\lambda} = \boldsymbol{\lambda}_0 = \mathbf{0}$, subsequently, $\boldsymbol{\lambda}$ is unconstrained in the fixed effects IRT model, denoted as $M$. Let $\boldsymbol{\Xi} = (\mathbf{a}, \mathbf{b}, \boldsymbol{\theta})$ and assume that $p(\boldsymbol{\lambda} = \mathbf{0}, \boldsymbol{\Xi} \mid \mathbf{y}, M) = p(\boldsymbol{\Xi} \mid \mathbf{y}, M_0)$. Then the marginal likelihood under model $M_0$ can be related to the marginal likelihood under the fixed effects IRT model $M$ (see, e.g., Chen, Shao, & Ibrahim, 2000; Verdinelli & Wasserman, 1995):

$$\begin{aligned} p(\mathbf{y} \mid M_0) &= \int p(\mathbf{y} \mid \boldsymbol{\Xi}, M_0) p(\boldsymbol{\Xi} \mid M_0) d\boldsymbol{\Xi} \\ &= \int \frac{p(\boldsymbol{\Xi} \mid M_0)}{p(\boldsymbol{\lambda} = \mathbf{0}, \boldsymbol{\Xi} \mid M)} p(\boldsymbol{\lambda} = 0, \boldsymbol{\Xi} \mid M) p(\mathbf{y} \mid \boldsymbol{\lambda} = \mathbf{0}, \boldsymbol{\Xi}, M) d\boldsymbol{\Xi} \\ &= p(\mathbf{y} \mid M) \int \frac{p(\boldsymbol{\Xi} \mid M_0)}{p(\boldsymbol{\lambda} = \mathbf{0}, \boldsymbol{\Xi} \mid M)} p(\boldsymbol{\lambda} = \mathbf{0}, \boldsymbol{\Xi} \mid \mathbf{y}, M) d\boldsymbol{\Xi}. \end{aligned} \tag{5.10}$$

As a result, the Bayes factor for testing the null-hypothesis $\boldsymbol{\lambda} = \mathbf{0}$ can be stated as:

$$\begin{aligned} BF &= \int \frac{p(\boldsymbol{\Xi} \mid M_0)}{p(\boldsymbol{\lambda} = \mathbf{0}, \boldsymbol{\Xi} \mid M)} p(\boldsymbol{\lambda} = \mathbf{0} \mid \boldsymbol{\Xi}, \mathbf{y}, M) p(\boldsymbol{\Xi} \mid \mathbf{y}, M) d\boldsymbol{\Xi} \\ BF &= \mathcal{E}\left[ \frac{p(\boldsymbol{\Xi} \mid M_0)}{p(\boldsymbol{\lambda} = \mathbf{0}, \boldsymbol{\Xi} \mid M)} p(\boldsymbol{\lambda} = \mathbf{0} \mid \boldsymbol{\Xi}, \mathbf{y}, M) \right], \end{aligned} \tag{5.11}$$

where the expectation is taken with respect to the marginal posterior distribution $p(\boldsymbol{\Xi} \mid \mathbf{y}, M)$. A single MCMC output denoted as $\boldsymbol{\Xi}^{(m)}$, $(m = 1, \ldots, M)$ from the posterior distribution $p(\boldsymbol{\Xi} \mid \mathbf{y}, M)$ can be used to compute the Bayes factor. That is,

$$\widehat{BF} = M^{-1} \sum_m \frac{p\big(\boldsymbol{\Xi}^{(m)} \mid M_0\big)}{p\big(\boldsymbol{\lambda} = \mathbf{0}, \boldsymbol{\Xi}^{(m)} \mid M\big)} p\big(\boldsymbol{\lambda} = \mathbf{0} \mid \boldsymbol{\Xi}^{(m)}, \mathbf{y}, M\big). \tag{5.12}$$

A special case occurs when $p(\boldsymbol{\Xi} \mid \boldsymbol{\lambda} = \mathbf{0}, M) = p(\boldsymbol{\Xi} \mid M_0)$. Via Equation (5.11) it follows that

$$\begin{aligned} BF &= \int \frac{p(\boldsymbol{\Xi} \mid \boldsymbol{\lambda} = \mathbf{0}, M)}{p(\boldsymbol{\lambda} = \mathbf{0}, \boldsymbol{\Xi} \mid M)} p(\boldsymbol{\lambda} = \mathbf{0} \mid \boldsymbol{\Xi}, \mathbf{y}, M) p(\boldsymbol{\Xi} \mid \mathbf{y}, M) d\boldsymbol{\Xi} \\ &= \int \frac{1}{p(\boldsymbol{\lambda} = \mathbf{0} \mid M)} p(\boldsymbol{\lambda} = \mathbf{0} \mid \boldsymbol{\Xi}, \mathbf{y}, M) p(\boldsymbol{\Xi} \mid \mathbf{y}, M) d\boldsymbol{\Xi} \qquad (5.13) \\ &= \frac{p(\boldsymbol{\lambda} = \mathbf{0} \mid \mathbf{y}, M)}{p(\boldsymbol{\lambda} = \mathbf{0} \mid M)}, \end{aligned}$$

which is known as the Savage-Dickey density ratio (Dickey, 1971). Note that the Bayes factor in Equation (5.13) is reduced to estimating the marginal posterior density $p(\boldsymbol{\lambda} \mid \mathbf{y}, M)$ at the point $\boldsymbol{\lambda} = \mathbf{0}$.

In a different way, Klugkist (2004) derived an expression for the Bayes factor, under comparable assumptions, that enables its computation via MCMC output under model M. In this approach the Bayes factor is expressed as a ratio of two proportions, a ratio of priors, and a ratio of posterior distributions, where the prior and posterior distributions are defined for the constrained and the unconstrained model. The ratios are estimated using the MCMC output.

## 5.7 Simulation Study

A simulation study was used to assess the performance of the MCMC algorithm and to illustrate the usefulness of the fixed effects IRT model. In simulation study 1, data were generated using a nonignorable missing data mechanism. In simulation study 2, data were generated given DIF items.

### *5.7.1 Simulation Study 1*

Analogous to Bradlow and Thomas (1998) and the example in the previous chapter, response data were simulated as if students were allowed to choose a subset of items. In this setup, for a subset of items, responses were simulated for pairs of items. This was done in such a way that for each person one response was generated for each paired item. The response mechanism was such that an item response was generated for the easier items within pairs with probability $p_1 = .95$ and the harder item with probability $p_2 = .05$ if the persons'

ability level was positive. If the persons' ability level was negative, an item response was generated for one of the items at random. So, the distribution of the response mechanism depends on the ability parameters $\boldsymbol{\theta}$ underlying the observed responses and the difficulty parameters.

Two groups were identified as follows: one group of respondents, denoted as the observed group, responding to splitter item 20, and the other group of respondents not responding to the splitter item, denoted as the missing group. So, the last item, $k = 20$, was considered as a splitter item and the corresponding responses (observed/-missing) were considered as a group indicator. It was expected that the item difficulties varied over groups since the distribution of abilities varied across groups. In the fixed effects IRT model, the observed group was considered as the baseline group. The fixed effects in Equation (5.3) represent item parameter differences between this baseline group and the missing group.

In this simulation study, 5000 abilities and 20 difficulty parameter values were generated from a standard normal distribution. Discrimination parameters were generated from a log-normal distribution. These parameter values were used to generate item response data according the 2PNO model. The last ten items were considered as paired items. The Gibbs sampling algorithm was used for estimating the parameters of the fixed effects IRT model. A total of 10,000 iterations were used for estimating the model parameters with a burn-in period of 1,000 iterations. The fixed effects IRT model was identified by fixing the scale of the latent variable with mean zero and variance one.

In Table 5.1 are the posterior means and standard deviations given of the difficulty parameters for different subsets of item response data. The posterior means of $p\big(\mathbf{b} \mid \mathbf{y}_{obs}\big)$ correspond to the estimated item difficulties given all observed item response data. It follows that for most paired item the difficult item is overestimated and the easy item is underestimated. The observed group consisted of the better respondents, making item 20 since it was the easier item. The true item difficulties are highly underestimated in the baseline group, that is, the respondents in the observed group make the items appear more easy. The fixed effects are all positive and significantly different from zero given the 95% HPD regions. As a result, the difficulty parameter estimates for the missing group are a factor $\hat{\boldsymbol{\lambda}}$ higher

in comparison to the difficulty parameter estimates in the observed group. The Bayes factor for testing the null-hypothesis $\boldsymbol{\lambda} = \mathbf{0}$ equals approximately zero. So, it can be concluded that the grouping of responses according to values of the splitter item affects the statistical inference. The difficulty parameter estimates vary across groups. The grouping of the data according to splitter item 20 resulted in significant fixed group effects indicating that the way of grouping the data (observed/missing) affects the results.

TABLE 5.1. Parameter estimates of the fixed effects IRT model using splitter item 20.

| | | | | Splitter Item 20 | | | |
|---|---|---|---|---|---|---|---|
| | | $p(\mathbf{b} \mid \mathbf{y}_{obs})$ | | $p_{obs}(\mathbf{b} \mid \mathbf{y}_{obs})$ | | $p(\boldsymbol{\lambda} \mid \mathbf{y}_{obs})$ | |
| Item | $\mathbf{b}$ | Mean | sd | Mean | sd | Mean | HPD |
| 1 | −1.37 | −1.37 | .03 | −1.71 | .06 | 1.02 | [.89, 1.16] |
| 2 | .05 | .07 | .02 | −.20 | .02 | .98 | [.87, 1.10] |
| 3 | −1.19 | −1.14 | .02 | −1.44 | .04 | .93 | [.80, 1.06] |
| 4 | −.42 | −.42 | .02 | −.68 | .02 | .90 | [.80, 1.00] |
| 5 | 1.24 | 1.22 | .02 | .93 | .03 | 1.14 | [.92, 1.41] |
| 6 | −.77 | −.78 | .02 | −1.07 | .03 | .93 | [.82, 1.05] |
| 7 | −.65 | −.72 | .02 | −1.00 | .03 | .92 | [.81, 1.04] |
| 8 | −.24 | −.25 | .02 | −.54 | .02 | .96 | [.86, 1.07] |
| 9 | .74 | .70 | .02 | .43 | .02 | .99 | [.85, 1.16] |
| 10 | −.33 | −.32 | .02 | −.57 | .03 | .83 | [.74, .94] |
| 11 | .08 | .21 | .04 | .00 | .07 | .72 | [.55, .92] |
| 12 | −.21 | −.24 | .02 | −.50 | .02 | .96 | [.83, 1.10] |
| 13 | −.04 | −.10 | .02 | −.38 | .02 | .98 | [.85, 1.13] |
| 14 | .93 | 1.16 | .05 | .97 | .08 | .58 | [.35, .81] |
| 15 | −.54 | −.58 | .02 | −.86 | .03 | 1.01 | [.88, 1.14] |
| 16 | −.24 | −.10 | .05 | −.39 | .08 | .78 | [.61, .98] |
| 17 | .53 | .75 | .05 | .52 | .07 | .70 | [.50, .91] |
| 18 | −1.05 | −1.09 | .03 | −1.33 | .04 | .86 | [.71, 1.02] |
| 19 | −.40 | −.32 | .05 | − | − | − | − |
| 20 | −1.00 | −1.05 | .03 | − | − | − | − |

### *5.7.2 Simulation Study 2*

In this numerical example, data were analyzed to investigate the performance of the fixed effects IRT model for detecting DIF items. In four different setups, response patterns, $\mathbf{y}$, were generated according to a fixed effects 2PL model for 2000 persons and 10 items. DIF was imposed on the item difficulties. The respondents were grouped by gender (Male, Female) denoted by $x_1$ and nations (Dutch, non-Dutch) denoted by $x_2$ where a female Dutch was coded as $x_1 = 1$ and $x_2 = 1$ respectively. It was assumed that the groups of respondents are homogenous with respect to the latent variable. Three data sets were generated: (1) no DIF items denoted as model $M_1$, (2) main effect of gender where $\boldsymbol{\lambda}_1 = .25$ for the last five items, denoted as model $M_2$, and (3) main effects of gender and nations where $\boldsymbol{\lambda}_1 = .20$, $\boldsymbol{\lambda}_2 = .20$ for the last five items, denoted as model $M_3$.

The MCMC algorithm was used to simultaneously estimate all model parameters given the generated item response data using the 2PL. The convergence of the MCMC chains was checked and it was concluded the all MCMC chains converged within 1000 iterations. Then, $10,000$ iterations were made to estimate the posterior means and standard deviations. Each model was identified by fixing the scale of the latent variable to make the outcomes comparable.

Table 5.2 presents the fixed effects IRT parameter estimates given data generated under model $M_1$ and $M_2$. The simulated difficulty parameters are given under the label $\mathbf{b}$. The difficulty parameter estimates and their standard deviations of the null-model with $\boldsymbol{\lambda} = \mathbf{0}$ are given under the label $p(\mathbf{b} \mid \mathbf{y}, \boldsymbol{\lambda} = \mathbf{0})$. It can be seen that for data generated under model $M_1$, the difficulty parameter estimates of the null model resemble the true parameter values since there are no DIF items simulated. The simulated data were used to estimate the parameters of a fixed effects IRT model where the fixed effects represent a main effect of gender. This model assumes that the item parameters differ across groups of males and females. The difficulty parameters estimates corresponding to the female group using this fixed effects IRT model also resemble the true values. Note that the estimated standard deviations are slightly higher in comparison to the corresponding estimates of the null model. This follows from the fact that the the estimates of the fixed effects IRT model are group specific, and so they are based on less observations. The mean of the fixed parameter estimates, $\boldsymbol{\lambda}$ is given under the label $p(\boldsymbol{\lambda} \mid \mathbf{y})$. The

estimated fixed effects are close to zero, and the 95% HPD regions show that none of the effects differ significantly from zero. This corresponds with the fact that the data were generated under model $M_1$ with no DIF items. The Bayes factor for testing the hypothesis $\boldsymbol{\lambda} = \mathbf{0}$ equals $\exp(8)$ and provides strong evidence that the null hypothesis should not be rejected.

The simulated difficulty parameters according to model $M_2$ are given under the label **b** and correspond to the baseline group (Female, $x_1 = 1$). For the last five items, a gender effect was imposed ($\lambda_k = .25, k = 6, \ldots, 10$), and it can be seen that the estimates of the difficulty parameters under the null model differ from the true values for these last five items. The parameter estimates of the baseline group according to the fixed effects IRT model resemble the simulated difficulty parameters since the model captures item parameter differences between groups. The true main effects are slightly overestimated by the estimated fixed effects parameters but they are all significant for last last items. The positive sign of the estimated fixed effects indicates that the item difficulties in the male group are more difficult. The estimated item difficulties in the male group are the sum of the estimated fixed effects and the estimated difficulties in the female group. Here, the Bayes factor equals $\exp(-34)$ and provides strong evidence that the null hypothesis should be rejected.

TABLE 5.2. Parameter estimates of the fixed effects IRT model given data generated under model $M_1$ and $M_2$.

| | | | $\mathrm{p}(\mathbf{b} \mid \mathbf{y}, \boldsymbol{\lambda} = 0)$ | | $p(\mathbf{b} \mid \mathbf{y}, x_1 = 1)$ | | $p(\boldsymbol{\lambda} \mid \mathbf{y})$ | |
|---|---|---|---|---|---|---|---|---|
| | Item | $\mathbf{b}$ | Mean | sd | Mean | sd | Mean | HPD |
| $M_1$ | 1 | −1.09 | −1.10 | .05 | −1.07 | .07 | .07 | [−.14, .29] |
| | 2 | 1.21 | 1.16 | .05 | 1.20 | .08 | .07 | [−.14, .29] |
| | 3 | 1.46 | 1.57 | .06 | 1.59 | .10 | .09 | [−.21, .37] |
| | 4 | −.41 | −.42 | .03 | −.37 | .05 | .09 | [−.06, .25] |
| | 5 | .60 | .66 | .04 | .74 | .06 | .15 | [−.05, .31] |
| | 6 | −.05 | −.05 | .03 | −.06 | .04 | −.02 | [−.17, .11] |
| | 7 | −.19 | −.24 | .03 | −.27 | .05 | −.06 | [−.22, .09] |
| | 8 | −.08 | −.15 | .03 | −.11 | .04 | .07 | [−.08, .22] |
| | 9 | −.34 | −.39 | .03 | −.35 | .05 | .09 | [−.06, .25] |
| | 10 | .20 | .19 | .03 | .14 | .04 | −.11 | [−.28, .03] |
| $M_2$ | 1 | −.28 | −.23 | .03 | −.29 | .05 | −.11 | [−.27, .05] |
| | 2 | −2.10 | −2.28 | .08 | −2.55 | .27 | −.61 | [−1.72, .12] |
| | 3 | −.38 | −.41 | .03 | −.38 | .05 | .08 | [−.09, .26] |
| | 4 | .59 | .63 | .04 | .61 | .05 | −.05 | [−.23, .12] |
| | 5 | .49 | .58 | .03 | .57 | .05 | −.02 | [−.21, .16] |
| | 6 | 1.28 | 1.59 | .06 | 1.37 | .09 | −.39 | [−.71, −.09] |
| | 7 | −1.32 | −1.16 | .05 | −1.32 | .09 | −.31 | [−.56, −.08] |
| | 8 | −.33 | −.47 | .03 | −.33 | .05 | −.30 | [−.48, −.14] |
| | 9 | −1.01 | −.92 | .04 | −1.06 | .07 | −.30 | [−.53, −.10] |
| | 10 | −.65 | −.49 | .03 | −.63 | .05 | −.25 | [−.41, −.09] |

TABLE 5.3. Parameter estimates of the fixed effects IRT model given data generated under model $M_3$.

| | | | $\mathrm{p}(\mathbf{b} \mid \mathbf{y}, \boldsymbol{\lambda} = 0)$ | | $p(\mathbf{b} \mid \mathbf{y}, x_1 = 0, x_2 = 0)$ | |
|---|---|---|---|---|---|---|
| | Item | $\mathbf{b}$ | Mean | sd | Mean | sd |
| $M_3$ | 1 | .04 | .02 | .03 | −.04 | .08 |
| | 2 | .07 | .09 | .03 | .09 | .07 |
| | 3 | .06 | .06 | .03 | .11 | .08 |
| | 4 | .06 | .07 | .03 | .11 | .07 |
| | 5 | .07 | .08 | .03 | .12 | .07 |
| | 6 | −.17 | .04 | .03 | −.19 | .08 |
| | 7 | .23 | .39 | .03 | .21 | .07 |
| | 8 | −.10 | .15 | .03 | −.10 | .07 |
| | 9 | −.06 | .11 | .03 | −.02 | .08 |
| | 10 | .00 | .22 | .03 | −.02 | .07 |

In Table 5.3 presents the parameter estimates given data generated under model $M_3$. The true simulated difficulty parameters for the baseline group (non-Dutch Males) are given under the label $\mathbf{b}$. The difficulty parameter estimates of the null-model, with fixed effects equal to zero, differ from the true values with respect to the last five items. The difficulty parameters of these DIF items are correctly estimated by the fixed effects IRT model. That is, the estimated difficulty parameters of the baseline group resemble the true parameters.

The fixed effects parameters are estimated for the four different groups. In Figure 5.1 are the estimated posterior distributions given of the group specific fixed effects parameters. The dotted lines correspond to the last five items of the test. In the group of Dutch-Females, the last five items are DIF items due to the main effect of gender with $\boldsymbol{\lambda}_1 = .2$. It can be seen that the fixed effects parameters of the DIF items are distributed around .2 but only two are significantly different from zero. The posterior distributions of the fixed effects parameters of the non-DIF items are centered around zero. The estimated posterior variances may seem large but they are based on the size of the groups and not the entire sample size. A main effect of nations, $\boldsymbol{\lambda}_2 = .2$, can be detected in the group of Dutch-Males. That is, three of the five posterior distributions of the fixed effects parameters corresponding to DIF items have a mean significantly

different from zero. The true difficulty parameters in the group of Dutch-Females are much higher due to main effects of gender and nations. It can be seen that the corresponding estimates of the fixed effects are approximately .4 for the DIF items, and around zero for the non-DIF items. The Bayes factor equals $\exp(-56)$ and supports the fixed effects IRT model without restricting the fixed effects to be zero. In conclusion, the fixed effects IRT model captures differences in difficulty parameters across groups and detects DIF items. As a result, the measurements of the latent variable are more reliable since differences in item parameters across groups are taken into account.

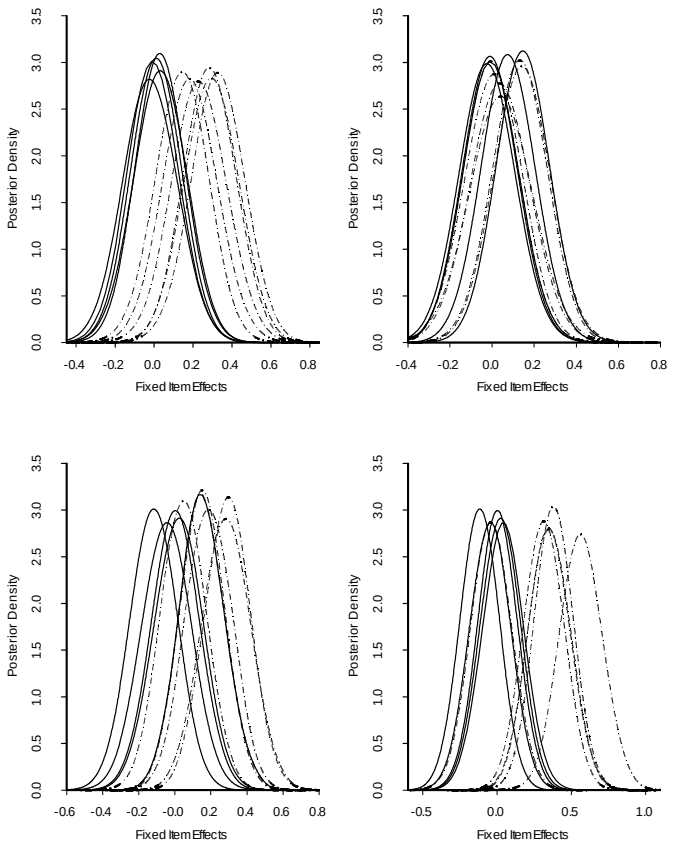



FIGURE 5.1. Posterior distributions of fixed effects parameters for the four groups. (Clockwise from top-left: Male-non-Dutch, Dutch-Male, Dutch-female, non-Dutch-Female)

## 5.8 Discussion

Fixed effects IRT models consisting of difficulty parameters that are allowed to vary across groups, are discussed. In contrast to random effects item parameters, interest is focused on the fixed effects and not on the variance in item parameters across groups. Two applications are considered: (1) detecting nonignorable missing data, and (2) detecting and/or modeling DIF items. It was shown that the fixed effects IRT model can be used for detecting nonignorable missing data in combination with the splitter item technique. That is, the observations of the splitter item (observed/missing) defines the grouping of observed item response data, and the fixed effects parameters model item parameter differences between these groups. Significant fixed effects parameters indicate item parameter differences between groups. In the second simulation study, it was shown that the fixed effects parameters can comprehend DIF items since differences in item parameters between groups are properly modeled. So, the fixed effects IRT model can be used to measure a latent variable in the presence of DIF items. It can also be used to detect DIF items in combination with a Bayes factor for testing the hypothesis that the fixed effects are zero.

It was shown that the proposed MCMC method for simultaneously estimating all parameters yields acceptable estimates. The estimation method can handle the 2PL and 2PNO model in three comparable sampling steps. This analogy makes the implementation easier. In general, the 2PNO model may be preferred since it has some computational advantages.

It has been shown that the Bayes factor for testing the null-hypothesis that all fixed effects are zero follows from evaluating the marginal posterior distribution of the fixed effects parameters in the point zero. This approach can be extended to facilitate the computation of Bayes factors for other hypothesis concerning problems of choosing between alternative models. For example, in the same way it can be tested whether all item discrimination parameters are equal. Bayesian inference concerning the fixed effects IRT model can also be based on HPD regions. Therefore, HPD region can be defined for the fixed effects IRT model to test hypotheses by deciding if a given point lies inside or outside the confidence region. Then, for example, testing the equality of difficulty parameters across groups,

all fixed effects are zero, can be done by computing the probability on a HPD region that just includes the point zero.

Finally, the extension of the fixed effects IRT model to capture differences in discrimination parameters across groups is easily done by extending the design matrix $\mathbf{x}$. In that case, the design matrix is extended with the latent variable and the fixed effects parameters represent difficulty and discrimination parameter differences across groups. Further research will also focus on population group differences in the distribution of the latent variable. The framework of the multilevel IRT model (Fox, 2004; Fox & Glas, 2001, 2003) can be used to model population differences on the latent variable but it assumes that item response curves are the same for all groups. Problems occur due to the fact that the fixed effects parameters and population parameters vary across the same groups, which results in an identification problem. A possible solution might be found in finding identifying constraints such that the scale of the latent variable is identified and common across groups, and item and fixed effects parameters can be estimated with respect to this scale.

# Synopsis

The handling of nonignorable missing data in psychometrics is not fully developed, but in recent years the attention for these problems in the application of latent variable modeling (see for instance Moustaki, 1996; O'Muircheartaigh & Moustaki,1999; Moustaki & Knott, 2000, Holman & Glas, 2005) is much increased. In educational measurement, most literature and software packages ignore missing data. However, this is inappropriate when the ignorability principle defined by Rubin (1987) does not hold. In these cases, the estimation of parameters ignoring the missing data often leads to biased results (Holman & Glas, 2005).

This thesis discusses methods to detect nonignorable missing data and methods to adjust for the bias caused by nonignorable missing data, both by introducing a model for the missing data indicator using item response theory (IRT) models.

In Chapter 2, a model based procedure that handles nonignorable missing data in the framework of IRT is presented. The relevant IRT model for the observed data is estimated in combination with an IRT model for the missing data process. The two IRT models are connected by invoking the assumption that their latent person parameters have a joint multivariate normal distribution. The model parameters are estimated using marginal maximum likelihood. As an example, the generalized partial credit model is used to model the

observed data while the Rasch model is used to model the missing data process. The simulation studies conducted with both dichotomous and polytomous data show that the bias in the item parameter estimates obtained ignoring the missing data process are reduced by using an explicit latent variable model for the missing data process in the estimation. The bias is further reduced when observed covariates are included in the IRT model for missing data in the estimation of the data.

The approach in Chapter 2 is further elaborated in Chapter 3 for a situation where a test is administered in a limited-time condition. The time limit condition leaves items at the end of the test unanswered by the examinee, i.e., the missing data in particular appear consecutively in the items at the end of a test. The cause of this missingness is usually related to the person's ability: the lower the ability the larger the number of items that are left unanswered at the end of the test. Thus, in this case the mechanism causing the missing data should not be ignored. Following the method in Chapter 2, the data are modeled using a combination of two IRT models: The observed response data are modeled by the generalized partial credit model (in particular, 2PL model) and the missing data are modeled by the sequential model also known as the steps model. Again, the two IRT models are connected by invoking the assumption that their latent person parameters have a joint multivariate normal distribution and the parameters are estimated using marginal maximum likelihood. Results of the simulation studies show that when the model for the missing data process is included in the estimation together with the model for the observed data, the bias in the item parameter estimates remains comparable to the base line obtained with ignorable missing data. Further, excluding the model for the missing data process leads to considerable bias, that increased with the extent of the violation of ignorability. A real data set was analyzed to assess the impact of the model in practice. Specifically, including the missing data process lead to an increase of the estimate of the global reliability of the test.

In Chapter 4, two methods based on the splitter item technique are proposed to detect a nonignorable missing data process. So the method aims at making decisions whether the missing data are ignorable or not. The sample of respondents is divided into two groups. The first group consists of respondents that have an observation

on the splitter item and the second group consists of respondents that do not have a response on the splitter item. Then, it is tested whether the item parameter estimates differ across the two groups. Two methods are considered. Both apply to IRT models for binary or ordinal responses estimated using a Bayesian method and MCMC. In the first method, all parameters of an IRT model for binary or ordinal responses are estimated given the subsets of item response data. Then, summary statistics of the estimated marginal posterior distributions of the item parameters are used for detection of differences. In the second method, values of IRT model parameters and, as an additional sampling step, values of so-called Bayesian modification indices (BMI) are sampled using MCMC. These BMI values provide information regarding any fluctuations in item parameter values across groups. They are estimated using MCMC and do not interfere with the estimation of the other model parameters. So the BMI values are obtained as a by-product of the MCMC algorithm for estimating the parameters of an IRT model. It is shown that the BMI distribution is a good approximation of the true marginal posterior distribution of the group-specific item parameters.

The last chapter, Chapter 5 of this thesis discusses fixed effects IRT models that include item parameters that are allowed to vary across groups. The models are used for modeling group specific item parameters. The proposed models were applied to detection of nonignorable missing data and for detecting and modeling differential item functioning (DIF). For the detection of nonignorable missing data, a splitter item defines the grouping of the item response data. The partitioning of the sample is based on whether or not there is a response on the splitter item. The fixed effects item parameters (as opposed to the often used random effects item parameters) model the group effects. Significant fixed effect parameters indicate that the item parameters differ between groups. The estimates are computed in a Bayesian framework using MCMC. The MCMC estimation method handles the 2PL and 2PNO model in three comparable sampling steps. The Bayesian inference concerning the fixed effects IRT model is based on HPD regions and Bayes factors. The HPD region is defined for the fixed effects IRT model in testing hypotheses in deciding if a given point lies inside or outside the confidence region. This way the null-hypothesis stating that all fixed effects are zero (the group-specific item parameters are equal) can be tested.

It is shown that a Bayes factor can be used for testing comparable null-hypotheses. Simulation studies are used to evaluate the performance of the procedure.

# Samenvatting

Methoden voor analyses van data met niet-negeerbare ontbrekende gegevens (non-ignorable missing data) zijn in de psychometrie nog niet volledig ontwikkeld, maar de afgelopen jaren is de aandacht voor het probleem van ontbrekende gegevens in relatie tot de schatting van parameters in modellen met latente variabelen sterk toegenomen (Moustaki, 1996; O'Muircheartaigh & Moustaki,1999; Moustaki & Knott, 2000, Holman & Glas, 2005). In de meeste literatuur over onderwijskundig meten en de meeste software die in dat kader gebruikt wordt, worden ontbrekende gegevens genegeerd. Dit is echter niet correct als niet aan het negeerbaarheidprincipe (ignorability principle, Rubin, 1987) voldaan is. In die gevallen zijn de parameterschattingen onder een model waarbij de ontbrekende gegevens genegeerd worden bijzonder onzuiver (Holman & Glas, 2005).

In dit proefschrift wordt een aantal methoden voor het ontdekken van niet-negeerbare ontbrekende gegevens en methoden voor het corrigeren van de schattingen gepresenteerd. In beide gevallen gebeurt dit door het postuleren van een item response theorie (IRT) model voor de indicator voor de ontbrekende gegevens.

In hoofdstuk 2 wordt een op een IRT model gebaseerde methode voor de het analyseren van data met niet-negeerbare ontbrekende gegevens gepresenteerd. Het IRT model voor de geobserveerde data wordt simultaan geschat met een IRT model voor de indicator

voor de ontbrekende gegevens. De twee IRT modellen worden verbonden via de veronderstelling dat hun latente persoonsparameters een gezamenlijke multivariaat normale verdeling hebben. De parameters van het complete model worden geschat met een marginale grootste-aannemelijkheid methode (marginal maximum likelihood estimation method). Als voorbeeld wordt het gegeneraliseerde partial credit model gebruikt voor de geobserveerde data en het Rasch model voor de indicator voor de ontbrekende gegevens. Met simulatiestudies wordt aangetoond dat zowel voor dichtoom als voor polytoom gescoorde antwoorden, de onzuiverheid in de schattingen gereduceerd wordt door de introductie van een IRT model voor de indicator voor de ontbrekende gegevens. De onzuiverheid wordt verder gereduceerd als er covariaten in dit laatste model worden opgenomen.

Deze aanpak wordt verder uitgewerkt in hoofdstuk 3, voor een test die is afgenomen onder tijdsdruk. Door tijdsdruk worden items aan het eind van de test niet beantwoord. Het patroon van de ontbrekende gegevens hangt meestal samen met het vaardigheidsniveau van de studenten: hoe lager het vaardigheidsniveau, hoe meer items aan het eind van de test niet gemaakt worden. Daarom mag het mechanisme dat de ontbrekende gegevens veroorzaakt heeft niet worden genegeerd. Net als in hoofdstuk 2 worden de data gemodelleerd met een combinatie van twee IRT modellen: de observaties worden gemodelleerd met het gegeneraliseerde partial credit model, terwijl de indicator voor de ontbrekende gegevens wordt gemodelleerd met het z.g. sequentiële model, c.q. het stapjesmodel. Ook hier worden de twee IRT modellen worden verbonden via de veronderstelling dat hun latente persoonsparameters een gezamenlijke multivariaat normale verdeling hebben en de schattingen worden berekend met een marginale grootste-aannemelijkheid methode. Met simulatiestudies wordt aangetoond dat de schattingen met behulp van dit model met data met niet-negeerbare ontbrekende gegevens dezelfde precisie hebben als schattingen met geobserveerde data met negeerbare ontbrekende gegevens via een IRT model zonder extra model voor de indicator variabele. Verder blijkt ook hier dat het negeren van niet-negeerbare ontbrekende gegevens leidt tot ernstige onzuiverheid in de parameterschattingen. Om de impact van de methode in de praktijk te evalueren wordt reële data van een toets, gemaakt onder tijdsdruk, geanalyseerd. Met deze data wordt getoond dat het mod-

elleren van de indicator voor ontbrekende gegevens kan leiden tot een belangrijke verandering van de schatting van de betrouwbaarheid.

In hoofdstuk 4 worden twee methoden voor het ontdekken van niet-negeerbare ontbrekende gegevens voorgesteld die gebaseerd zijn op de splitter-item techniek. Dus het doel van de methode is om vast te stellen of de ontbrekende gegevens negeerbaar zijn, of niet. Hiertoe wordt de steekproef van studenten verdeeld in twee groepen. De eerste groep bestaat uit studenten die een antwoord gaven op het splitter-item, de tweede groep bestaat uit studenten waar het splitter-item niet beantwoord is. Daarna wordt getoetst of the schattingen van de itemparameters verschillen voor de twee groepen. Dit wordt gedaan met twee methoden. Beide methoden hebben betrekking op IRT modellen voor binaire of ordinale responsie in een Bayesiaans raamwerk. De schattingen worden berekend met een iteratief simulatieproces dat bekend staat onder het acroniem MCMC. In de eerste methode worden alle itemparameters van een model voor binaire of ordinale responsie geschat op de twee deelsteekproeven. Daarna worden functies van geschatte marginale a-posteriori verdelingen van de itemparameters gebruikt om verschillen vast te stellen. In de tweede methode worden in de MCMC procedure via simulatie zogenaamde Bayesiaanse modificatieindices (BMI) gegenereerd. Deze indices geven informatie over de verschillen tussen de itemparameters tussen de groepen. De simulatie van de indices heeft geen invloed op de simulatie van de modelparameters; de gesimuleerde indices zijn een bijproduct van de MCMC simulatie. Er wordt aangetoond dat de verdeling van deze indices een goede benadering is van de verdeling de groepsafhankelijke itemparameters.

In het laatste hoofdstuk, hoofdstuk 5, wordt een zogenaamd fixed-effects IRT model besproken waarin de itemparameters kunnen variëren tussen groepen. Deze modellen worden gebruikt voor het modelleren van groepsspecifieke itemparameters. De modellen kunnen worden toegepast voor het opsporen van niet-negeerbare ontbrekende gegevens en voor het opsporen van vraagonzuiverheid (differential item functioning, DIF). Wanneer het doel is het opsporen van niet-negeerbare ontbrekende gegevens wordt opnieuw een splitter-item gebruikt voor het groeperen van de responsiedata. Ook hier is de indeling van de steekproef gebaseerd op het al of niet geven van een response op het splitter-item. Fixed-effects itemparameters (in tegenstelling tot de vaak gebruikte random effects itemparameters) mod-

elleren de groepseffecten. Significante waarden van de fixed-effects itemparameters geven aan dat de itemparameters variëren tussen groepen. De parameter schattingen worden berekend met een MCMC methode. Het toetsen van de hypothese dat alle fixed-effects gelijk zijn aan nul (de itemparameters variëren niet tussen de groepen) kan worden gedaan op basis van een betrouwbaarheidsinterval en op basis van een Bayesiaanse toets die bekend staat als Bayes factor. De methoden worden geëvalueerd met behulp van onderzoek op basis van simulaties.

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# Local attitudes and sightings of crocodiles in Ligawasan Marsh and its tributaries: a survey

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*National Museum Papers Vol. 14 (2008), 7 pages, 2 tables*

# Local Attitudes and Sightings of Crocodiles in Ligawasan Marsh and its Tributaries: a Survey

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AND
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ABSTRACT

The study of crocodiles in the Philippines focuses on the two species that are present in the area, *Crocodylus mindorensis* Schmidt, also known as the Philippine Crocodile and *Crocodylus porosus* Schneider, the Indopacific Crocodile. *Crocodylus porosus* is common in the crocodile industry for its meat and skin and the species is not only widespread in the Philippines but also in other areas of the Asia-Pacific rim. On the other hand, *C. mindorensis* is endemic in the Philippines, and it is believed that its number is decreasing due to the destruction of its natural habitat and being captured as pets or food. Further the actual number of these crocodiles in the wild is unknown. Ortega (1998) states that less than 100 individuals survive in the wild. This number is commonly quoted and has never been challenged. Ross and Alcala (1983) estimated the wild population at 500 to 1000 individuals and acknowledged that this guessed at populations in some areas not surveyed (then or now), such as Samar Island and the Ligawasan Marsh.

There are claims of their presence in some areas in the wild but these reports are mostly unverified. In Mindanao, *Crocodylus mindorensis* are mostly found in Ligawasan marsh and its tributary rivers although baseline information about their presence is limited due to the location and the political situation of the area. This paper aims to present a survey of the situation of these crocodiles based on the actual sightings of people who are resident in the area and comment on their attitude towards the crocodiles.

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## The Ligawasan Marsh

The Ligawasan Marsh is the most extensive and largest marsh in the Philippines. It is suspected to be home to a considerable population of *Crocodylus porosus* Schneider and *Crocodylus mindorensis* Schmidt. This extensive swamp region has an area of 288,000 hectares along the Pulangi River and south central Mindanao. There are fertile rice-growing areas and mangrove forests in the marsh. A game refuge and bird sanctuary around 440 sq km in size was established in 1941. The Marsh, although generally known as Ligawasan, actually consists of two adjoining marshy basins, the Ligawasan and Libungan. Ligawasan lies at the influence of the Pulangi River from Bukidnon, Maganoy and Buluan Rivers from Maguindanao Province and Allah River from South Cotabato Province while Libungan lies at the influence of Libungan River in North Cotabato Province. All these rivers converge in Cotabato City and are known as the Rio Grande de Mindanao which empties into the Moro Gulf.

The majority of the inhabitants within the marsh are Maguindanaoan Muslims although, due to migration, some Ilocano, Cebuano and Ilongo Christian groups are also found in the villages surrounding the marsh. The marsh produces a huge quantity of fresh water fishes like mud fish (locally known as Dalag), tilapia, cat fish (native and hybrid Hito), carps, freshwater eels, crabs and shrimps, that are delivered daily in the cities of Cotabato, Kidapawan, Koronadal and Tacurong and the provinces of Sultan Kudarat, Maguindanao, South Cotabato, North Cotabato and Lanao del Sur. During the period of heavy rains, when the water level is high due to the swelling of its tributaries, most of inhabitants temporarily migrate to neighboring highland areas in search for alternative livelihood such as upland farming and daily labor in some commercial farms and businesses. The marsh is also home to some rebel factions such as the Moro National Liberation Front (MNLF) and the Moro Islamic Liberation Front (MILF), which demand an independent Islamic state in the Southern Philippines, and, the feared criminal group, the Pentagon kidnapping gang. At present the MNLF has a peace agreement with the Philippine government while MILF has an ongoing negotiation with the Philippine government.

## Survey of sightings and Attitudes

We selected respondents or subjects that actually sighted crocodiles in the area of Ligawasan marsh and its tributaries as well as respondents who owned captive crocodiles. Using a written questionnaire interviews were conducted (Tables 1 and 2).

Table I. Respondents to Questionaires and Interviews.

| Subject | Age | Place | Yrs. live | Job | Tribe | Seen | Times |
|---|---|---|---|---|---|---|---|
| 1 | 10 | Cuyapon | 10 | Student | Maguindanao | Water | 5 |
| 2 | 12 | Cuyapon | 12 | Student | Maguindanao | Water | 1 |
| 3 | 22 | Cuyapon | 22 | Student | Igorot | Captured | 5 |
| 4 | 47 | Cuyapon | 25 | Farmer | Ilonggo | Water, land | 3 |
| 5 | 47 | Cuyapon | 47 | Farmer | Ilocano | Water | 10 |
| 6 | 43 | Cuyapon | 30 | Farmer | Ilocano | Water | 5 |
| 7 | 34 | Kabacan | 34 | Business | Ilocano | Captured, bought | 2 |
| 8 | 64 | Kabacan | 64 | Business | Ilocano | Captured, Malmar area | 3 |
| 9 | 36 | Omonay | 36 | Farmer | Cebuano | water, attacked | 5 |
| 10 | 50 | Omonay | 50 | Farmer | Maguindanao | Water | 10 |
| 11 | 30 | Roxas | 30 | Caretaker | Ilongo | captured, bought | 2 |
| 12 | 13 | Tambad | 12 | Student | Maguindanao | Water | 1 |
| 13 | 35 | Tambad | 35 | Farmer | Maguindanao | Water | 10 |
| 14 | 27 | Tambad | 27 | Farmer | Maguindanao | Water | 10 |
| 15 | 29 | Omonay | 29 | Farmer | Ilongo | Water | 5 |
| 16 | 30 | Omonay | 1 | Soldier | Cebuano | land | 5 |
| 17 | 65 | Roxas | 65 | farmer, former mayor | Ilongo | captured | 3 |

Table ll. Sightings of Crocodiles by Respondents.

| Subject | Group | Size | Food | Response | Saw Egg | Time Appered | Sound of Crocs | Attitude |
|---|---|---|---|---|---|---|---|---|
| 1 | Pairs | 7 feet | Dog,fish, ducks, chicken | Run | No | 4:00PM | Yes, uuaa | Leave alone |
| 2 | Alone | 2 feet | Fish, dog | Run | No | 4:00PM | No | Leave alone |
| 3 | Alone | 2 feet | Fish | Does not know | No | Noon | No | Leave alone |
| 4 | Alone | 7 feet | Fish, frog | Does not know | No | 8:00AM, 3:00PM | Like Carabao | Help eat pest |
| 5 | Pairs | 18 feet | Fish | Ignored | No | 5:30PM | Like Carabao | Enemy & Friend |
| 6 | Alone | 12 inches | Fish | Does not know | No | 4:00PM | Sound | Leave alone |
| 7 | Alone | 16 inches | Chicken | Ignored | No | 4:00PM | Yes, uuaa | Pet, good luck |
| 8 | Alone | 5 feet | Pork, fish | Run | No | Noon | No | Pet, good luck |
| 9 | Alone | 15 feet | Fish | Ignored | Yes | 8:00AM, 3:00PM | No | Throw stone |
| 10 | Alone | 9 feet | fish, dog | capture, eaten | Yes | Noon | No | capture, eat |
| 11 | Alone | 7 feet | Pork | capture | No | captive | No | Leave alone |
| 12 | Alone | 14 feet | Fish | Ignored | No | Noon | No | Leave alone |
| 13 | Alone | 15 feet | Fish | Ignored | Yes | Noon | No | Leave alone |
| 14 | Alone | 15 feet | Fish | Ignored | Yes | Noon | No | Leave alone |
| 15 | Alone | 5 feet | Fish, chicken | capture | Yes | afternoon | No | eat |
| 16 | Alone | 7 feet | Chicken | shot | No | 10:00PM, 2:00AM | No | Drive away. |
| 17 | Alone | 19.5 feet | Fish | capture | No | Captive | No | put in zoo |

Surveys were conducted in Barangay Cuyapon, Kabacan, which is an entrance barangay of the Ligawasan Marsh and Tambad, Carmen. Interviews were also conducted in the towns of Kabacan, President Roxas and Carmen, Cotabato Province. President Roxas and Carmen are adjacent to the Pulangi River, a tributary of the Ligawasan Marsh. The survey was also done in barangay Omonay of Damulog, Bukidnon Province, which is a neighboring barangay of Tambad. Both are on the Muleta river, a tributary of the Pulangi River and where crocodiles have been sighted.

## Survey Results

The result of the survey showed that there were 85 sightings of crocodiles, 70 are in the wild and 15 in captivity. Most appeared in noon time as well in the afternoon and most were alone. The size of the crocodiles ranged from 1 foot to 19.5 feet. Most of the crocodiles diet are found in the fresh water particularly fish, which are in abundance in the Ligawasan Marsh.

### Barangay Cuyapon and Poblacion, Kabacan

Crocodiles were caught by the local poachers in Barangay (village) Cuyapon, Kabacan, Cotabato Province, which is in the vicinity of the Ligawasan Marsh, and were sold in the town Poblacion area for a price from 750 to PhP 1500 ($30) for a 12 inch crocodile. All of these crocodiles were *Crocodylus mindorensis*. We also found that middlemen are selling these 12 inch crocodiles in the cities and provinces for raising on crocodile farms and to pet lovers for a price of PhP 3500 ($71). We have also seen a captive *C. mindorensis* in the Poblacion, Kabacan, that was captured in the Malmar area (Malitubog-Maridagao rivers), also a tributary of the Ligawasan Marsh, as a juvenile that is now 7 feet long.

### Tambad, Carmen and Omonay, Damulog

In the boundary between barangay Omonay and Tambad along Muleta River, a man (one of our respondents) was attacked while in the river and bitten in the right leg in 2005. One respondent also reported that they shot a 200 kilogram, 9 foot black crocodile in September 2006. This crocodile was slaughtered and eaten. In Tambad area, along the Muleta River which is surrounded by bamboo trees and corn fields, a nesting ground around 6 feet in length was seen by the researchers. Crocodile marks were seen near the banks of the river. It was reported that the 22 eggs of this crocodile was taken by one of our respondents (a crocodile poacher). He sold those eggs to the local people for PhP 20 ($0.45)

each. We recovered two eggs from one of the locals (our respondent) who said he wanted to hatch them but was unsuccessful. The eggs measured almost 3 inches (7 cm) in length.

The Philippine Army detachment in barangay Omonay also reported that crocodiles were seen in the vicinity of the camp. The commanding officer said that in daytime they saw crocodiles in the Muleta River. During the night around 10 p.m. and also at dawn around 2 a.m., crocodiles were seen crawling in the slope of the hill where the detachment is located. The army's response is to drive them away by shooting them with their guns.

**President Roxas Town**

In President Roxas Town, we saw 2 captive *Crocodylus mindorensis* which are under the custody of the local government. The length of both crocodiles is around 7 feet. We also interviewed the former mayor who bought the longest crocodile so far reported from the Philippines, a *Crocodylus porosus* whose length was 19 feet and 6 inches, 3 feet wide, and weighed 800 kilograms. This crocodile died in 1994 due to gunshot wound (Alconaba and Maulana 1994, Duque 1994, Maulana 1994). The skull of this huge reptile which measures almost 2 feet, is now in the collection of the University of Southern Mindanao, Kabacan.

**Myths and Beliefs**

Myths and beliefs of local people in the sanctuaries of crocodiles are contributory to their survival. Some myths and beliefs are good but some are considered a threat to crocodiles. In our survey, the local inhabitants had some forms of myths and beliefs about crocodiles. The Maguindanaoan people for instance do not want to harm the crocodiles since they argued that the spirit of the crocodiles sometimes becomes human (Mangansakan 2008). Others said that they want to capture crocodiles and raise them as pets since they will give good luck in terms of financial prosperity to the family. Others said crocodiles protect humans by absorbing sickness and diseases. Others just leave them alone since crocodiles they say will not harm them if they are not harmed.

Some respondents said the sexual organ of the male crocodile if eaten can give then more vigor in their sex life while others said that if they kept the sexual organ as an amulet will make them more attractive to women. A respondent also said that crocodiles have some sort of center teeth whose function he said will tear humans. Finally one of our respondents said that crocodiles are friends since they help eating pest.

## Conclusions

The results of this survey gave us baseline information that *Crocodylus mindorensis* are in the wild in the Ligawasan Marsh and its tributaries in Mindanao. We recommend that a conservation area be established in the vicinity with the University of Southern Mindanao in Kabacan, Cotabato, that will serve as an area for research, conservation, protection and education, for this endangered but unique crocodile species endemic to the Philippines.

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# Reply to Pitogo et al.: No single silver bullet to simply understand war-biodiversity conflict in the Philippines

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Sociopolitical conflicts have significant but often overlooked impacts on biodiversity. In our reply, we reaffirm key findings from our previous work and directly address the *Matters Arising* raised by Pitogo and colleagues. Additionally, we present fine-scale analyses that further support our original conclusions. We emphasise the need for continued research to fully unravel the complex relationship between conflict and environmental impacts.

In Hilario-Husain et al.[1], we explored the potential link between sociopolitical conflict and biodiversity knowledge shortfalls in Mindanao in the Southern Philippines using publicly available datasets on conflict and biodiversity records. We found that species occurrence records were related to the frequency and distance of conflicts at the provincial scale. We argue that security risks, logistical challenges, and political restrictions associated with conflict zones often deter biodiversity research, leading to underdocumented regions despite their ecological significance. Our study provides an initial discussion highlighting that sociopolitical and environmental conflicts represent an underestimated threat to biodiversity in the Philippines and suggests potential solutions to address them. In response to our work, Pitogo and colleagues raised some interesting concerns, specifically regarding the choice of dataset analysed and our analytical decisions. While they acknowledge the significance of our work, they suggest that it is premature and warrants reconsideration. We welcome their interest in our work and are open to their criticisms. The impressive number of authors from diverse academic institutions, including those from the Philippines, China, Taiwan, and the United States, who showed interest in our study, indicates the significance of this issue.

First, we would like to clarify that our work neither claims to investigate nor hypothesizes the direct effect of sociopolitical conflict on species richness per se (i.e., increasing conflict decreases levels of biodiversity) or on biodiversity as a whole, but our approach was exploratory, aimed at providing a basis for understanding the link between sociopolitical conflict and biodiversity knowledge shortfalls[2]. We frame biodiversity shortfalls by focusing on gaps in our understanding of species distribution, especially in geographic areas with limited or no data, and on knowledge accessibility gaps caused by challenges in accessing or sharing data due to conflict and war. We reiterate our overarching goals, acknowledge the preliminary nature of our study, and recognise the inherent caveats in pioneering research that lacks prior empirical groundwork[1]. Thus, the interpretation of our initial results requires care and caution. However, we believe that Pitogo and colleagues may have overlooked our objectives and chosen analyses, which could have led to a misunderstanding of the message we aim to convey.

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Second, Pitogo and colleagues raised concerns about our data sources for analysis and stated the use of 'vague definitions'. In Hilario-Husain et al., we used publicly available data from the United Nations Office for the Coordination of Humanitarian Affairs (UN-OCHA)[3] and MOBIOS + [4] datasets, which represent conflict events and biodiversity occurrence data, respectively. Pitogo and colleagues criticised that using UN-OCHA data did not adhere to their ideal definition of 'conflict.' We argue that the conflict events reported by UN-OCHA were defined according to the ACLED (Armed Conflict Location and Event Data Project) Code Book[5] following the internationally agreed-upon definition. Therefore, data from the UN-OCHA remain the most extensive and standardised source of information currently available and can be utilised as a proxy for sociopolitical conflicts and to remove potential bias in collating data from multiple sources.

Pitogo and colleagues also raised concerns about the potential bias in our dataset selection and the representativeness of taxonomic groups. While we acknowledge the limitations of the data coverage, we refute the claims of bias. Pitogo and colleagues recommended incorporating the entire GBIF dataset into our study, a suggestion that was also raised by an anonymous peer reviewer, but we opted to limit our data based on literature records. Our reliance on publication-based biodiversity records was intentional, as it reflects research efforts and knowledge gaps, aligning with our objective of examining changes in knowledge generation through standardised research.

The taxonomic limitations of the database have been clarified and are consistent with the available data, and our analysis was at the 'class' level, where errors at the species level should not affect our analysis. Similar to other biodiversity databases, some errors are inevitable, often originating from inaccuracies in species records and the reporting of occurrences in the original mobilised data sources. As with all GBIF data, these records undergo continuous verification and correction[6–8]. Regardless of these inconsistencies, each of the 'species occurrence data' we used in our analysis represents 'biodiversity knowledge', which is consistent with our intended analysis. In addition, they also raised concerns about the limited taxonomic representation in our analysis, and we emphasise that our analysis included major taxonomic groups known to be reliable environmental proxies or indicators[9,10]. Achieving 'complete' data for all groups is a common challenge in macroecological research, particularly for elusive species[11,12].

Pitogo and colleagues also questioned our choice for spatial analysis. We used the simple 'join attributes using the nearest function in QGIS, which measures the distance of the attributes of one layer (species occurrence) from another (conflict records) based on feature's proximity. Using this function, we set the maximum nearest neighbours to 1 to ensure that only the single closest conflict record was considered for each biodiversity occurrence record. For the analysis, distance measurements for each taxonomic category were averaged across each province. It should be noted that all distances were measured in kilometres, including those presented by Hilario-Husain et al.[1] in Figure 4b. To ensure clear and interpretable results, we prioritised the nearest conflict records to minimise spatial noise from distant or less relevant data.

Consequently, Pitogo and colleagues reanalysed the same dataset and found slight variations and differences compared to our outcomes; in particular, they indicated a decline in observed species occurrence records with increasing distance from conflict events, which is the opposite of our findings. These differences may have been due to the variability in our analytical choices and decisions (for example, Botvinik-Nezer et al.[13], Gould et al.[14], and Silberzahn et al.[15]). Firstly, Pitogo and colleagues, they treated taxonomic groups as a random effect rather than a fixed effect, which does not align with our primary goal and is different from our analysis. We reiterate that our aim was to estimate and compare the specific effects of each taxonomic group. We reason that if taxonomic groups are treated as random effects, the model will not estimate the individual coefficients for each group. Instead, it estimates a single variance parameter that describes variability among groups. This would obscure the specific effects of each group, which is contrary to our goal of assessing the differences in response to distance and frequency. Secondly, Pitogo and colleagues did not disclose their critique of the data treatment they made on independent variables prior to analysis (i.e., log-transformation of the distance and frequency values), which may have altered the values of our results and produced patterns that differed from ours.

We recognise the critiques regarding the conservative estimates of our models, which may stem from the uneven sampling effort in the real-world data that could have distorted the data distribution, reduced variability, and potentially masked the true strength of the observed relationship between conflict and biodiversity records. To validate and support our previous findings in Hilario-Husain et al., we filtered 'synonymous' and 'doubtful' records and reanalysed the data using a taxon-specific generalised linear mixed model with a negative binomial distribution (i.e. taking account of zero-inflated data) at the municipal scale. Our results and the observed patterns converged and remained consistent with our previous findings, but had better and refined estimate values (see Fig. 1). We suggest that the stronger effects at the municipal scale highlight the importance of a scale-dependent analysis of socioecological interactions, reflecting the mechanisms and impacts being studied. We also recognise that the provincial scale may be too coarse to detect distinct patterns that are more apparent at finer, more localised resolutions. Nonetheless, both of our analyses consistently filtered the 'noise' and highlighted the important 'signal': *sociopolitical conflict could hamper biodiversity research efforts*. We also incorporated additional findings showing a significant difference in the distance of occurrence records from conflict areas based on species conservation status, with under-assessed species often found closer to critical areas (Fig. 2). This could potentially undermine the effective conservation status of under-assessed or even undescribed species in conflict zones, which may face risks similar to those of well-studied groups[16]. However, this observation warrants further investigation to fully comprehend the underlying relationships.

More importantly, both our findings shed light on the critical need for careful contextualisation of our conclusions to prevent potential misinterpretation by the public. Conversely, from the opposing findings of Pitogo and colleagues, the following question arises: Why does species richness drop as you move farther from conflict zones? To make their case

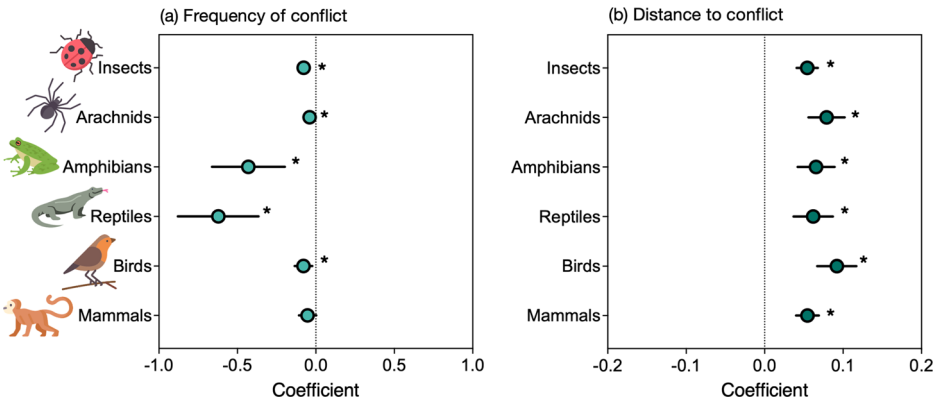



**Fig. 1 | Municipal scale analysis of biodiversity-conflict relationship.** Dot plot showing the estimates from the taxon-specific generalised linear mixed model (GLMM) with a negative binomial distribution (i.e. to account for zero-inflated data) explaining species occurrence records influenced by frequency (**a**) and distance (**b**) from conflict events. Each dot represents the estimates from separate GLMMs, and the whiskers represent the 95% CI. The figure was generated using GraphPad Prism 9[21]. Note: the image icons used from https://www.flaticon.com/ under the Creative Commons Attribution-NonCommercial (BY-NC) License.

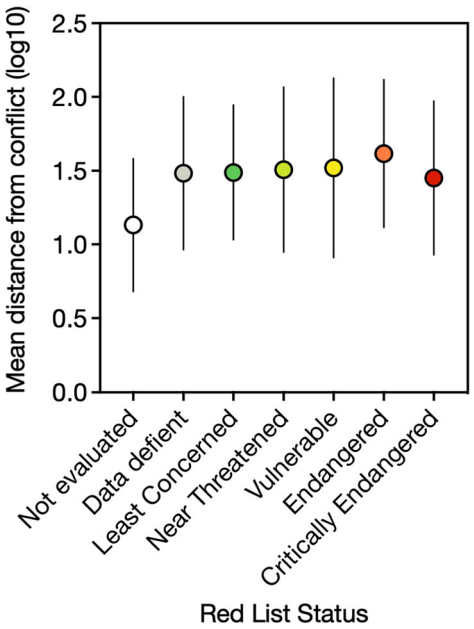



**Fig. 2 | Conservation status of species in conflict areas.** Comparison of the distance of occurrence of species records from conflict events according to their Red List conservation status. Our analysis showed a significant difference (Kruskal-Wallis test: $H = 4760$, df = 7, $p < 0.0001$) in the distance from conflict areas. The whiskers represent the 95% CI. Figure was generated using GraphPad Prism 9[21].

more compelling, Pitogo and colleagues could strengthen their argument and raise informed dialogue by offering alternative explanations to reconcile opposing findings and include alternative conflict datasets for deeper analysis. This would provide a balanced perspective and allow conservation biologists and policymakers to develop practical solutions grounded in a thorough understanding of sociopolitical conflict and biodiversity.

Sociopolitical conflicts disrupt biodiversity surveys, data collection, and conservation efforts, creating gaps in our understanding of biodiversity dynamics in the affected areas[17,18]. When linked to habitat loss or subsistence hunting, such conflicts can lead to biodiversity loss, which is often unmeasured because of limited data generation. Conversely, they may create areas where humans cannot access and allow wildlife populations to grow[19]. As awareness grows, conflicts emerge as an underestimated yet critical indirect driver of global biodiversity loss[18,20]. In the Philippines, the impact of warfare on biodiversity remains underexplored, and our research pioneers this field while acknowledging the need for further scientific and political priority settings to fully understand this relationship.

Finally, we reassure Pitogo and colleagues that our study is not a panacea, but only serves as an initial step to stimulate dialogue on this important yet often overlooked environmental issue. Acknowledging that our analysis does not offer a single silver bullet solution to the ongoing socioecological challenge, we welcome and anticipate further studies that will deepen our understanding and address the complex issues between the impact of sociopolitical conflict on biodiversity in the Philippines. We urge readers to consider the broader context of our study to understand the importance of our findings in addressing key ecological concerns and enhancing awareness of the negative impact of sociopolitical conflict on biodiversity. As mentioned earlier, we appreciate the criticisms from Pitogo and colleagues, as they highlight the need for further efforts in this area of environmental research. There is still much to discuss about the best way to do it. We hope that this *response* not only clarifies the procedures they questioned but also contributes to advancing the field and highlights the significance of our work in driving meaningful progress in biodiversity conservation efforts in Mindanao and other regions facing similar challenges due to war and conflict.

## Data availability

No datasets were generated in the current work.

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## Author contributions

K.C.T. led the response and performed additional spatial analysis. A.C.H. supervised the spatial analysis and responses. B.A.H., S.J.S., F.G.G., T.L., M.E.G., R.J.A.-E., M.M.-R., S.M., L.C., J.D.R., K.D.C., S.A., S.M.P.B., J.M.R., A.L., M.T.C., J.P., F.J.T., A.A., A.C.H. contributed to the discussion and agreed to the submission of our Reply.

## Competing interests

The authors declare no competing interests.

## Additional information

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# Nonignorable data in IRT models: Polytomous responses and response propensity models with covariates

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**Abstract**

Missing data usually present special problems for statistical analyses, especially when the data are not missing at random, that is, when the ignorability principle defined by Rubin (1976) does not hold. Recently, a substantial number of articles have been published on model-based procedures to handle nonignorable missing data due to item nonresponse (Holman & Glas, 2005; Glas & Pimentel, 2008; Rose, von Davier & Xu, 2010; Pohl, Gräfe & Rose, 2014). In this approach, an item response theory (IRT) model for the observed data is estimated concurrently with an IRT model for the propensity of the missing data.

The present article elaborates on this approach in two directions. Firstly, the preceding articles only consider dichotomously scored items; in the present article it is shown that the approach equally works for polytomously scored items. Secondly, it is shown that the methods can be generalized to allow for covariates in the model for the missing data. Simulation studies are presented to illustrate the efficiency of the proposed methods.

Keywords: item response theory, latent traits, missing data, nonignorable missing data, observed covariates

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## Introduction

Missing data are always a source of concern for statistical analyses. It raises the level complexity of statistical inference. Many researchers, methodologists, and software developers resort to editing the data, although ad hoc edits may do more harm than good by producing results that are substantially biased, inefficient and unreliable (Schafer & Graham, 2002). One way to address the bias in parameter estimates is the identification of the variables that explain the cause of missing data. Below, these explanatory variables will be called "mechanism or process" variables. By including a model for this missing data mechanism in the estimation we can reduce or eliminate the bias in parameter estimates.

Theoretically, if all the process variables associated with a particular piece of missing data can be identified and modeled accurately as controls, the impact of the missing data can be statistically adjusted to the point where it is ignorable (Little & Rubin, 1987). In practice, it is difficult to identify these process variables for all cases of missing data. However, if the given data set contains missing observations, the mechanism causing this missingness can be characterized by its variety of randomness (Rubin, 1976) as missing completely at random (MCAR), missing at random (MAR) and not missing at random (NONMAR).

In this article, we focus on responses of persons to items and on item nonresponse. Suppose $\theta$ and $\zeta$ are the parameters of the observed data and the missing data process, respectively, and $D$ is the missing data indicator with elements $d_{ik} = 1$ if a realization $x_{ik}$ was observed and $d_{ik} = 0$ if $x_{ik}$ was missing for persons $i$ and items $k$. Following Rubin's definition, missing data is MAR if the probability of $D$ given the observed data $x_{obs}$, missing data $x_{mis}$, and observed covariates $y$ does not depend on the missing data $x_{mis}$, that is, if

$$p(D \mid x_{obs}, x_{mis}, \zeta, y) = p(D \mid x_{obs}, \zeta, y). \tag{1}$$

Furthermore, the parameters θ and $\zeta$ are distinct if there are no functional dependencies between them, that is, restrictions on the parameter space (frequentist version) or if the prior distributions of $\zeta$ and $\theta$ are independent (Bayesian case). (It should be noted that this is a somewhat rough definition, for technical details refer to Rubin (1976), Heitjan (1994, 1997), Heitjan and Rubin (1991), and Jaeger (2005)). If MAR and distinctness hold, the missing data is said to be ignorable, otherwise the missing data are nonignorable. If ignorability holds, we do not have to take the distribution of D and $\zeta$ into account, and the consistency of the estimates is not threatened by the occurrence of the missing data.

In the framework of IRT, missing data can be split into four types (Lord, 1974). The first consists of missing observations which result from a priori fixed incomplete test administration and calibration designs. In this case, the missing data are a priori fixed and ignorability trivially holds. That is, $p(D \mid x_{obs}, x_{mis}, \zeta, y) = p(D \mid x_{obs}, \zeta, y) = 1$. The second type consists of classes of response-contingent designs such as two-stage and multistage

testing designs and computerized adaptive testing (Lord, 1980). These designs produce ignorable missing data, because the design variables *D* are completely determined by the observed responses (see, for instance, Mislevy & Wu, 1996). The third type is ignorable missing data that results from unscalable responses such as items missing from booklets and responses such as "do not know" or "not applicable"'. Missing pages can be reasonably viewed as missing at random; "do not know"' or "not applicable" are already suspicious and might fall in the next category of missing data. The reason is that it is not automatically clear whether the given response (don't know or not applicable) is accurate or an instance of avoidance behavior. The fourth and last type of missing data results from a nonignorable missing data mechanism. These will, for instance, occur when low-ability respondents fail to give responses to specific items as a result of discomfort or embarrassment. In the framework of a medical survey, Holman and Glas (2005) report that patients with a relatively high functional status boosted the estimate of their ability level by failing to respond to items of a physical disability scale. Of course, whether ignorability holds or not, needs to be tested in these cases.

Moustaki (1996, see also Bartholomew & Knott, 1999) developed a general latent trait and latent class model for mixed observed variables which applies to Lord's fourth type of missing data. Three methods for dealing with nonignorable missing discrete data were proposed (O'Muircheartaigh & Moustaki, 1999; Moustaki & O'Muircheartaigh, 2000; Moustaki & Knott, 2000). In the first method for the treatment of nonresponse, the missing value is treated as a separate response category. So the method includes the missing values in the analysis of the observed items. That is, it is assumed that responses and nonresponses are related to the same attitude dimension or dimensions.

The second method to deal with nonresponse is computing response propensities. The idea is to use a propensity score to weight item responses and respondents to account for item and unit nonresponse and to obtain adjusted estimates. This response propensity method uses a logistic or probit regression model which is fitted to a binary item response-nonresponse variable for the item of interest with a set of covariates. The third method is to use a latent variable model with two latent dimensions, one to summarize the response propensity and the other to summarize the individual position on the dimension of interest (such as ability or attitude). As an example, O'Muircheartaigh and Moustaki (1999) used a latent variable model for the treatment of item nonresponse in attitude scales. This latent variable approach allows missing values to be included in the analysis and, equally important, allows information about attitude to be inferred from nonresponse. Their method handles binary (dichotomous), metric and mixed (binary and metric) manifest items with missing values. The second and third methods are closely related: both entail the estimation of a response propensity distribution and can be seen as an elaboration of methods of adjustment by propensity scoring proposed by Heckman (1979).

Working within the third approach, Holman and Glas (2005) proposed an IRT model for skipped items that allows concurrent estimation of IRT item parameters for both a model for the observed dichotomous responses and the missing data indicators. Other applications pertain to items that are not reached, such as missing item responses at the end of a speeded test (Glas & Pimentel, 2008). Rose (2013) generalized these approaches to allow

for multidimensional IRT models for the missing data indicators and showed that disregarding the multidimensional latent structure can lead to a failure to correct for nonignorable item nonresponse. Further, Rose (2013) considered an alternative approach where the average number of missing responses is used as a covariate in the distribution of the latent variables of an IRT model for the observed responses. This approach has both advantages (works well with small sample sizes and small numbers of missing responses) and disadvantages (attenuated estimates when the reliability of the covariate is low). Finally, Pohl, et al. (2014) studied a version of the model with a combination of propensity distributions for omitted and not-reached items. Also, they presented examples of empirical applications where a propensity distribution was not needed to correct for bias in parameter estimates. In such cases, the actually observed responses contain enough information to account for missing responses.

In this article, we generalize model-based adjustment using IRT in two directions: IRT models for polytomously scored items and covariates for the propensity distribution. This article consists of five sections and is organized in the following manner. After this introduction, the model will be outlined and a maximum marginal likelihood (MML) estimation procedure will be sketched. Then some simulation studies will be presented to illustrate the performance of the procedure. The next section presents an empirical simulation, that is, a simulation using real data, to give an example of how the model can work in practice. The article concludes with a discussion.

## IRT models

### General IRT model for missing data

Suppose, for respondents labeled $i = 1,...,N$ and items labeled $k = 1,...,K$, that $x_{ik} \in \{0.,1,2,...,m\}$ is the observed item response and let $p(x_{ik} \mid d_{ik} = 1, \theta_i, \alpha_k, \beta_k)$ be the IRT model for the observed item response with item parameters $\alpha_k$ and $\beta_k$ and person parameters $\theta_i$. If $d_{ik} = 0$, we assume that $x_{ik}$ is equal to an arbitrary constant $c$ and so $p(x_{ik} = c \mid d_{ik} = 0, \theta_i, \alpha_k, \beta_k) = 1$. Let $p(d_{ik} \mid \zeta_i, \gamma_k, \delta_k)$ be the IRT model for the missing data indicator defined above, with item parameters $\gamma_k$ and $\delta_k$ and person parameters $\zeta_i$. All parameters may be vector-valued. It is assumed that the person's latent variables have a multivariate normal distribution with density $g(\zeta_i, \theta_i \mid \Sigma, H, y_i)$, where $y_i$ are observed covariates, $H$ are regression coefficients for the regression of $\lambda_i = (\theta_i, \gamma_i)$ on $y_i$, and $\Sigma$ is the covariance matrix of the residuals. Note that if the covariates are lacking, $\Sigma$ reduces to the covariance matrix of the latent person variables. Usually, $\Sigma$ is restricted to a correlation matrix to identify the model. The likelihood of the model is expressed as

$$\prod_{i=1}^{N}\prod_{k=1}^{K} p(x_{ik} \mid d_{ik}, \theta_i, \alpha_k, \beta_k)\, p(d_{ik} \mid \zeta_i, \gamma_k, \delta_k)\, g(\zeta_i, \theta_i \mid \Sigma, H, y_i) \quad . \tag{2}$$

Note that by the definition of $p(x_{ik} \mid d_{ik}, \theta_i, \alpha_k, \beta_k)$, the unobserved values of $x_{ik}$ are ignored in the likelihood given by (2). The idea behind the method is that ignorability is violated when the covariance between $\theta$ and $\zeta$ is non-zero. In these cases, the estimation procedure must be based on the complete model, so including the model for the response propensities.

Next, we will specify the three densities in formula (2). The propensity distribution $p(d_{ik} \mid \zeta_i, \gamma_k, \delta_k)$ must be chosen to reflect the process that caused the missing responses. For instance, Korobko, et al. (2008) choose an IRT model with single peaked response curves to model the choice of examination topics. In the present application, we assume the simpler situation of skipped items, and choose a propensity model that is a multidimensional generalization of the model by Holman and Glas (2005). So also "not reached" is not considered here. To model the missing data process, we use a $Q_1$-dimensional IRT model proposed by Reckase (1985, 1997) and Ackerman (1996a & 1996b). The probability of an observation is given by

$$p(d_{ik} = 1 \mid \zeta_i, \gamma_k, \delta_k) = \frac{\exp\left(\sum_q^{Q_1} \gamma_{kq}\zeta_{iq} - \delta_k\right)}{1 + \exp\left(\sum_q^{Q_1} \gamma_{kq}\zeta_{iq} - \delta_k\right)} . \qquad (3)$$

The model becomes the two parameter logistic (2PL) model (Lord & Novick, 1968) when $Q_1 = 1$ and the Rasch model (Rasch, 1960) when, in addition, $\gamma_{kq} = 1$.

For the observed responses, we consider the multidimensional generalized partial credit model (GPCM; Muraki, 1992). The probability of responding in a category $g$ of item $k$ by person $i$ is given by

$$p(x_{ik} = g \mid d_{ik} = 1, \theta_i, \alpha_k, \beta_k) = \frac{\exp\left(g\sum_{q=1}^{Q_2} \alpha_{kq}\theta_{iq} - \beta_{kg}\right)}{1 + \sum_{h=1}^{m} \exp\left(h\sum_{q=1}^{Q_2} \alpha_{kq}\theta_{iq} - \beta_{kh}\right)} . \qquad (4)$$

Note that the model has a $Q_2$-dimensional latent person parameter $\theta_i = (\theta_{i1}, ..., \theta_{iq}, ..., \theta_{iQ_2})$, $\alpha_k = (\alpha_{k1}, ..., \alpha_{kq}, ..., \alpha_{kQ_2})$ are discrimination parameters and $\beta_k = (\beta_{k1}, ..., \beta_{kq}, ..., \beta_{kQ_2})$ are location parameters for the item response categories.

The latent person parameters $\lambda_i$ (defined above as the concatenation of $\theta_i$ and $\zeta_i$ are assumed to have a $Q$-variate normal distribution ($Q = Q_1 + Q_2$), that is,

$$g(\lambda_i \mid \Sigma, H, y_i) = (2\pi)^{-Q/2} \mid \Sigma \mid^{-1/2} \exp\left(-1/2(\lambda_i - H^t y_i)^t \Sigma^{-1}(\lambda_i - H^t y_i)\right), \qquad (5)$$

where $H$ is a $P$ $X$ $Q$ matrix of regression coefficients and $\Sigma$ is a $Q$ $X$ $Q$ variance-covariance matrix. Equivalently, the general model for the latent variables can be expressed in matrix form as

$$\lambda = YH + E, \tag{6}$$

where $\lambda$ is the $N$ $X$ $Q$ matrix of latent variables, $Y$ is a $N$ $X$ $P$ matrix of observed covariates, and $E$ is the $N$ $X$ $Q$ matrix of residuals.

### MML estimation

In the present article, the parameters of the model are estimated by maximum marginal likelihood (MML, see, Bock, Gibbons & Muraki, 1988). The likelihood given by formula (2) is marginalized by integrating over the person parameters, such that the marginal likelihood given by

$$\prod_{i=1}^{N} \int \cdots \int \prod_{k=1}^{k} p(x_{ik} \mid d_{ik}, \theta_i, \alpha_k, \beta_k)\, p(d_{ik} \mid \zeta_i, \gamma_k, \delta_k)\, g(\zeta_i, \theta_i \mid \Sigma, H, y_i)\, d\theta_i d\zeta_i \tag{7}$$

is a function of item parameters $\alpha_k, \beta_k, \gamma_k, \delta_k$, regression parameters $H$ and the covariance matrix $\Sigma$ only. Glas (1992, 1999, 2010a) shows that the likelihood equations can easily be derived using Fisher's identity (Efron, 1977; Louis 1982). The procedure boils down to deriving the likelihood equations assuming $\lambda$ known, and then taking the posterior expectation of both sides of the equation. For instance, applied to the regression parameters, the likelihood equations assuming $\lambda$ known are given by

$$\widehat{H} = (Y^t Y)^{-1} Y^t \lambda \tag{8}$$

and taking posterior expectations results in

$$\widehat{H} = (Y^t Y)^{-1} \sum_{i=1}^{N} y_i\, E(\lambda_i \mid x_i, d_i, y_i)^t, \tag{9}$$

where the Q-dimensional column-vector $E(\lambda_i \mid x_i, d_i, y_i)$ is the posterior distribution of $\lambda_i$ given all observations on respondent $i$. Estimation equations for the other parameters are derived analogously (refer to Glas 1992, 1999, 2010a, also see, Adams, Wilson, & Wang, 1997, for an alternative derivation).

## Simulation studies

Simulation studies were undertaken to assess the effect of the presence of nonignorable missing data on the estimates of item parameters and the effectiveness of the proposed methods to improve the estimates. It must be noted in advance that these simulations did not have the pretention of being exhaustive, because there are too many possible configurations of multidimensional IRT models for polytomously scored items and possible

propensity models to meet such a goal. So the primary aim was to check whether the approach behaved as expected in a limited set of conditions.

The simulation study consisted of two parts. The first part extended the study by Holman and Glas (2005) to a situation where the model for the missing data indicators is multidimensional, and studied the effects of including no, part of, or all latent dimensions of this model in the estimation. The motivation for this part of the study was that Rose (2013) showed that ignoring the multidimensional structure of the response propensity model can lead to substantial bias. In the present study, it was investigated whether this phenomenon also appeared here. The second simulation study pertained to the effects of adding observed covariates to the model. All simulations reported in this article were conducted with the public domain program MIRT (Glas, 2010b) using an MML estimation procedure.

## Multidimensional response propensity models

To study the effects of including no, part of, or all latent dimensions of the missing data process in the estimation procedure, latent person parameters were drawn from a three-variate normal distribution. The sample size was $N = 500$ persons. The variance of the latent variables was always equal to one. The correlation between the latent trait variables $\theta_i$ and $\zeta_i$, say, $\rho(\theta,\zeta)$, was varied as 0.0, 0.4 and 0.8. The values 0.0, 0.4 and 0.8 are chosen to clarify the effect of no, a small and a large violation of ignorability. The simulations by Holman and Glas (2005) showed that violations of ignorability related to latent correlations less than 0.4 had little impact and that the effects became manifest starting from a value of 0.4. Models with $\rho(\theta,\zeta)=0.0$ will be referred to as MAR models because they assume that the covariance between the latent variables pertaining to the item responses and the latent variables pertaining to the response propensities are zero. Other models will be called NONMAR models for the analogous opposite reason. Also the correlations between the two dimensions of the missing data process $\rho(\zeta_1,\ \zeta_2)$ were varied as 0.0, 0.4 and 0.8. The items were either dichotomously and polytomously scored. The test consisted of $K = 10$ items. The values $x_{ik}$ and $d_{ik}$ were drawn from $p(x_{ik}\,|\,d_{ik},\theta_i,\alpha_k,\beta_k)$ and $p(d_{ik}\mid\ \zeta_i,\gamma_k,\delta_k)$, respectively. The data were used to compute MML estimates of the item parameters under the various assumptions. Then the values of item parameters estimates over replications $r$ *(r=1,...,R,* with *R=100)*, say $\hat{\phi}_r$, were compared with the values of the parameters used to generate the data using the mean absolute error, that is

$$MAE=\frac{1}{R}\sum_{r=1}^{R}\left|\hat{\phi}_r-\phi\right| \ . \tag{10}$$

For the dichotomous case, two conditions were used. In the first condition, the item parameters for all items were $\alpha_k=\gamma_k=1.0$, $\beta_k=0.0$, and $\delta_k=-1.0$. These values resulted in about 25% missing data. In the second condition, we used $\alpha_k=\gamma_k=1.0$ and, $\beta_k=\delta_k=0.0$, which resulted in about 50% missing data. The motive for fixing the item parameters was that it leads to a clear interpretation of the MAE relative to the fixed

value. Not reported here are simulation studies carried out where the item parameters were drawn from normal distributions with the reported fixed values as expectation and standard deviations of 0.5. They produced results similar to the results reported below. The MAE of the item parameter estimates are given in Table 1. For the polytomous case, items with three response categories were used in the simulation. The location parameters of the IRT model for the response propensities were varied as $\delta_k = 0.0$, and $\delta_k = -1.0$ for all $k$. The other item parameters were the same across conditions, that is, $\alpha_k = \gamma_k = 1.0$, $\beta_{k1} = -1.0$, and $\beta_{k2} = 1.0$, for all $k$. The MAEs of the parameter estimates are given in Table 2.

Both tables have the same format. The first column, labeled $\delta$, refers to the location parameter for the IRT model for the response propensities used for generating the data. The first row pertains to a baseline condition where $\rho(\theta,\zeta) = 0.0$. So there ignorability holds. The values of the *MAE(α)* and *MAE(β)* are given in the two columns labeled $\alpha$ and $\beta$; they are the mean absolute errors over the 100 replications, and they serve as a baseline. The next three rows pertain to data generated using $\rho(\theta,\zeta) = 0.4$ and $\rho(\zeta_1, \zeta_2) = 0.0$. These data were analyzed using no, one and two dimensions for the missing data indicator, as indicated in the column labeled $Q_2$. The lines below, give the results for further values of $\rho(\theta,\zeta)$, $\rho(\theta_1,\theta_2)$, and $Q_2$. In Table 1, the columns labeled α, β, δ and γ give the MAEs for the respective item parameters. For polytomous case, reported in Table 2, there are two columns for the mean absolute error of the location parameters, referred as $\beta_1$ and $\beta_2$.

The simulations showed that the MAE values of the item parameter estimates were inflated when no model for missing data process was used in the parameter estimation. The effect increased as correlation between the latent variables for both observed data and the missing data process increased. For instance, if we consider Table 1, when $\delta = 0$, (that is, when there were 50% missing data) the baseline simulation of MAR data resulted in *MAE(α) = 0.225* and *MAE(β) = 0.120*. When $\rho(\theta,\zeta) = 0.4$ and $\rho(\zeta_1, \zeta_2) = 0.0$, and the missing data were ignored ($Q_2 = 0$), the MAE for α and β had values 0.245 and 0.148, respectively. This main effect was generally present both for dichotomously and polytomously scored items. So the first conclusion is that ignoring the missing data process lead to inflated estimation errors.

When the model for the missing data process was included in the analysis, that is, when the NONMAR model was used, the MAE values dropped to 0.228 for $\alpha$ and 0.133 for $\beta$ when $Q_2 = 1$, and to *MAE(α) = 0.223* and *MAE(β) = 0.128* when $Q_2 = 2$. In general, a decrease in the values of the MAE of the item parameters was observed and this decrease was positively related to the number of dimensions included. Similar results were also observed for the values of MAE of the item parameters $\delta$ and $\gamma$ for missing data process. So the second conclusion is that invoking the missing data process leads to a reduction of estimation errors, even if not all dimensions are invoked.

The third conclusion that can be drawn from the tables is that when the missing data process was completely modeled, that is, when $Q_2 = 2$, the estimation errors could even fall below the errors of the baseline. For instance, in Table 1 we see that for $\delta = 0$, the

**Table 1:**
MAE of item parameter estimates under MAR and NONMAR model;
Dichotomously scored items

| Generating Values | | | Analysis | Mean Absolute Error | | | |
|---|---|---|---|---|---|---|---|
| $\delta$ | $\rho(\theta, \zeta)$ | $\rho(\zeta_1, \zeta_2)$ | $Q_2$ | $\alpha$ | $\beta$ | $\delta$ | $\gamma$ |
| -1.0 | 0.0 | - | - | 0.168 | 0.102 | | |
| | 0.4 | 0.0 | 0 | 0.169 | 0.118 | | |
| | | | 1 | 0.163 | 0.113 | 0.126 | 0.467 |
| | | | 2 | 0.163 | 0.113 | 0.106 | 0.162 |
| | | 0.4 | 0 | 0.169 | 0.107 | | |
| | | | 1 | 0.164 | 0.102 | 0.109 | 0.205 |
| | | | 2 | 0.165 | 0.102 | 0.108 | 0.149 |
| | | 0.8 | 0 | 0.170 | 0.110 | | |
| | | | 1 | 0.165 | 0.104 | 0.100 | 0.137 |
| | | | 2 | 0.165 | 0.104 | 0.104 | 0.140 |
| | 0.8 | 0.4 | 0 | 0.176 | 0.140 | | |
| | | | 1 | 0.156 | 0.105 | 0.101 | 0.151 |
| | | | 2 | 0.160 | 0.104 | 0.099 | 0.135 |
| | | 0.8 | 0 | 0.170 | 0.137 | | |
| | | | 1 | 0.153 | 0.103 | 0.099 | 0.136 |
| | | | 2 | 0.156 | 0.103 | 0.103 | 0.137 |
| 0.0 | 0.0 | - | - | 0.225 | 0.120 | | |
| | 0.4 | 0.0 | 0 | 0.245 | 0.148 | | |
| | | | 1 | 0.228 | 0.133 | 0.081 | 0.568 |
| | | | 2 | 0.223 | 0.128 | 0.089 | 0.154 |
| | | 0.4 | 0 | 0.229 | 0.142 | | |
| | | | 1 | 0.209 | 0.124 | 0.079 | 0.194 |
| | | | 2 | 0.209 | 0.125 | 0.084 | 0.147 |
| | | 0.8 | 0 | 0.222 | 0.144 | | |
| | | | 1 | 0.214 | 0.121 | 0.086 | 0.126 |
| | | | 2 | 0.214 | 0.122 | 0.088 | 0.126 |
| | 0.8 | 0.4 | 0 | 0.257 | 0.210 | | |
| | | | 1 | 0.187 | 0.128 | 0.078 | 0.158 |
| | | | 2 | 0.186 | 0.129 | 0.083 | 0.136 |
| | | 0.8 | 0 | 0.245 | 0.220 | | |
| | | | 1 | 0.192 | 0.133 | 0.083 | 0.121 |
| | | | 2 | 0.194 | 0.133 | 0.083 | 0.121 |

**Table 2:**
MAE of item parameter estimates under MAR and NONMAR model;
Polytomously scored items

| Generating Values | | | Analysis | Mean Absolute Error | | | | |
|---|---|---|---|---|---|---|---|---|
| $\delta$ | $\rho(\theta, \zeta)$ | $\rho(\zeta_1, \zeta_2)$ | $Q_2$ | $\alpha$ | $\beta_1$ | $\beta_2$ | $\delta$ | $\gamma$ |
| -1.0 | 0.0 | - | - | 0.137 | 0.135 | 0.194 | | |
| | 0.4 | 0.0 | 0 | 0.142 | 0.129 | 0.194 | | |
| | | | 1 | 0.139 | 0.126 | 0.192 | 0.127 | 0.470 |
| | | | 2 | 0.138 | 0.125 | 0.193 | 0.103 | 0.167 |
| | | 0.4 | 0 | 0.138 | 0.139 | 0.198 | | |
| | | | 1 | 0.136 | 0.129 | 0.194 | 0.109 | 0.192 |
| | | | 2 | 0.135 | 0.129 | 0.194 | 0.107 | 0.162 |
| | | 0.8 | 0 | 0.136 | 0.137 | 0.206 | | |
| | | | 1 | 0.133 | 0.126 | 0.193 | 0.098 | 0.138 |
| | | | 2 | 0.132 | 0.127 | 0.192 | 0.100 | 0.139 |
| | 0.8 | 0.4 | 0 | 0.152 | 0.153 | 0.247 | | |
| | | | 1 | 0.140 | 0.129 | 0.200 | 0.100 | 0.154 |
| | | | 2 | 0.138 | 0.126 | 0.200 | 0.103 | 0.138 |
| | | 0.8 | 0 | 0.138 | 0.150 | 0.241 | | |
| | | | 1 | 0.129 | 0.125 | 0.196 | 0.098 | 0.130 |
| | | | 2 | 0.128 | 0.125 | 0.197 | 0.102 | 0.130 |
| 0.0 | 0.0 | - | - | 0.187 | 0.156 | 0.239 | | |
| | 0.4 | 0.0 | 0 | 0.182 | 0.173 | 0.259 | | |
| | | | 1 | 0.175 | 0.148 | 0.250 | 0.080 | 0.548 |
| | | | 2 | 0.174 | 0.145 | 0.242 | 0.088 | 0.155 |
| | | 0.4 | 0 | 0.189 | 0.173 | 0.257 | | |
| | | | 1 | 0.182 | 0.150 | 0.241 | 0.078 | 0.182 |
| | | | 2 | 0.182 | 0.150 | 0.243 | 0.084 | 0.143 |
| | | 0.8 | 0 | 0.188 | 0.182 | 0.274 | | |
| | | | 1 | 0.183 | 0.151 | 0.246 | 0.087 | 0.131 |
| | | | 2 | 0.183 | 0.151 | 0.246 | 0.090 | 0.129 |
| | 0.8 | 0.4 | 0 | 0.197 | 0.228 | 0.367 | | |
| | | | 1 | 0.165 | 0.148 | 0.245 | 0.081 | 0.152 |
| | | | 2 | 0.167 | 0.143 | 0.247 | 0.086 | 0.137 |
| | | 0.8 | 0 | 0.195 | 0.241 | 0.411 | | |
| | | | 1 | 0.170 | 0.154 | 0.250 | 0.088 | 0.120 |
| | | | 2 | 0.171 | 0.153 | 0.249 | 0.090 | 0.123 |

*MAE(α) = 0.225* for the baseline and *MAE(α) = 0.194* for $\rho(\theta,\zeta) = \rho(\zeta_1, \zeta_2) = 0.8$ and $Q_2 = 2$. The tentative conclusion is that invoking a model for the missing data indicator resulted in additional collateral information which improved the estimates.

The fourth conclusion pertains to the extent to which MAR was violated. Inspection of the tables shows that if we ignored the missing data process ($Q_1 = 0$), the magnitude of the estimation error for $\rho(\theta,\zeta) = 0.8$ was greater than the magnitude for $\rho(\theta,\zeta) = 0.4$. For instance, in Table 1 we see that conditionally on $\rho(\zeta_1, \zeta_2) = 0.4$, the MAEs for $\alpha$ were 0.229 and 0.257, respectively. Finally, there was no clear effect of $\rho(\zeta_1, \zeta_2)$. That is, the effect was clear insofar that for the item parameters of the latent ability, the actual dimensionality of the missing propensity was irrelevant.

**Response propensity models with observed covariates**

The simulation procedure used was analogous to the simulation procedure in the previous section, but with the added feature of including observed covariates. To achieve comparability with the previous section, the regression coefficients were chosen as follows. Let $\Sigma_\lambda$ be the covariance matrix of both the latent variables for the observed responses and the missing data indicator. As in the previous section, there was one dimension for the observed responses and there were two dimensions for the missing data process. Only the case $\rho(\zeta_1, \zeta_2) = 0.8$ was considered here. Further, either $\rho(\theta,\zeta) = 0.4$ or $\rho(\theta,\zeta) = 0.8$. Let $\Sigma_\varepsilon$ be the diagonal matrix of the variances of the error terms. These variances were all equal to 0.15. For the data generation, the variables $y_i$ had Q-variate independent standard normal distributions, and the regression coefficients $H$ were chosen such that

$$\Sigma_\lambda = HH^t + \Sigma_\varepsilon \ , \tag{11}$$

where $H$ is a lower-triangular matrix. As before, the sample size was $N = 500$. Again the test length was $K = 10$ and the item parameters were also as used above. One hundred replications were made for every combination of $\delta$, $\rho(\theta,\zeta)$ and $Q_2$.

The results are given in the tables 3 and 4. The format of the tables is analogous to the previous two tables, except for an added column *P*, which refers to the number of covariates included in the parameter estimation. Note that also the baseline model where $\rho(\theta,\zeta) = 0.0$ (the MAR model) includes a covariate. This was done to enable the comparison with the NONMAR models.

Referring to Table 3, the baseline MAR model resulted in *MAE(α) = 0.145* and *MAE(β) = 0.117* for the case $\delta = 0$, i.e., 50% missing data, as compared to the analogous simulation reported in Table 1, where *MAE(α) = 0.225* and *MAE(β) = 0.120*. This increase in precision is due to the inclusion of a covariate. When we increased the correlation to $\rho(\theta,\zeta) = 0.4$ and $\rho(\theta,\zeta) = 0.8$, results showed that when the missing data process was ignored and only the covariate for $\theta$ was included, the values *MAE(α) = 0.170* and *MAE(β) = 0.164* were obtained. When a response propensity model with one latent di-

**Table 3:**
MAE of item parameter estimates under MAR and NONMAR model with covariates. Dichotomously scored items

| Generating Values | | | Analysis | | Mean Absolute Error | | | |
|---|---|---|---|---|---|---|---|---|
| $\delta$ | $\rho(\theta, \zeta)$ | $\rho(\zeta_1, \zeta_2)$ | $Q_2$ | $P$ | $\alpha$ | $\beta$ | $\delta$ | $\gamma$ |
| -1.0 | 0.0 | - | - | 1 | 0.121 | 0.095 | | |
| | 0.4 | 0.8 | 0 | 1 | 0.157 | 0.117 | | |
| | | | 1 | 2 | 0.131 | 0.097 | 0.102 | 0.150 |
| | | | 2 | 3 | 0.120 | 0.097 | 0.095 | 0.104 |
| | 0.8 | 0.8 | 0 | 1 | 0.188 | 0.142 | | |
| | | | 1 | 2 | 0.159 | 0.110 | 0.092 | 0.127 |
| | | | 2 | 3 | 0.126 | 0.101 | 0.089 | 0.100 |
| 0.0 | 0.0 | - | - | 1 | 0.145 | 0.117 | | |
| | 0.4 | 0.8 | 0 | 1 | 0.170 | 0.164 | | |
| | | | 1 | 2 | 0.150 | 0.116 | 0.083 | 0.134 |
| | | | 2 | 3 | 0.140 | 0.114 | 0.078 | 0.094 |
| | 0.8 | 0.8 | 0 | 1 | 0.313 | 0.228 | | |
| | | | 1 | 2 | 0.204 | 0.134 | 0.092 | 0.125 |
| | | | 2 | 3 | 0.155 | 0.126 | 0.084 | 0.097 |

**Table 4:**
MAE of item parameter estimates under MAR and NONMAR model with covariates. Polytomously scored items

| Generating Values | | | Analysis | | Mean Absolute Error | | | | |
|---|---|---|---|---|---|---|---|---|---|
| $\delta$ | $\rho(\theta, \zeta)$ | $\rho(\zeta_1, \zeta_2)$ | $Q_2$ | $P$ | $\alpha$ | $\beta_1$ | $\beta_2$ | $\delta$ | $\gamma$ |
| -1.0 | 0.0 | - | - | 1 | 0.109 | 0.118 | 0.175 | | |
| | 0.4 | 0.8 | 0 | 1 | 0.139 | 0.133 | 0.212 | | |
| | | | 1 | 2 | 0.115 | 0.120 | 0.179 | 0.099 | 0.149 |
| | | | 2 | 3 | 0.108 | 0.119 | 0.177 | 0.093 | 0.103 |
| | 0.8 | 0.8 | 0 | 1 | 0.143 | 0.159 | 0.254 | | |
| | | | 1 | 2 | 0.130 | 0.125 | 0.198 | 0.103 | 0.132 |
| | | | 2 | 3 | 0.107 | 0.119 | 0.180 | 0.096 | 0.104 |
| 0.0 | 0.0 | - | - | 1 | 0.126 | 0.146 | 0.217 | | |
| | 0.4 | 0.8 | 0 | 1 | 0.166 | 0.180 | 0.309 | | |
| | | | 1 | 2 | 0.138 | 0.143 | 0.211 | 0.090 | 0.143 |
| | | | 2 | 3 | 0.128 | 0.140 | 0.211 | 0.084 | 0.101 |
| | 0.8 | 0.8 | 0 | 1 | 0.191 | 0.241 | 0.417 | | |
| | | | 1 | 2 | 0.159 | 0.148 | 0.238 | 0.089 | 0.126 |
| | | | 2 | 3 | 0.127 | 0.140 | 0.219 | 0.084 | 0.097 |

mension predicted by two covariates was used, results were *MAE(α) = 0.150* and *MAE(β) = 0.116*. Further, when two dimensions with three covariates were used as a propensity model, results obtained were *MAE(α) = 0.140* and *MAE(β) = 0.114*. An analogous pattern appeared in all conditions. It can be seen that increasing the correlation of the latent variables $\theta$ and $\zeta$, that is, increasing the violation of ignorability, resulted in more bias in the parameter estimates when the covariates are ignored. Including them reduced the bias to a value close to the baseline.

## An empirical simulation

In the previous studies, all response data were simulated under IRT models. In practice, IRT models do not perfectly fit response data. For instance, the assumptions regarding local reliability, dimensionality and subgroup invariance might not be perfectly met. Therefore, an empirical simulation was carried out to investigate the effectiveness of the method with real data. The data were collected in the LISS panel of CentERdata, a representative internet panel for Longitudinal Internet Studies in the Social sciences. The panel consisted of households which are randomly selected from the municipal registers in the Netherlands. Participants filled out questionnaires on a monthly basis. The empirical simulation was based on data from two questionnaires. The first was the Mental Health Continuum-Short Form (MHC-SF, abbreviated here to MHC) (Keyes et al., 2008, Lamers et al., 2011). The MHC consisted of 14 items measuring positive mental health on a Likert scale from 0 to 5. The Dutch version of the MHC has shown good psychometric properties (Lamers et al., 2011) and the item parameters proved stable over time (Lamers et al., 2012). The second questionnaire was the Brief Symptom Inventory (BSI; Dutch version) (de Beurs & Zitman, 2006). The 53 items pertained to psychological symptoms which are scored on Likert scales ranging from 0 to 4. The sample consisted of 1,932 adults who filled out the questionnaires on four measurement occasions in nine months. The autocorrelation between response occasions was 0.653 for the MHC and 0.663 for the BSI. The distribution of item responses across the categories for both tests was quite different. The MHC had an average score of 42.55 with a maximum possible score of 70 and all response categories attracted a substantial number of responses. For the BSI the average score was low, that is, 19.30 with a maximum possible score of 212, and most responses were in the zero categories. The reason is that the BSI assesses negative psychological symptoms ranging from depression and anxiety up to paranoia and psychoticism. Such symptoms are relatively rare in the general population.

For the simulation, missing item responses were created by removing responses from the fourth occasion. This was done as follows. The probability of an observation was determined by the posterior expected estimate of θ on the previous occasion. In one condition, constant item location parameters were used to produce proportions of missing responses of 0.30 or 0.50. In a second condition, similar proportions of missing responses were created, but in this case the item location parameters were chosen in such a way that the response propensity uniformly decreased from 0.625 on the first item to 0.375 on the last item. The first condition will be labeled the Uniform condition, while the second will be labeled the Progressive condition. The means and variances of all latent dimensions were

equal to zero and one, respectively; the correlations were equal to the autocorrelations mentioned above.

100 replications were made and the item parameter estimates obtained using the complete data were the reference values to which mean absolute errors were computed using formula (9). The models used to analyze the data are listed in Table 5. All estimates were made using MML. Model 1 in Table 5 does not include a response propensity model and is expected to produce the largest bias. Model 2 has the total scores of the previous administration as covariate for $\theta$. Model 3 has the 2PL model for the distribution of the missing data indicators but no covariates. The same held for Model 4, but here, the total

**Table 5:**
Summary of analysis models for empirical simulation

| | **Model** | **Legend** |
|---|---|---|
| 1 | $p(x\mid\theta)g(\theta)$ | Incomplete response data only. |
| 2 | $p(x\mid\theta)g(\theta\mid y_{t-1})$ | Incomplete response data with total scores of the previous administration as covariate. |
| 3 | $p(x\mid\theta)p(d\mid\zeta)g(\theta,\zeta)$ | Incomplete response data and missing data indicator. |
| 4 | $p(x\mid\theta)p(d\mid\zeta)g(\theta,\zeta\mid y_{t-1})$ | Incomplete response data and missing data indicator, with total scores of the previous administration as covariate. |
| 5 | $p(x\mid\theta)p(d\mid\zeta)g(\theta,\zeta\mid\theta_{t-1})p(x_{t-1}\mid\theta_{t-1})$ | Incomplete response data, missing data indicator, and responses on previous administration as covariate. |

**Table 6:**
**MAE** of item parameter estimates under various MAR and NONMAR models

| **Measure:** | **MHC** | | | | **BSI** | | | |
|---|---|---|---|---|---|---|---|---|
| **Pattern:** | **Uniform** | | **Progressive** | | **Uniform** | | **Progressive** | |
| **Missing:** | **30%** | **50%** | **30%** | **50%** | **30%** | **50%** | **30%** | **50%** |
| **Model** | | | | | | | | |
| 1 | 0.826 | 1.111 | 0.768 | 1.184 | 0.914 | 1.283 | 0.871 | 1.283 |
| 2 | 0.331 | 0.397 | 0.308 | 0.444 | 1.038 | 1.110 | 0.862 | 1.199 |
| 3 | 0.341 | 0.415 | 0.326 | 0.466 | 1.000 | 1.200 | 0.974 | 1.245 |
| 4 | 0.331 | 0.396 | 0.293 | 0.400 | 1.003 | 1.104 | 0.899 | 1.095 |
| 5 | 0.310 | 0.388 | 0.285 | 0.396 | 0.905 | 1.088 | 0.900 | 0.972 |

scores of the previous administration were a covariate for $\zeta$. Finally, in Model 5, the latent variable pertaining to the previous administration was a covariate for $\zeta$, while this latent variable was measured by the item response on the previous administration. Model 4 is expected to produce better results than the models 2 and 3, since this model can be seen as a combination of the two previous models. Further, Model 5 is expected to do even better. However, this expectation may be jeopardized by the increasing numbers of parameters in the models and the related increase in estimation errors.

The results are given in Table 6. For the MHC, the models 2 through 5 all created a substantial decrease in MAE from the baseline in Model 1. For instance, in the uniform condition with 30% missing data, the MAE decreased from 0.826 in Model 1 to 0.310 in Model 5. Model 3 produced the smallest decrease while Model 5 produced the highest decrease. For the MHC, this pattern was visible in all conditions. In general, the pattern was as expected: adding information decreased the effect of the violation of ignorability. Further, Model 2 was always slightly better than Model 3, so using the total score on the previous administration worked slightly better than invoking a latent variable for the pattern of missingness. Model 5 using the complete observations on the previous administration, always worked best, but overall it must be concluded that the differences between the performances of the models 2 through 4 were small.

For the BSI, the pattern was far less clear. Model 5 did produce a decrease of MAE compared to Model 1, but the decrease was small. Further, in some conditions the MAE actually increased. So here accounting for the violation of ignorability was far less successful. A tentative explanation may be related to the low average score on the BSI and the highly skewed distribution of responses across response categories with most responses in the zero categories. This seriously harms the precision of the item parameter estimates. So a conclusion here is that the effectiveness of the methods proposed here depends on the characteristics of the data.

## Discussion

This article is meant as a contribution to the growing literature on modeling nonignorable missing item responses. This field of research is very broad and therefore, this article has several limitations. First of all, the focus is on the estimates of the item parameters. This topic is of interest when the item parameters are of interest, for instance in the calibration of an item bank for a computerized adaptive test or a large-scale education survey. Equally interesting are the consequences of violation of ignorability for the estimates of the person parameters, especially when these estimates have serious consequences for the tested persons. This topic deserves further study. Further, the study was limited to the generalized partial credit model, while other models such as the graded response model (Samejima, 1969) and the sequential model (Tutz, 1990) are also often used in educational and psychological measurement. The problems addressed in this article are definitely also relevant in non-parametric IRT (Sijtsma, 1998). However, it is well known that results obtained using different models and approaches to modeling polytomously scored item responses do not produce very different results (see, for instance,

Verhelst et al.), so the approach advocated here is expected to be relevant for these models also.

The next remark pertains to the implications of the presented methods for practitioners. All computations were done by MML using public domain software (MIRT, Glas, 2010b). However, this is not essential. The computations can be also done with the much-used computer program Mplus (Muthén & Muthén, 2012) or in a Bayesian framework using the computer program OpenBugs (Lunn, Spiegelhalter, Thomas & Best, 2009). Therefore, after adding missing data indicators to the data file, practitioners can perform all needed analyses within the well-known framework of latent variable and IRT modeling. The global fit of MAR and NONMAR models can be compared using the well-known statistics such as likelihood ratio statistic and its modifications, the AIC and BIC. However, also local, item oriented fit statistics dedicatedly developed for IRT models (see, for instance, Glas, 2005) can be used to evaluate the appropriateness on the models. If adding a response propensity model, possibly with covariates, leads to a substantial improvement of model fit, taking the propensity model into account is highly recommended. A final remark pertains to the fact that the simulations presented here show that applying model-based corrections to violations of ignorability can work in data-analyses with polytomously scored items, and that the propensity models can include multidimensional IRT and observed covariates. However, what the, admittedly limited, empirical study also shows, is that there is no guarantee that the approach is always effective. A tentative conclusion drawn from the empirical simulation was that unfavorable characteristics of the data, such as poor support for parameter estimates, may seriously threaten the procedure.

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# Overview of priorities, threats, and challenges to biodiversity conservation in the southern Philippines

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ABSTRACT

Human activities have severely impacted many species and ecosystems. Thus, understanding the local biodiversity situation is crucial for implementing effective biodiversity conservation interventions. Mindanao in the southern Philippines is home to various unique species, particularly in its pristine ecosystems. However, the available biodiversity data for many terrestrial vertebrates and key areas remain incomplete. To address this issue, we synthesized published literature related to biodiversity from 2000 to 2022 in Mindanao. Moreover, this analysis used four key terrestrial vertebrates (amphibians, reptiles, birds, and mammals) in Mindanao as research objects. According to our findings, there was a significant and positive correlation between the number of biodiversity studies and species recorded. In terms of species richness, birds were the most recorded group ($n = 334$ spp.), followed by reptiles ($n = 108$ spp.), mammals ($n = 70$ spp.), and amphibians ($n = 52$ spp.). We also found that the number of endemic and threatened species varies geographically and across taxonomic groups. Yet, we discovered a significant disparity in the information available on biodiversity in different provinces of Mindanao. For example, the western provinces of Mindanao have had not records of biodiversity for more than two decades. Furthermore, we found that the changes in tree cover loss were consistent with biodiversity records, but this correlation is only significant for birds. Finally, we highlighted some critical threats and challenges to biodiversity, including deforestation, agricultural expansion, mining, and their impact on biodiversity conservation in Mindanao. Our findings suggested that biodiversity

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conservation should focus not only on areas with high levels of biodiversity but also on areas lacking biodiversity information. To do this, we call for strengthening collaboration among various institutions and digitizing and centralizing of information related to biodiversity. By gaining a deeper understanding of biodiversity in Mindanao, we can better and sustainably protect critical ecosystems in this region from the increasing threats posed by human activities.

## 1. Introduction

The decline in environmental quality is an inevitable consequence of rapid population growth and increased consumption of natural resources, which threatens global and local biodiversity at an alarming rate (Malcolm et al., 2006; Pimm et al., 2014). Balancing economic development and biodiversity protection must be achieved, but it is often challenging and requires understanding and setting biodiversity conservation priorities (Hughes, 2017a). Identifying regions with relevant biodiversity features is a primary step in crafting and strengthening existing biodiversity conservation frameworks (Benayas and Montaña, 2003). However, now global biodiversity conservation priorities are often too broad to be effectively implemented at the national and local levels (Mace et al., 2010; Zhu et al., 2021). Additionally, the current economic situation and the lack of capacity to implement these priorities locally pose a challenge to their implementation (Sarvajayakesavalu, 2015). Considering the significant differences in threat levels, response measures, and government capabilities in different places, it is necessary to develop adaptive biodiversity conservation priorities (Tanalgo and Hughes, 2018, 2019). One of the crucial steps in developing biodiversity conservation priorities at the regional or local scale is to understand its status (e.g., species richness and occurrence) and identify biodiversity hotspots, threatening processes, research directions, and existing biodiversity policies (Cannon et al., 2007). Consolidation of available and accessible biodiversity information from various networks and literature is the basis for establishing conservation goals and programs (Cannon et al., 2007; Di Minin et al., 2017). However, many biodiversity data are still unpublished and incomplete, particularly in developing countries (Troudet et al., 2017; Tantipisanuh and Gale, 2018) such as the Philippines.

The Philippines is a tropical country with biodiversity, characterized by its pristine rainforest and archipelagic environment (Heaney and Regalado, 1998). It is considered as one of the wealthiest countries in Southeast Asia in terms of the high number of endemic and unique species (Heaney and Regalado, 1998; Posa and Sodhi, 2006; Boquet, 2017). However, the country faces many anthropogenic threats (Posa and Sodhi, 2006; Mallari et al., 2016). The decline in forest area caused by logging and agricultural expansion has led to a dramatic change in land cover, especially in the lowlands in the Philippines (Apan et al., 2017). In contrast, approximately 61.00% of the land in the Philippines is devoted to agriculture, contributing to at least 9.00% of the Philippines' Gross Domestic Product in 2018 (Statista, 2020). The 2021 Philippine Forest Management Bureau (FMB) published that the remaining forest cover in the Philippines in 2020 was $7.23 \times 10^4$ $hm^2$, which is equivalent to 24.09% of the total land area of the Philippines (FMB, 2021).

The threatened species of the Philippines are widely distributed among its more than 7000 islands. Mindanao covers at least 34.00% of the country's total land area of 300,000 $km^2$, with six economic regions, 24 provinces, 14 cities, and 400 towns (PhilAtlas, 2022). There are numerous key important areas for biodiversity conservation in Mindanao, and they share a high level of endemism and have several threatened species. For example, Mount Apo Natural Park (2954–3144 m a.s.l.), the Philippines's highest mountain, is in Mindanao and is a critical home range for the critically endangered Philippine Eagle (*Pithecophaga jefferyi*) (Molina, 2020). Mount Kitanglad in northeastern Mindanao, the second highest peak in the Philippines, also has several endemic species, including *Limnomys* sp., a Mindanao-only rodent genus (Rickart et al., 2003).

Meanwhile, some species occur only in a single region in Mindanao, such as Comb-crested Jacana (*Irediparra gallinacea*), which is endemic to the Liguasan Marsh, a game refuge and bird sanctuary in southcentral Mindanao. Moreover, Agusan Marsh is a habitat for more than 200 species of migratory birds from China, Russia, and Japan. Both wetlands are important habitats for the endangered Philippine crocodile (*Crocodylus mindorensis*) and the estuarine crocodile (*C. porosus*) (Hinlo et al., 2014). Agusan Marsh was the largest home of captive salt crocodiles in the world, which spanned up to 6.17 m. Despite these wetland ecosystems and natural resources are abundant, threats and disturbances always exist (Hinlo et al., 2014; Sarmiento, 2021). Other important protected areas in Mindanao include the Protected Landscape and Seascapes of the Siargao Islands and the Turtle Islands Wildlife Sanctuary. The Siargao Islands comprise the largest contiguous mangrove area in the Philippines and are home to 14 endemic species of mammals, 55 endemic species of birds, and nine endemic species of frogs (Key Biodiversity Areas Partnership, 2023). The Turtle Islands in the southwest Philippines is an important nesting site for the green sea turtle (*Chelonia mydas*) and the hawksbill turtle (*Eretmochelys imbricata*) (Senga, 2001).

Mindanao is the second largest group of islands in the Philippines after Luzon, facing challenges in limited research and funding for biodiversity (Amoroso, 2000; Alcala, 2004). While some exploration of protected areas in Mindanao has been conducted in the past, comprehensive studies have not been carried out in several regions and habitats of Mindanao in recent years. Furthermore, information on species distribution and the extent of threats to biodiversity is still limited (Amoroso, 2000; Alcala, 2004; Tanalgo et al., 2019). This study aims to gain a comprehensive understanding of the status and trends of biodiversity in Mindanao and identify the priority areas for conservation. Specifically, we aimed to identify important trends and gaps in biodiversity information from 2000 to 2022, evaluate the conservation status and endemism of key terrestrial vertebrates, and examine their relationship with provincial-level tree cover loss.

Additionally, our goal was to analyze the major and potential threats to terrestrial biodiversity in Mindanao. Our findings presented here will provide a reference for biodiversity research in Mindanao and help develop effective conservation measures. In addition, our study is significant as it involves a crucial area for biodiversity conservation in the Philippines. Furthermore, the framework we developed has the potential to be applied to other regions of the Philippines, providing a valuable tool for evaluating and understanding

the status of biodiversity. This framework would facilitate comparative analyses for biodiversity scientists and aid in identifying biodiversity conservation priorities across the country.

## 2. Materials and methods

We extensively explored the literature related to biodiversity in Mindanao published from 2000 to 2022, using Google Scholar (https://scholar.google.com.ph/) and self-archiving ResearchGate (https://www.researchgate.net/). This analysis focused on four key terrestrial vertebrates (amphibians, reptiles, birds, and mammals) as biodiversity proxies. We used the following keywords and their combinations to search for published literature: 'Mindanao', 'Wildlife', 'Fauna', 'Biodiversity', 'Mountain*', 'Surveys', 'Protected areas and key biodiversity areas (KBA)', 'Long-Term Ecological Research (LTER)', 'Protected Areas', and 'Key Biodiversity Areas'.

We filtered and classified the downloaded literature according to relevance using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) procedure (McInnes et al., 2018). We reviewed the abstract, which only included biodiversity studies focusing on terrestrial vertebrates in Mindanao. We excluded studies that exclusively dealt with laboratory experiments, domesticated wildlife, and review articles. All search results were reduced and classified twice for relevance. The first dataset from the published literature was checked and cross-referenced. We then curated all information, such as taxonomic groups, scientific names, endemism, conservation status, geographical distribution, and location, and noted threats following the research of Tanalgo and Hughes (2018). Then we classified all literature based on research themes, diversity, taxonomy and systematics, ecology, wildlife disease, and conservation (see Tanalgo and Hughes (2018) for a detailed description of each theme). We counted those studies separately with overlapping themes. Then we classified based on four major terrestrial vertebrates (e.g., amphibians, reptiles, birds, and mammals) and locality. We assessed species conservation status and endemism using the International Union for the Conservation of Nature (version 2022–1) (IUCN Red List, 2022).

We used a simple linear regression to analyze the spatiotemporal trends of biodiversity research in Mindanao from 2000 to 2022. Subsequently, we used the non-parametric Kruskal-Wallis test to determine the differences in the number of biodiversity studies and species recorded according to groups of terrestrial vertebrates. We then identified key potential threats in Mindanao based on the database we built. Initially, we identified habitat loss and modification as the primary threats to species and ecosystems. To determine the extent of tree cover loss in various provinces and key biodiversity hotspots in Mindanao, we extracted data on tree cover loss from Global Forest Watch from 2000 to 2021 (Global Forest Watch, 2022). We used Kendall's Tau-b to determine the correlation between the number of species recorded per province and tree cover loss, thereby determining the relationship between threat and biodiversity.

We conducted all our statistical tests and visualization using open-source Jamovi software (Jamovi project team, Sydney, Australia) and GraphPad Prism (GraphPad Software company, Boston, USA), respectively. All statistical significance was set at $P < 0.05$ level.

## 3. Results and discussion

### 3.1. Trends of biodiversity in Mindanao

A total of 99 published literature was collated from 2000 to 2022. We found that numerous studies have overlapped thematic classifications and taxonomic records. Moreover, most of the studies are in the areas of 'diversity' (mean value = 5.7/a) and are followed by studies related to 'conservation' (mean value = 1.4/a), with very few studies focused on 'wildlife disease' and 'ecology' (Fig. 1) (Kruskal-Wallis: H = 16.180, $df = 4$, $P = 0.003$).

In terms of the number of biodiversity studies per taxonomic group, there are 36 researches for amphibians, 26 for reptiles, 39 for birds, and 30 for mammals (Fig. 2) (Kruskal-Wallis: H = 0.391, $df = 3$, $P = 0.954$). According to our findings, there was a significant and

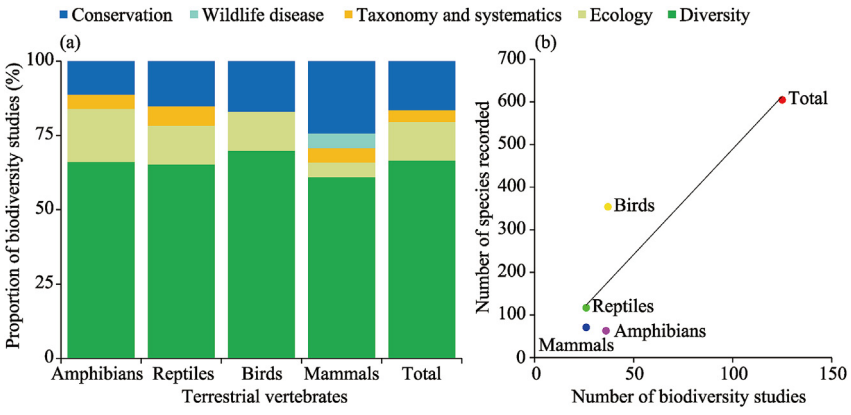



**Fig. 1.** Proportion of four major terrestrial vertebrates (amphibians, reptiles, birds, and mammals) (a) and the relationship between the number of biodiversity studies and species recorded in Mindanao from 2000 to 2022 (b).

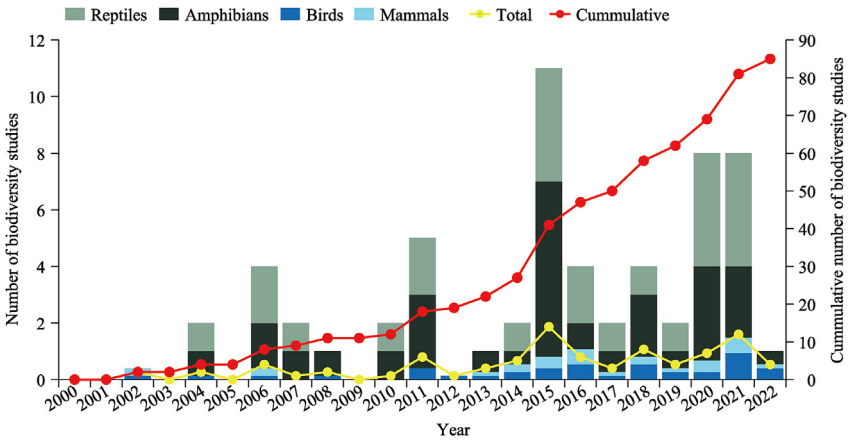



**Fig. 2.** The number of biodiversity studies and the variation trend of the four major terrestrial vertebrates (amphibians, reptiles, birds, and mammals) in Mindanao from 2000 to 2022.

positive correlation between the number of biodiversity studies and species recorded ($R^2 = 0.792$, $P = 0.043$) (Fig. 1b). The annual trend in the number of total terrestrial vertebrates (amphibians, reptiles, birds, and mammals) varied, peaking only in 2015 and 2021 (Fig. 2). Notably, there has been a positive increase in the total number of biodiversity studies across all terrestrial vertebrates (amphibians: $R^2 = 0.295$, $P = 0.007$; reptiles: $R^2 = 0.268$, $P = 0.011$; birds: $R^2 = 0.334$, $P = 0.004$; mammals: $R^2 = 0.338$, $P = 0.004$). Moreover, we observed a strong and significant correlation between the number of studies and species recorded in four terrestrial vertebrates (Table 1).

Although biodiversity studies in Mindanao remained relatively low (mean value = 4/a), we recorded 609 terrestrial vertebrate species based on published literature in Mindanao from 2000 to 2022. In terms of species richness, birds were the most recorded group ($n = 334$ spp.), followed by reptiles ($n = 108$ spp.), mammals ($n = 70$ spp.), and amphibians ($n = 52$ spp.) (Fig. 3). Moreover, we found a significant difference in the means of species recorded per year between terrestrial vertebrates (Kruskal-Wallis: $H = 11.038$, $df = 4$, $P = 0.026$) from 2000 to 2022.

A total of 565 species of terrestrial vertebrates were recorded from published literature from 2000 to 2022. We found reasonably high endemism in the four combined terrestrial vertebrates (total species: 49.00%; $n$=276 spp.) (Fig. 4a). The highest proportion of endemism within terrestrial vertebrates was recorded in amphibians (62.00%; $n$=33 spp.), followed by reptiles (58.00%; $n$=63 spp.), birds (46.00%; $n$=152 spp.), and mammals (40.00%; $n$=28 spp.). In terms of conservation status, 13.00% of all species are in the threatened status. Mammals and reptiles topped the highest proportion of endangered species (23.00% and 15.00%, respectively) (Fig. 4b), while 88.00% of the total species were in the least concerned species.

Biodiversity patterns in Mindanao were mainly concentrated in biodiversity hotspots and within intact habitats (e.g., Key Biodiversity Area, but this could be associated with the fact that research efforts are skewed towards these sites. Among these sites, Agusan del Sur Province recorded the highest number of species, followed by the North Cotabato and Agusan del Sur provinces (Fig. 5a). However, the Bangsamoro Autonomous Region in Muslim Mindanao (BARMM), formerly the Autonomous Region in Muslim Mindanao (ARMM), and most of the areas in the Zamboanga Peninsula in western Mindanao lacked biodiversity records for more than 20 years (Fig. 5a), so they are identified as key priority regions in terms of future biodiversity research and conservation.

We quantified the tree cover loss in different provinces of Mindanao to represent the environmental status of each province and then studied the relationship between tree cover loss and the number of species recorded per province. Only 19 (79.00%) provinces in Mindanao have had terrestrial vertebrate biodiversity data recorded since 2000 (Fig. 5a). Furthermore, we found that the changes in tree cover loss were consistent with biodiversity records (i.e., the number of species recorded), but this correlation is only significant for birds (Fig. 5b and c). Mammals showed fewer records than other terrestrial vertebrates, which may be related to researchers' low detectability and investigation of terrestrial vertebrates.

**Table 1**
Kendall's Tau-b correlation coefficient for species recorded in four major terrestrial vertebrates.

| Terrestrial vertebrates | Amphibians | Reptiles | Birds | Mammals |
|---|---|---|---|---|
| Amphibians | – | – | – | – |
| Reptiles | 0.845 | – | – | – |
| Birds | 0.670 | 0.526 | – | – |
| Mammals | 0.589 | 0.548 | 0.676 | – |

Note: All values are significant at $P < 0.05$ level. "-" means no data.

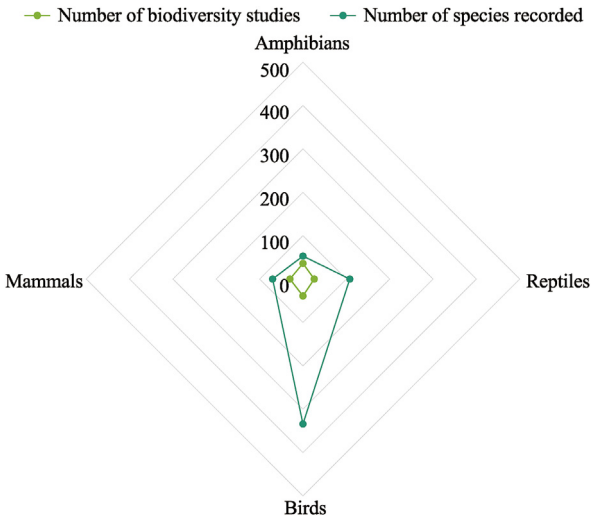



**Fig. 3.** Number of biodiversity studies and species recorded in four major terrestrial vertebrates (amphibians, reptiles, birds, and mammals) in Mindanao from 2000 to 2022.

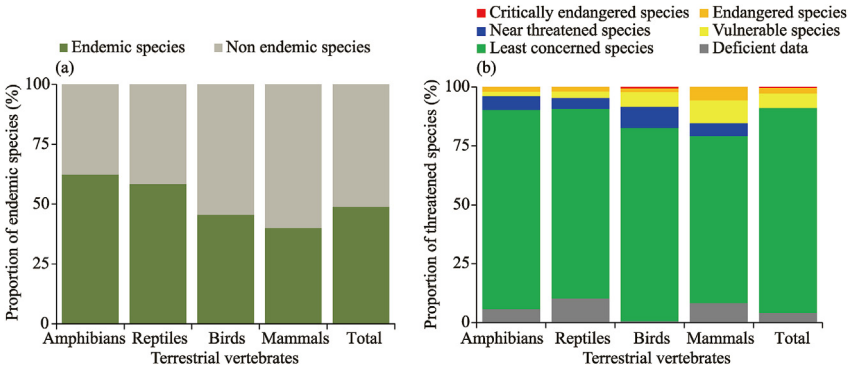



**Fig. 4.** Proportion of endemic species (a) and threatened species (b) of four major terrestrial vertebrates (amphibians, reptiles, birds, and mammals) in Mindanao from 2000 to 2022.

### *3.2. Threats and challenges of biodiversity in mindanao*

#### *3.2.1. Deforestation and habitat loss*

In Mindanao, the total tree cover loss was approximately 5.11 $\times$ $10^6$ $hm^2$ from 2000 to 2021 (Global Forest Watch, 2022) (Fig. 5b). An immense problem facing local farmers and indigenous people today is illegal logging (Shively and Pagiola, 2004; Suarez and Sajise, 2010). Massive illegal logging practices were reported despite the government's striving to ban wood harvesting (Vitug, 1998; Lasco et al., 2001; Reboredo, 2013). In Mindanao, the loss of tree cover is more significant in the northeast region than in other areas due to deforestation, agricultural expansion, and mining in these areas. In this study, we found that the highest loss of tree cover occurs in regions where the species recorded is also high, especially those areas inhabited by amphibians, reptiles, and birds (Fig. 5). The effects of deforestation on the number of species in Mindanao are poorly studied, and even understanding how species respond to deforestation is limited for many terrestrial vertebrates in the Philippines, for example, bats (Tanalgo and Hughes, 2019). In some local-scale bird studies, Tanalgo et al. (2019) found that species and functional diversity decreased in southern Mindanao, where the habitat is less intact or degraded. This suggested that endemic and specialist species prefer areas with more intact forests than areas with high disturbance and low vegetation (Maala, 2001; Achondo et al., 2014). Although wildlife species are likely to decrease in highly modified areas, Quibod et al. (2021) found some important species may continue to thrive in some fragmented sites.

#### *3.2.2. Agricultural development and expansion*

The increase in demand for food production has put tremendous pressures on terrestrial biodiversity (Wilcove et al., 2013; Hughes, 2017b). Deforestation and land conversion lead to changes in ecosystems and loss of biodiversity (Dudley and Alexander, 2017). For example, slash-and-burn agriculture, locally known as '*kaingin*', is a significant land use type, especially in the highlands of underdeveloped tropical countries (Mukul et al., 2016), including Mindanao. The degree of shifting cultivation and previous land use patterns

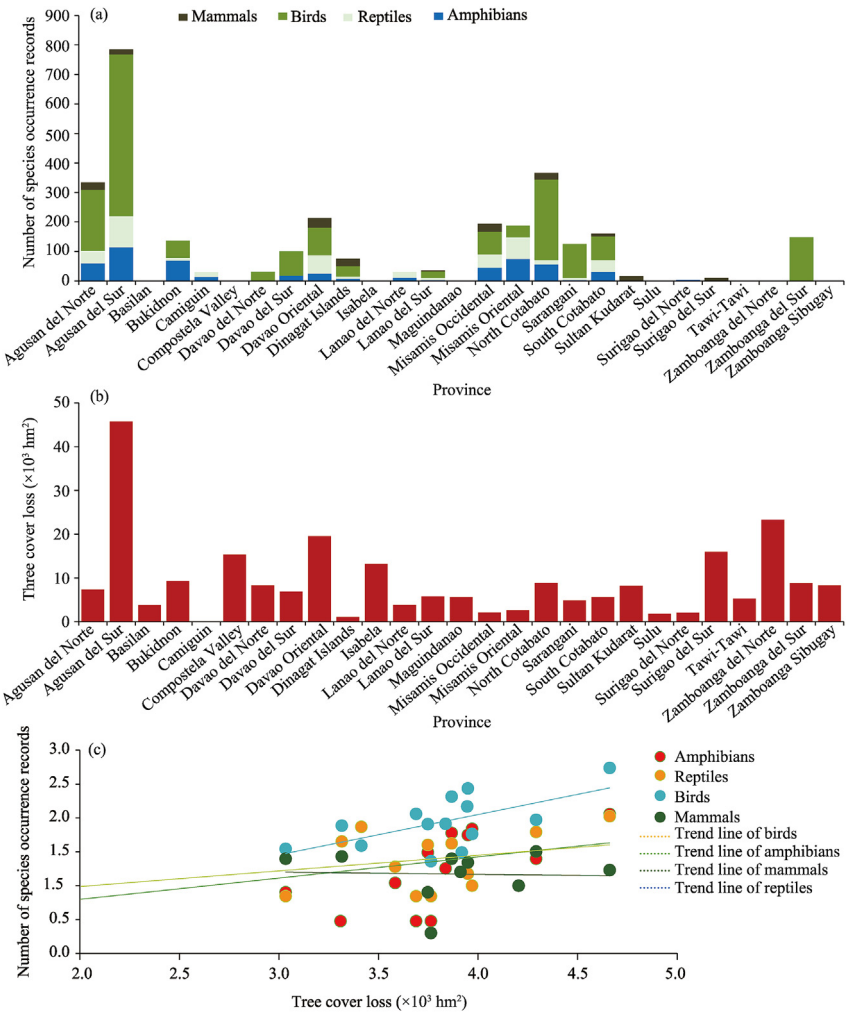



**Fig. 5.** Spatial distribution of four major terrestrial vertebrates (amphibians, reptiles, birds, and mammals) (a), tree cover loss (b), and the relationship between tree cover loss and number of species occurrence records (c) in Mindanao from 2000 to 2022.

can affect the ability of tropical forests to regenerate (Jakovac et al., 2015). It may take more than five years for woody species to become prominent secondary woods after rotation (Raharimalala et al., 2010).

The Philippine economy is growing, and agricultural production is expected to expand in the next decade (Ortiz and Torres, 2020), particularly in Mindanao due to its rich soils and available arable land areas. Mindanao is the leading production region of agricultural and raw materials in the Philippines, such as corn (*Zea mays*), durian (*Durio* sp.), coconut (*Cocos nucifera*), mango (*Mangifera indica*), and rice (*Oryza sativa*) (Varela et al., 2013; Dejarme-Calalang et al., 2015). The soils and climate of Mindanao are suitable for many monoculture plantations, including the oil palm plantations (*Elaeis guineensis*). Among the eight central oil palm plantations in the Philippines, six are in Mindanao. Oil palm plantations have produced approximately $7.70 \times 10^7$ kg annually from 2000 to 2018 (Index Mundi, 2018). The most extensive oil palm plantations, estimated to be around $9.00 \times 10^4$ hm$^2$, are concentrated in the Caraga and Soccsksargen regions (Varela et al., 2013; Batugal, 2014) and are predicted to increase drastically over the next few years (Tanalgo et al., 2019). Similar to oil palm plantation, rubber plantations (*Hevea brasiliensis*) are also expanding, covering at least $2.19 \times 10^5$ hm$^2$ (in 2015), particularly in western Mindanao and Soccsksargen (Tanalgo and Hughes, 2019). Banana (*Musa* sp.) and pineapple (*Ananas* sp.) plantations are also currently expanding in Mindanao. Recent spatial analysis of about 250 banana and pineapple plantations in Mindanao and its surrounding impact zones revealed an overlap with at least 83 important habitats of flora and fauna habitats threatened and affected by intensive agriculture (Ortiz and Torres, 2020).

It is increasingly recognized that agricultural areas are possible temporary habitats for wildlife in Mindanao (Sanguila et al., 2016). However, there is still limited information on the effects of agriculture on wildlife in the Philippines, particularly in Mindanao. Although there are several studies on biodiversity within agricultural areas, for example, Agduma et al. (2011) showed that rubber plantations can promote the growth of some native vegetation valuable to wildlife in southern Mindanao. A study of bird communities between rubber and oil palm plantations also showed that the former could have higher species richness and endemism compared to the latter (Achondo et al., 2011), but the diversity of both plantations was still lower than in heterogeneous and intact habitats such as protected areas and

restored sites (Achondo et al., 2014; Tanalgo et al., 2019). In a similar case, long-term monitoring of fruit bats in southern Mindanao showed a significant difference in the ability of oil palm and rubber plantations to promote the growth of common species (Tanalgo et al., 2021). The abundance of bats and more specialized species is significantly higher in rubber plantations compared to oil palm plantations. This could be associated with taller and more diverse vegetation in rubber plantations (Agduma et al., 2011).

#### 3.2.3. Mining and extraction industry

Mining affects natural ecosystems on multiple spatial scales. For example, surface mining can remove topsoil and vegetation (Wickham et al., 2013) and lead to habitat degradation on a local scale (Naeem et al., 1999). Mindanao holds over a third of the country's mineral reserves, 50.00% of gold and 65.00% of nickel (Bautista, 2016; Paz et al., 2016). The Caraga recorded gross mineral sales of $1.33 \times 10^9$ USD in 2020 and $1.44 \times 10^9$ USD in 2021 (Jocson, 2022). On the other hand, Tampakan in South Cotabato has the most significant untapped copper-gold reserve in Southeast Asia. There is a value of $5.90 \times 10^9$ USD copper and gold mining project stalled in Tampakan, which is one of the largest projects of its kind in the world. Once developed, the average yield can reach $3.75 \times 10^8$ kg of copper and 289.00 kg of gold (Sarmiento, 2022). Other key mining areas in Mindanao are T'boli, South Cotabato, Bagumbayan, Sultan Kudarat, Gango, Libona, Bukidnon, Tumpagon and Pigsag-an, Cagayan de Oro City, Nangcaon, Opol, Misamis Oriental, Rogongon, and Iligan City (Canencia et al., 2015; Paz et al., 2016).

The Department of Environment and Natural Resources of the Philippines recently identified $9.00 \times 10^8$ $hm^2$ of potential mining areas (Recuenco, 2022), most of which have intact forests, and mining may damage the biodiversity of these areas. Tampakan had $18.60 \times 10^3$ $hm^2$ of natural forest in 2010, extending over 63.00% of its land area, but it experienced a net change of 766.00 $hm^2$ (−2.70%) of tree cover loss from 2000 to 2020. T'boli in South Cotabato experienced a net change of 925.00 $hm^2$ (−1.60%) of its tree cover loss, while Libona in Bukidnon had $1.88 \times 10^2$ $hm^2$ of tree cover loss, equivalent to a 2.00% decrease in tree cover loss since 2000. Cagayan de Oro City, on the other hand, experienced a net change of 351.00 $hm^2$ (−1.20%) in tree cover loss from 2000 to 2020, while Opol, Misamis Oriental, experienced a net variation of 142.00 $hm^2$ (2.00%) in tree cover (Global Forest Watch, 2022).

Although mining was a significant driver of biodiversity loss, previous studies showed that it could also promote an increase in the number of various species, but mainly common species and a few species dependent on forest (Paz et al., 2016). Profiling of terrestrial biodiversity across multiple mining areas in Mindanao identified 11 critically endangered flora, 13 endangered flora and fauna, and 58 vulnerable species in Caraga, Soccsksargen, and northern Mindanao (Paz et al., 2016). Furthermore, some identified threatened and endemic birds preferred mature forests, which required immediate conservation of these forests in these mining areas (Paz et al., 2016). Despite restoration attempts and a push for sustainable mining in Mindanao (Manila Standards, 2020; BMB-DENR, 2022), the adverse effects of mining on biodiversity and habitats were irreversible (Tropek et al., 2012; Sonter et al., 2018).

### 3.3. Role of indigenous peoples in biodiversity conservation of mindanao

Conservation of biological resources has received a lot of attention (Xu et al., 2021; Zhu et al., 2021). Indigenous peoples are the primary stewards of the natural environment and play a vital role in safeguarding biodiversity (Ogar et al., 2020). At least a quarter of the land area on Earth is occupied and inhabited by indigenous peoples (Sneed, 2019). Indigenous peoples live in areas where forest integrity is well-protected or highly protected (Grantham, 2022). The vital role of indigenous peoples in preserving and restoring biodiversity in different regions of the world has been well demonstrated (Ogar et al., 2020). For example, 30.00% of the primates and 71.00% of the species live on indigenous peoples' lands (Estrada et al., 2022). Furthermore, over 25.00% of Amazon bats overlap with the areas where indigenous peoples live (Fernández-Llamazares et al., 2021).

Approximately 85.00% of key biodiversity areas in the Philippines are embedded within indigenous peoples' lands (Global Environment Facility, 2019). In Mindanao, about 18–25 indigenous groups (i.e., Lumad ethnolinguistic groups) (Clariza, 2022) mostly thrive in areas with high biodiversity. The indigenous peoples of Mindanao believed that wildlife and the natural environment are part of their culture and lifestyle (Buenavista et al., 2022). Therefore, academics and non-governmental organizations often study biodiversity in indigenous peoples' lands (Manuel et al., 2021; Buenavista et al., 2022). For example, Buenavista et al. (2022) studied the ethnobotanical practices of the Higaonon tribe and obtained valuable information on wild food plants that are potential alternatives but are often neglected.

Similarly, Nuneza et al. (2021) revealed various flora with ethnomedicinal values from the Mamawa tribe in Surigao del Norte and Agusan del Norte provinces, suggesting the important role of indigenous peoples in conveying knowledge of biodiversity. In Mount Apo Natural Park, populations of endemic brown deer (*Rusa marianna)* within the ancestral domains are widely protected by indigenous peoples, suggesting the importance of indigenous peoples in the preservation of biodiversity (Villegas et al., 2022). The indigenous peoples of Mount Apo Natural Park considered that wildlife and forests are sacred. Therefore, they must protect the species and their habitats (Villegas et al., 2022). Although there are some reports of hunting species, such as flying foxes, wild boars, and large reptiles, they only hunt for subsistence and cultural practices, which is allowed by law in the Philippine government. Indigenous peoples are also crucial to protecting flagship species; for example, environmentalists collaborate with residents to protect the famous Philippine eagle within its range of activities (Salvador and Ibanez, 2006; Panopio et al., 2021).

Indigenous peoples, governments, and non-government have taken some encouraging measures, such as integrating community planning with current goals for community development, cultural reaffirmation, and environmental preservation. In Pangasananan, Bislig City, Surigao del Sur Province, Caraga, the Manobo tribes participated in the Community Conservation Plan (CCP), which aims to promote the reforestation of denuded areas and water sources areas and increase biodiversity protection in these regions. Indigenous people are also sought as Bagani or wildlife enforcement officers (Indigenous Peoples and Local Communities Conserved Territories and Areas (ICCA) ICCA Registry, 2019). Initiatives such as ICCA aimed to promote and maintain the leadership of indigenous peoples in the

conservation of critical habitats and prevent the cultural development and industrialization of these critical areas from being affected (Global Environment Facility, 2019). In the Liguasan Marsh, most fishermen followed traditional fishing and farming practices, emphasizing sustainability and considering the wetlands as a sacred site, which helps prevent overexploitation. The indigenous laws strictly prohibited electric and chemical fishing and the catching of young fish to protect them. The heads of local neighborhoods, traditional religious leaders, and Bangsamoro mujahideen leaders were able to work harmoniously despite their diverse backgrounds, which helps to maintain peace. Magindanawan Bangsamoro held exclusive control over the access and utilization of the marshes, demonstrating their dominance in this area (Sinolinding and Porciuncula, 2005). Due to the relationship between conservation values and the promotion of wildlife conservation (Low et al., 2023), understanding and preservation of indigenous beliefs should be prioritized.

In addition to these situations, there was ample evidence to suggest that indigenous peoples have contributed to the protection of biodiversity in Mindanao and the entire Philippines. However, many indigenous peoples are concerned about the ongoing degradation of forests and face difficulties due to the rapidly changing environment. For example, the Teduray tribes of Upi, Maguindanao, were severely affected by the El Nino phenomenon in 2016, particularly in agriculture (Greenpeace, 2016). But the most worrying issue for indigenous peoples in Mindanao is that they are forced to leave their ancestral territories. To protect already declining biodiversity and achieve genuine sustainability in Mindanao, protecting various indigenous peoples and their lands (i.e., where biodiversity is concentrated) should be one of the priorities in environmental policy-making in the country (Sneed, 2019). In addition, it is crucial to involve indigenous people just and equally, in conjunction with conservation biologists and ecologists, to enhance our knowledge and appreciation of biodiversity in Mindanao.

### *3.4. Advancing biodiversity research in mindanao*

Our study showed several gaps and inequities in biodiversity research in Mindanao, which provides an opportunity to address them. An important step forward is to make biodiversity information publicly available (Guerrero et al., 2021). Much valuable biodiversity data have not yet been published and were often kept in many academic and research institutions (Tantipisanuh and Gale, 2018; Guerrero, 2020; Khapugin and Silaeva, 2020). One way to address the inequities of biodiversity research in Mindanao is to emphasize the crucial role of these academic and research institutions in unravelling biodiversity information. Expanding biodiversity research and paying more focus on ecological conservation rather than simple and rapid inventory is an important first move toward advancing biodiversity conservation in Mindanao. To achieve this, academic institutions must establish fair collaboration and equitable partnerships, complement, and strengthen their research capabilities, and promote further exploration by sharing research results. Many academic institutions in the Philippines lack support for open-access journals, which is another challenge in accessing biodiversity information. Support for this should be one of the measures that academic institutions need to take. Moreover, a facility of centralized biodiversity data is needed to record and monitor the information on species and habitats within Mindanao. This information can be used for ecological research to formulate biodiversity-related policies.

## 4. Conclusions and recommendations

Biodiversity studies are sparse in the Philippines, especially for certain terrestrial vertebrates, habitats, and regions (such as Mindanao). Understanding biodiversity trends and gaps is crucial in designing effective intervention measures, which should be carried out at various scales, ideally locally, to reflect the local situation and respond to environmental changes. Our current analysis showed apparent trends and knowledge gaps in biodiversity that require careful attention. We used species recorded in key terrestrial vertebrates at the taxonomic and species levels. Although this study was limited to these groups, they effectively indicated the biodiversity in Mindanao. We found that most researches from 2000 to 2022 have exceedingly focused and were limited to community composition and diversity studies with very scarce information on the habitats and numbers of species, e.g., how species respond to the changing environment and how species interact with the environment. Additionally, when developing biodiversity conservation priorities, it should include those endemic and threatened species and those understudied species. At the spatial level, priorities for future biodiversity research and funding in Mindanao should include more provinces and areas with poor biodiversity records, especially in the western part of Mindanao. Furthermore, some protected areas in Mindanao, although there have been previously known studies, have limited published information (e.g., Mt. Apo Natural Park).

A better understanding of biodiversity in Mindanao showed that we can better safeguard critical ecosystems, especially those threatened by direct human activities and habitat loss, which are currently expanding to respond to growing populations. However, our understanding of how these threats affect the biodiversity of Mindanao also remains limited and requires more attention and work. More importantly, actively involving indigenous peoples and communities around protected areas to strengthen biodiversity conservation is crucial to maintaining biodiversity integrity and improving ecosystem services in Mindanao.

The approach we developed in this work would contribute to the assessment and understanding of biodiversity in other regions of the Philippines. This study would ultimately help to achieve the goals of the Post-2020 Global Biodiversity Framework.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Some Biases in Likert Scaling Usage and its Correction

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**Abstract**

In this paper, common biases or errors in the construction of intervals under the Likert scaling methodology in both odd and even scales are shown. Examples of researches that uses this scales especially in survey that requires perception response from the respondents will be presented, discussed and analyzed for possible biases. Suggestions for the corrections of these biases are presented in order to minimize the bias leading for a better labeling and interpretations of the results.

***Keywords:*** Bias; difference; even; Likert scale; odd; scoring.

## 1. Introduction

Likert scaling is a psychometric concept commonly used in survey research that uses guide questionnaires that need responses in scales from the subject or respondents of interest. It is a widely used scale in many field of discipline most particularly in the social science research like in education and psychology. It is a scaling method in which a statement can be responded positively or negatively. This scaling was invented by a psychologist named Rensis Likert in 1932, whose purpose is to look for an effective and efficient means of describing attitudes of humans and its influences that affected them. Likert developed that scale that carried his name until this present time. This scale is also considered a rating scale. As an example, if a respondent wanted to respond a Likert questionnaire item, this respondent specifies a level of agreement or an order/ranking to a particular statement which can be describe in a description which in turn has an equivalent numerical values. Likert scale has many uses in psychology like on the study of behavior, marketing surveys as to the preferences of a product, education, medicine, nursing, finance, engineering and human study and the likes [1, 4, 8, 15].

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Further, it is used either as a total score that is when the scale is sum up for all items and in this case the result produced an index, this is used most commonly in psychology researches or a single ordinal score that is common among survey research like in marketing and business where they need to have an exact rank of a certain product. If a statement being ask on which a person need to respond of any criteria whether subjective or objective criteria, that statement is called a Likert item. Generally, Likert scaling is a measure on the level of say agreement or a disagreement on that particular statement.

Most social science researchers preferred to use response categories that are on odd scale (example three, five, seven or nine) because they are interested the scenario in the middle response. Further, psychometricians preferred to used seven and nine response categories [3] for reasons that they can stretch the interval on which they can describe the respondent's preference or view. Most common practiced on the other hand used five response categories while others used seven response categories, however according to reference [5] in his recent empirical study, the use of five or seven response categories produced slightly mean scores higher relatively to a possible maximum score and their difference is significant. Others researchers preferred to use the response categories that are even (example 4,6, 8 or 10), which are termed force-number Likert scale in some literature for some reasons that they want to escape the in between scenario of response scale like the three (neutral/undecided) in the five Likert scale. Most common in this case is the scale that have four response categories. Other data characteristics on the use of Liker scales shows a very little difference among the scale formats in terms of variation about the mean, skewness or kurtosis.

Among researchers, some controversies and confusion sometimes arises due to some several causes on the use of Likert scaling. Hence, an elaboration is needed. First is the treatment of the scale whether it is an ordinal scale or a measurement scale (ratio or interval) [13, 16]. This sometimes need an explanation and perhaps requires an assumption so the method applied is valid and consistent. In this study we include this an assumption so to avoid debates and confusion. Second question is the existence of central tendency bias due to the subject's avoidance of extreme response choice that is most respondents tend to go to the middle to escape form the outlying responses. Third is the presence of acquiescence bias that is an agreement with the given statements as presented, well this to refrain controversy. Fourth question is known as the social desirability bias that is putting a subjects or their group or establishments in a more favorable way hence the resulting conclusion is questionable due to this kind of error. The thought of designing a balance keying (an equal number of positive and negative statements) is always a challenge in the choice of selecting a scale that can minimize those biases and problems that may appears. Reference [12] also mentioned on the criticism among reviewers on the application of statistical methods which is not appropriate when dealings with Likert scales. Some of the criticism are well founded. For instance, violations to the assumptions of the use of some of parametric methods for example the analysis of variance, regression analysis and correlations which requires the normality assumptions on the distributions of the data or per see the appropriateness of using parametric statistical methods for Likert scaling. However, reference [12] argued that many studies since the 1930s consistently show that parametric studies can handle with respect to violations of these assumptions. Thus, those claims of inappropriateness are unfounded according to him hence, parametric methods can still be utilized without the concern of answers being invalidated. In this study, we do not attempt to apply any parametric statistical method but to do some trial and error method to further improve the method introduced by reference [13] that is to

enhanced specifically the use of Likert scaling for both even and odd number categories as well as its final equivalent description and interpretations. In this study, the researcher will illustrate in practice, for example the format of a typical five- point level Likert item on agreement coded with a numerical notation and a description legend being labeled. For example, we agree assigning 1 to strongly disagree, 2 to disagree, 3 to neither agree nor disagree (undecided or neutral), 4 to agree and 5 to strongly agree.

### *1.1 Limitation of the Study*

This study probably is applicable only to those study that uses instruments that needs responses in scales either in an ordinal or maybe a measurement (interval or ratio) scales. The study assumes that the data in an ordinal scale can be considered to be in continuous scale that is either in interval or in ratio scale so that labeling a weighted mean (which is considered continuous) to its corresponding verbal description after it was computed is valid.

## 2. Methodology

### *2.1 Scoring and analysis*

If items in a survey questionnaire instrument is responded using a Likert scale, each item maybe in two ways first it is analyzed separately given the response scale of the respondents or responses is totaled to create a score for a group of items. This is usually a common practice in some psychology and educational researches (the score is considered an index). One issue being raised is whether Likert items is an ordinal data or an interval data. This is always a subject of disagreement among researcher and users of this scale. In most practice however, they are considered ordinal or interval level data depending on the assumptions being considered. In this study we consider this as an assumption. Whether individual Likert items be considered as ordinal level data, many researchers regard such items only as an ordinal data because, in a special case when using only five levels, one cannot assume that respondents perceive all pairs of adjacent levels as equidistant. On the other hand, often the wording of response levels clearly considered both directions (symmetry) of its response levels assuming there is a middle category at. In that situation such item would fall between ordinal and an interval level measurement since treating the item as just merely ordinal, we would lose information. In the case of a scale where we consider an equal spacing of its response levels, we can immediately treat this case as an interval level data which we can argue is more strongly. Now, if we consider Likert responses an ordinal data, This responses can be displayed in a graph particularly bar charts or can describe in a median or mode, ,used as a center but not the average, or the variability using the range across quartiles but not the standard deviation since the average and standard deviation are inappropriate measures for ordinal data as mentioned by reference [6]. Other alternative for the analysis of the data is using a non-parametric tests on which it depends on the researcher's objective are the chi-square test (a categorical statistical method usually or count data), Mann–Whitney test, Wilcoxon signed-rank test, or Kruskal–Wallis test (comparison of mean ranks). If guaranteed by the Central Limit Theorem that ordinary averages of the Likert scale data behaves or can be assumed as an approximately normally distributed in which parametric statistical analysis can be performed. In psychological researches, Answers to several Likert questions are summed up (totaled) with the assumptions that all questions

use the same Likert scale and the scale is a defendable approximation to an interval scale, for which case it may be treated as an interval data that measures an unobserved (or latent) variable. Thus, parametric statistical tests such as the analysis of variance may be applied. Further, one consideration the method can be applied is to have more than five Likert item questions that are totaled.

### *2.2 Level of measurement*

The five response categories are often believed to represent an interval level of measurement but this can only be in the case if the intervals between the scale points correspond to empirical observations in a metric sense. We bear in mind that the consideration of ordinal scales as an interval scales has a lot of controversial issues as mentioned previously, however in matter of agreement of its principle, it will be used as a basis for obtaining estimates in an interval level consideration on a continuum by applying item response models in which the data can be obtained in the form of an ordinal scale that fit the model particularly known as the polytomous Rasch model [20,21, 22],. The researcher recommends that the data has to be thoroughly checked to fulfill the strict formal axioms of the model before it is being analyzed.

## 3. Results and Discussions

Following reference [13], When items are Likert scaled and is assumed to have an interval measurement, often in practice, the information to all the respondents are summarized in the form of a weighted mean that is the scale is used as a weight multiplied to the frequency divided by the total frequency as to obtain the weighted average or mean. After this procedure the resulting weighted mean is being interpreted using an interval where in return had a corresponding verbal description. Most of these Likert scales are used in giving the level of agreement, level of frequency, level of importance or the likelihood of appearance in most application. To give an illustration in its use. Reference [17] in their paper, used four point Likert scales in the assessment of students' self-level of motivation in mathematics. Seven survey question items were constructed for their assessment. From these items, one item was selected for discussion, the item "How much have you liked mathematics this year" which was answered using the four point Likert Scale with corresponding verbal description: 1. Not much, 2. A little, 3. some, 4. A lot. Now, some researchers in these kind of surveys, in practice created an interval of means in order to give interpretations for the weighted mean. Results of most studies would treat the problem as practiced by some researchers in which we will illustrate below

**Table 1:** Four point Likert Scale

| Likert Scale | Interval | Verbal Description |
|---|---|---|
| 1 | 1.00-1.50 | Not much |
| 2 | 1.51-2.50 | A little |
| 3 | 2.51-3.50 | Some |
| 4 | 3.51-4.00 | A lot |

Table 1 shows a table of a four point Likert scale with the constructed interval and its corresponding equivalent verbal description. This kind of interval is bias in the sense that the difference in the upper interval and lower interval of the first and last intervals is much lower as compared to the two middle intervals (see table 2 below). This observation will result items with the computed weighted mean considered more in the middle. This creates an unbalanced difference as we observed.

**Table 2:** Four point Likert Scale

| Likert Scale | Interval | **Difference** | Verbal Description |
|---|---|---|---|
| 1 | 1.00-1.50 | **0.50** | Not much |
| 2 | 1.51-2.50 | 0.99 | A little |
| 3 | 2.51-3.50 | 0.99 | Some |
| 4 | 3.51-4.00 | **0.49** | A lot |

To correct this problem, we attempt to reduce or eliminate the bias using trial and error, by making the difference in each interval having a uniform difference. A new interval was constructed as shown in Table 3 having a majority uniformed difference in each interval (except for one interval). This new interval may improve the description of the weighted mean.

**Table 3:** Four point Likert scale

| Likert Scale | Interval | Difference | Description |
|---|---|---|---|
| 1 | 1.00-1.75 | **0.75** | Not much |
| 2 | 1.76-2.51 | **0.75** | A little |
| 3 | 2.52-3.27 | **0.75** | Some |
| 4 | 3.28-4.00 | 0.72 | A lot |

The correction of the bias in the interval makes the difference “uniform” except for one and perhaps might change the descriptive interpretation of the item. Going back to the study of reference [17], As shown in the results of the overall weighted mean of the 5 student survey items by which yielded a values of 3.47 for IWB group and 3.22 for the control group. A description of this weighted mean leads to the interpretation for the verbal description of “some” for IWB group when using the table 2 but will have different description “a lot” when using the table 3 in which there is the presence of the correction. For the control group it has the same “some” interpretation when table 2 and table 3 are both used.

For the next tables which will be presented below, we will show the improvements for some even and odd scale categories specifically for the three, four (as shown above), five six, and seven response categories. Intervals with two decimal places are recommended below given their differences and possible description (Note

description can be change for appropriate description depending on the objective of the study)

**Table 4:** Three point Likert scale

| Likert Scale | Interval | Difference | Description |
|---|---|---|---|
| 1 | 1.00-1.66 | **0.66** | Bad |
| 2 | 1.67-2.33 | **0.66** | Undecided |
| 3 | 2.34-3.00 | **0.66** | Good |

Table 4 shows that for a three point Likert scale, we can create an interval in which their differences are uniform which is what we wanted to achieved.

**Table 5:** Five point Likert scale

| Likert Scale | Interval | Difference | Description |
|---|---|---|---|
| 1 | 1.00-1.79 | **0.79** | Never |
| 2 | 1.80-2.59 | **0.79** | Rare |
| 3 | 2.60-3.39 | **0.79** | sometimes |
| 4 | 3.40-4.19 | **0.79** | Often |
| 5 | 4.20-5.00 | 0.80 | Always |

Next is the table 5 above for a five point Likert scale in which an intervals were created with majority of the differences are similar except for one which is wider and has slighter difference of 0.1 among the rest.

For table 6 below, in a six point Likert scale an intervals were created with majority differences are similar except for one interval which is wider and has a difference of 0.03 among the rest.

**Table 6:** Six point Likert Scale

| Likert Scale | Interval | Difference | Description |
|---|---|---|---|
| 1 | 1.00-1.82 | **0.82** | Very bad |
| 2 | 1.83-2.65 | **0.82** | Rather bad |
| 3 | 2.66-3.48 | **0.82** | Bad |
| 4 | 3.49-4.31 | **0.82** | Slightly good |
| 5 | 4.32-5.14 | **0.82** | Good |
| 6 | 5.15-6.00 | 0.85 | Rather good |

Lastly, table 7, a seven point Likert scale with created intervals produced a majority of similar difference except the upper interval which is shorter but a very small difference of 0.01 among the rest. These per observation is a good table.

**Table 7:** Seven point Likert scale

| Likert Scale | Interval | Difference | Description |
|---|---|---|---|
| 1 | 1.00-1.85 | **0.85** | Very bad |
| 2 | 1.86-2.71 | **0.85** | Rather bad |
| 3 | 2.72-3.57 | **0.85** | Bad |
| 4 | 3.58-4.43 | **0.85** | Neither good nor bad |
| 5 | 4.44-5.29 | **0.85** | Good |
| 6 | 5.30-6.15 | **0.85** | Rather good |
| 7 | 6.16-7.00 | 0.84 | Very good |

For all the tables created above which shows the intervals, differences and verbal descriptions, These tables can be used as a guide or a tool for describing the verbal description of a computed weighted mean for presentation which can perhaps be considered valid and has less error (bias) preventing which is usually common in practice to some intervals created by some researchers whose intervals usually are concentrated at the middle of the scale leading to more items whose weighted assigned mostly in the middle verbal description.

## 4. Conclusions

Using this suggested interval perhaps will appropriately improve proper labeling for the descriptive interpretation of the computed weighted mean or average for any researches that considered Likert rating scales in each items for the whole responded questionnaires according to the number of response categories of the items.

## 5. Recommendations

The researcher recommends that more study should be done to refine the interval in each table by any statistical methods so to obtain uniform difference in each point Likert Scale table similar to column difference in table 4. It is also worth to discover the intervals for 8,9 and 10 point Likert scale.

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# The Effect of Classical Music on Spatial Intelligence

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## Abstract

The present study was to examine the effect of classical music on the spatial intelligence in college students. Standard Progressive Matrices (SPM), a 60-item spatial intelligence test was employed to measure the level of spatial intelligence. There were two groups — 20 participants in each group ages 16-18 which were observed during the study. The experiment consisted two sessions. On the first session, both groups were exposed, in ten minutes, into silence and answered the SPM after the exposure. On the other hand, on the second session, one group was still exposed, in ten minutes, into silence and the other group was exposed, in the same duration of time, into classical music. They answered the same test just after the exposure to the said conditions. Results showed that classical music has a highly significant effect on the level of spatial intelligence of the participants.

**Keywords:** Classical Music, Spatial Intelligence

## INTRODUCTION

It has always been believed that Intelligence quotient bitterly known as IQ is the measure for one's success, however many other modern standardized tests used today fail to tap into a cognitive ability known as spatial ability that is, the capacity to mentally generate, rotate and transform visual images (Park, Lubinski, &Benbow, 2010).

Many research studies has established that music is beneficial in improving learning, building self-esteem, reducing stress, supporting physical exercise and facilitating a host of other health-related activities (Heidersheit, 2009). Because of this music therapy was born.

It was after the World War II when a throat, nose, and lung physician Alfred Tomatis used music to treat his patients with developmental delays, ADHD, Autism, and other health related issues (soundlistening.com, 2006). Additionally, other researchers were also involved in the study of music cognition which involved the study of several aspect of music particularly how music was processed in the mind and the resulting mental processes (Maglione, 2005).

Can music influence the mental abilities of individuals? One of the findings in music is that classical music helps in synchronizing emotions, eases tension and depression, and enhances critical thinking through its rhythm. Its melody on the other hand, was found to catalyse creative process in our mind (Maglione, 2005).

In 1993, Gordon Shaw et al. studied and proved the effect of Mozart music on the spatial intelligence of some college students in the University of California, However, some others study contradicted.

But why is there a need to study the effect of such music in spatial intelligence? In traditional school settings, educators commonly fail to facilitate students in developing spatial ability — one of the specific cognitive abilities (other are quantitative and verbal abilities) — plays a critical role in architecture, engineering, mathematics, arts, games, and scientific disciplines; it is relatively one of the most important cognitive ability for developing expertise in learning and work settings (Benbow, Lubinski, & Park, 2010), but is often unrecognized and neglected.

Given the contradicting previous studies and limited amount of local research on the subject, one can only be encouraged to pursue such study. Hence, the

Mozart effect on the increase of spatial intelligence in a local setting was conducted conducted.

This study centers on the increase impact of Mozart music on the spatial intelligence. According to Park et al. spatial intelligence is the capacity to mentally generate, rotate and transform visual images (Park, Lubinski, &Benbow, 2010). In addition to these, as said by (Christensen, 2011), it is the ability to comprehend three-dimensional images and shapes which involves interpreting dimensions of invisible space, the ability to picture the shapes and spaces of objects mentally and to retain the form of an object in the mind's eye. Specifically, to where the spatially intelligent person good at? According to mypersonality.info (2011), a spatially intelligent person is good at solving visual puzzles, geometry, drawing, visualizing pictures in head, noticing colors and shapes, photography, deciphering directions, remembering places vividly, and good at artistic composition. He/she is also more inclined at using metaphor and talk fast (Christensen, 2011).

What we expect in this study and its benefit. School institutions which are responsible for shaping the student's future might find information from the research that will provide more insight among educators. They can encourage students to listen to classical music, that will in turn, help students improve their academic performance. Second, students, enriched their academic feat for it will bestow knowledge that Mozart music might boost their spatial intelligence.Theoretically, such act may help them perform better various

subjects such as, geometry, arts (mypersonality.info, 2011), writing compositions, physical science, engineering, mathematics, and computer sciences (Park, Lubinski, &Benbow, 2010). Additionally, this might also prepare them for future professions in, but only some, Artistry, Architecture, Graphic Designing, Engineering, Fashion Designing, Interior Design, Photography (mypersonality.info, 2011) and in Film Industry (Christensen, 2011). Furthermore, this might also assist them in enhancing their scores in entrance examinations and other examinations, placement, promotion, and licensure tests since these examinations include a lot of test on spatial intelligence and non-verbal reasoning. Third, to athletes, this may be helpful for it will lend a hand to progress their performance — since the classical music increases the likelihood of one in picturing the positions, shapes and spaces of objects in the mind and in gauging field from different angles. Fourth, to writers, the study can help them because spatial intelligence is central to writing: that is the ability to discern similarities across diverse domains, metaphor and can communicate new thoughts by linking the unknown with the known. Further, spatial intelligence is required in organizing idea when writing through outlining or displaying the organizational structure of a thought (Grow, 2007). Finally, to the greater population, the study will impart them awareness, since spatial intelligence is a cognitive skill that is often neglected and unrecognized in the traditional classroom settings but plays a vital role in engineering and scientific disciplines, that Mozart music can

heighten their spatial intelligence (Park, Lubinski, &Benbow, 2010). For this reason, they will be able to use such awareness as early as now.

The purpose of this study is to determine the effectiveness of listening to Mozart music in spatial intelligence using Progressive matrices. Particularly, determine the significant effect of classical music on the spatial intelligence of the participants.

This study is expected to measure the spatial intelligence of the participants in the experimental and the control group. This study was limited only on the determination of the effectiveness of Mozart music specifically Sonata of Two Pianos in D Major (K. 448) on the spatial intelligence of the participants from the two selected curricular programs which have an admission policy of a percentile rank of at least 500 in the USMCEE.

The selection of these two programs was drawn from the five identified curricular programs (BS. Chemistry, BS.Biology, BS. Civil Engineering, BS. Accountancy sections A, B, and C, and Doctor of Veterinary Medicine) which have the said admission policy. However, though, the participants had undergone the random procedure, there selection had been limited to the identified programs. The selection of the experimental group's participants was only limited to the first identified program; on the other hand, the selection of

the control group's participants was only limited to the second identified program.

Another limitation of this study was that, the researcher had used 40 participants — 20 from the experimental group and 20 from the control group. In addition to this, the participants only were 16-18 years of age. However, numbers 16, 17 and 18 year old participants from the experimental group were not equal to numbers 16, 17, and 18 year old participants from the control group.

Moreover, two sessions were implemented in the experiment-pre-test was administered on the first session and post-test was on the next session. In line with this, there was an hour gap between the first and the second sessions. However, the participants were not monitored during this gap.

**Sonata of Two Pianos in D Major (K.448)** is a classical music which is one of the compositions of Wolfgang Amadeus Mozart.This was being played within 10 minutes during the second conduct of the experiment on the experimental group. On the other hand, spatial intelligence of the participants was measured to both the experimental group and the control group participants' on the first and second sessions of the experiment using Standard Progressive Matrices.

| RS | RIQ Equivalent | QD |
|---|---|---|
| 60 | 118 | Superior |
| 59 | 117 | |
| 58 | 116 | |
| 57 | 114 | |
| 56 | 113 | |
| 55 | 112 | |
| 54 | 110 | |
| 53 | 109 | Above-Average |
| 52 | 108 | |
| 51 | 107 | |
| 50 | 105 | |
| 49 | 104 | |
| 48 | 103 | High-Average |
| 47 | 102 | |
| 46 | 100 | |
| 45 | 99 | |
| 44 | 98 | Average |
| 43 | 96 | Low-Average |
| 42 | 95 | |
| 41 | 94 | |
| 40 | 93 | |
| 39 | 91 | |
| 38 | 90 | |
| 37 | 89 | Below-Average |
| 36 | 87 | |
| 35 | 86 | |
| 34 | 85 | |
| 33 | 84 | |
| 32 | 82 | |
| 31 | 81 | |
| 30 | 80 | |
| 29 | 78 | |
| 28 | 77 | |
| 27 | 76 | |
| 26 | 75 | |
| 25 | 73 | |
| 24 | 72 | |

| | | |
|---|---|---|
| 23 | 71 | |
| 22 | 70 | |
| 21 | 68 | |
| 20 | 67 | |
| 19 | 66 | |
| 18 | 64 | |
| 17 | 63 | |
| 16 | 62 | |
| 15 | 61 | |
| 14 | 59 | |
| 13 | 58 | |
| 12 | 57 | **Disadvantage** |
| 11 | 55 | |
| 10 | 54 | |
| 9 | 53 | |
| 8 | 52 | |
| 7 | 50 | |
| 6 | 49 | |
| 5 | 48 | |
| 4 | 46 | |
| 3 | 45 | |
| 2 | 44 | |
| 1 | 43 | |

Where:

RS—Raw Score

RIQ Equivalent—Raven I.Q Equivalent

QD—Qualitative Description

Further, **Standard Progressive Matrices** was being used to measure the participants' level of spatial intelligence and was being administered to both the experimental group and the control group participants' on the first and second sessions of the experiment.

The study was anchored on the “Mozart Effect” theory of Alfred Tomatis. The Mozart effect theory accounts that the spatial intelligence may increase when listening to Mozart music. Spatial intelligence as defined by (Park, Lubinski, Benbow, 2010) is the capacity to mentally generating, rotating and transforming visual images; one of the cognitive skills (learningrx.com. 2011).

The Mozart effect theory vindicates that, by listening to Mozart music, hormonal system will be influenced and this will affect the breathing rate and electrical resistance of the skin. Additionally, this brings pupils to dilate and increases blood pressure and heart rate. These allow the brain to concentrate more easily, and to assimilate more information in less time. This happens because music stimulates the left and the right hemispheres of the brain at the same time. The contemporaneous activation of the two hemispheres of the brain boosts learning and information intake, therefore augmenting cognitive skills (Maglione, 2005).

The Mozart effect is a term coined by Alfred A. Tomatis for the alleged increase in brain development that occurs in children under age 3 when they listen to the music of Wolfgang Amadeus Mozart (skepdic.com, 2007).

In 1988, Shaw and Leng first attempted to model the brain activity on a computer by stimulating it using conventional printouts. They found that the

way nerve cells were connected to one another predisposed groups of cells to adopt certain specific firing patterns and rhythms. These patterns form the basic exchange of mental activity. By this observation, they turn the printout that they used in stimulations into sounds. They were surprised to the result as they found that the rhythmic patterns sounded somewhat familiar, with some of the characteristics of baroque, new age, or Eastern music (Anderson, 2000)

Not until the year 1993 when a study about the effect of Mozart music on spatial intelligence was conducted by Gordon Shaw and colleagues. In this research, they assigned thirty six Cal-Irvine students to one of three groups and gave them a pre-test. One group then listened to Mozart music (Sonata in D major for Two Pianos, K488), the second group listened to what was called a "relaxation tape," and the third group was subjected to ten minutes of silence. They were then given a test which measures spatial IQ. A relative increase in spatial IQ was found to those who listened to Mozart's sonata (Jones, 20003).

In 1994, Stough, Kerkin, Bates, and Mangan, at the University of Aukland conducted the same experiment but they failed to produce "Mozart effect". This was may be because, in this study, they used Raven's Advanced Progressive Matrices to measure spatial IQ. Rauscher et al. study used the Stanford-Binet Intelligence Scale (Stough, Kerkin, Bates, &Mangan, 1994).

In the same year however, Kenealy and Monseth studied thirty subjects. They were subjected to Mozart, disco music, and silence. Participants were then given the same test (Stanford-Benet) but there were no significant differences in the scores (Kenealy&Monseth, 1994).

## METHODOLOGY

This study is an Experimental Research Design specifically Pre-test-Post-test Between-Within Two Group Design. The diagram below shows the design of the study.

| | | | |
|---|---|---|---|
| EG | O 1 | X | O 2 |
| - - -

The respondents were the freshmen students from the five identified curricular programs ( BS.Biology and BS. Accountancy sections A, B, and C) which have admission policy of a percentile rank of 500, at least, at the University of Southern Mindanao College Entrance Examination (USMCEE) at the University of Southern Mindanao Kabacan, Cotabato. The participants were 16-18 years old. Drawing of lots was employed in selecting the two programs from the five identified curricular programs which have the said admission policy. The first selected program was chosen to be the experimental group and the second selected program was chosen to be the control group. 20 participants from the selected experimental group and 20 participants from the selected control group were randomly picked using the drawing of lots.

The instrument used was the Sonata of Two Pianos in D Major K. 448, A classical music composed by Wolfgang Amadeus Mozart in 1781and the Standard Progressive Matrices (SPM) which is a group of individually administered test that non-verbally assesses intelligence in children and adults through abstract reasoning tasks. It is sometimes called Raven's, although the SPM is only one test of three tests that together comprise Raven's Progressive Matrices.

The SPM consists of 60 problems 5 sets of 12 all of which involve completing a pattern or figure with a part missing by choosing the correct missing piece from among six alternatives. Patterns are arranged in order of

increasing difficulty. From the original studies on SPM, Ravens (1948) and Fouls and Raven (1948) found reliabilities ranging from 0.83 to 0.93, with the higher values being associated with younger subjects (under 30). A summary of the early evidence has been provided by Desai (1952) and a later summary by Burke (1958). The content validity of SPM, measures by the internal consistency of the test, varies markedly when different test items are considered (Keir, 1949; Hornowski, 1959; Kalina, 1967). Banks and Sinha (1951) with children, found an average biserial correlation between SPM items and the combined results of three IQ tests, of +0.45; for different test items, correlations ranged from +0.2 to +0.8. For a large sample of adults, Sheppard et al. (1949), Banks and Sinha (1951) and Sheppard et al. (1968) suggest that there may be a preponderance of medium difficulty items and a need for significant shift in the order of some items.

The following procedure has been served in the administration of this research

1. The researcher, together with his assistant had undergone training in conducting Standard Progressive Matrices (SPM).
2. The researcher had identified all the curricular programs which have an admission policy of a percentile rank of at least 500 in the USMCEE test. Among identified Programs, using randomization, the researcher had assigned the first selected curricular program as the experimental group and the second selected curricular program as the control group.

3. Using randomization, the researcher had selected 20 participants from the identified experimental group and 20 participants from the identified control group.
4. A secured permission to conduct the study was also sent to the Dean's Office of the selected programs.
5. The experiment consisted two sessions. The first session was conducted at 8:30 AM on September 23, 2011. The second session was conducted at 10:30 AM on the same date.
6. On September 21, 2011, the participants were blindly informed through text messages about the experiment.
    a. Participants blind script. Text message 1: *"Good morning, I'm the UGC test administrator trainee. I would like to inform you that on Friday, September 23, 2011 you will be excused from your 8:30 AM and 10:30 AM classes to take two different tests (*actually, two the same test will be administered-Standard Progressive Matrices or SPM*) required by the UGC."* Text message 2:*"First test will be administered at exactly 8:30 AM and the next test will be at exactly 10:30 AM."*Text message 3*: "I would also like to inform you, guys, that late comers will not be entertained but have to take the special test at the UGC. To those who willabsent, you also have to take the special test at the UGC."* Text message 4:*"Special test will have to be paid P300.00.*

*There is no need to study.The venue of the test will be at _____.”*

7. The testing venues were the Conference room of the University Extension Office and the Speech Laboratory of the College of Arts and Sciences. These two venues are soundproof rooms; this is to avoid any unnecessary noise that might affect the experiment. The rooms are fully air-conditioned. Each of these venues was randomly assigned for the groups. The first selected venue (Conference room) was assigned for the Experimental group and the second selected venue (Speech Laboratory) was assigned for the control group.
8. All students from the identified groups were invited to participate in the experiment, though; only those selected 20 participants from each group were monitored.
9. On the experiment proper, both the researcher and the assistant were contacting each other in simultaneously starting and ending the experiment procedure.
10. On the first session (8:30 AM) of the experiment, both groups were being in silence within 10 minutes inside the room before the SPM test had been administered.
    a. Blind script. *“I would like to ask everybody to, please, turn off his/her phone. While we’re waiting for the test questionnaire to arrive, let us stay here for a while, be in silence, and relax.”*

The researcher and the assistant waited until the 10 minutes had been consumed.

11. SPM was administered to both groups and was answered within 20 minutes.
12. On the second session (10:30 AM) of the experiment, the control group was still being in silence within 10 minutes inside the room. However, the experimental group was being exposed, within 10 minutes, to Mozart music (Sonata of Two Pianos in D Major K 448) inside the room.
    a. Control group's blind script. *"Again, I would to ask everybody to turn off his/her phones and while we're waiting for the test questionnaire, let us again be in silence, and relax."*

Waiting until the 10 minutes had been consumed.

    b. Experimental group's blind script. *"Again, I would like to ask everybody to turn off his/her phones and while we're waiting for the test questionnaire, let us again be in silence, and relax. Hmmmm, I think it would be better if we play music for a while. Would that be okay to you?"*

Waiting until the 10 minutes had been consumed.

    c. Blind script for the experimental and the control groups before the second administration of the SPM. *"Excuse me guys, I would like to tell you that the UGC office has just called me and said*

*that your answer sheets mixed up with the other files of the UGC. I don't know exactly what happened, but the office instructed me to re-administer the test that you have just taken. The other test that I told you last time will be rescheduled next week."*

13. SPM test was administered again.
14. The "blind" of the experiment had been revealed.

The data was subjected to t-test for independent samples and paired t-test respectively.

## RESULTS AND DISCUSSION

Determining the effect of the classical music on the spatial intelligence of the participants. Two groups were compared according to their pre-test and post-test scores result on the Standard Progressive Matrices.

**Table1.** Difference between the mean scores of pre-test and post-test to both the experimental and control groups

| *Test* | *Group* | *n* | *mean* | *sd* | *t* | *df* | *p-value* | *mean differences* |
|---|---|---|---|---|---|---|---|---|
| **Pre-test** | Experimental | 20 | 51.55 | 3.252 | 0.751 | 38 | 0.0457$^{ns}$ | 0.09 |
| | Control | 20 | .50.65 | 4.258 | | | | |
| **Post-test** | Experimental | 20 | 54.75 | 2.023 | 6.020 | 22.7 | 0.000** | 9.10 |
| | Control | 20 | 45.65 | 6.450 | | | | |

Legend: $^{ns}$-not significant at 5% level
**- significant at 1% level

The result of the analysis shows (refers to table 1) that for the pre-test, there is no significant difference between the experimental group and the control groups' mean pre-test scores ($p > 0.05$). This is expected since initially, respondents were randomly selected based on the admission policy (at least a percentile rank of 500 in the USMCEE) of the program where they were enrolled at. Hence, statistically, their mean pre-test should not vary.

Further, on the post-test, the result of the analysis shows that there is a high significant difference between the experimental group and the control

groups' mean post-test ($p \leq 0.01$). The experimental group has a higher mean score compared to the control group. This implicates that the intervention had increased the level of spatial intelligence.

On the other hand, for the difference between the pre-test and post-test results of both the experimental and the control groups, table 2 provides the analysis of the data.

**Table2.** Difference between the scores of pre-test and post-test of both the experimental and the control groups

| *Group* | *test* | *mean* | *n* | *sd* | *mean differences* | *sd* | *t* | *df* | *p-value* |
|---|---|---|---|---|---|---|---|---|---|
| Experimental | Post-test | 54.75 | 20 | 2.023 | 3.2 | 2.397 | 5.969 | 19 | 0.000** |
| | Pre-test | 51.55 | 20 | 3.254 | | | | | |
| Control | Post-test | 45.65 | 20 | 6.450 | -5.0 | 5.813 | -3.847 | 19 | 0.001** |
| | Pre-test | 50.65 | 20 | 4.258 | | | | | |

Table 2 reveals that there is a highly significant difference ($p \leq 0.01$) on the mean pre-test and post-test scores in the experimental group. The respondents mean post-test score is higher compared to their mean pre-test score. This result signifies that the intervention of the treatment improved the scores of the respondents in the Standard Progressive Matrices, thus it has a positive effect on the respondents' level of spatial intelligence.

On the other side, in the control group, there was also a high significant difference between the respondents mean pre-test and post-test scores. The post-test has a lower mean score compared to the mean pre-test score. Thus,

for the control group, on the average, there was a deterioration of level of spatial intelligence among participants. It is perhaps, due to mental fatigue. Mental fatigue can be caused by continual mental effort and attention on a particular task (betterhealthusa.com, 2010) and may increase simple mistakes and inability to stay on task (Upson, 2011).

Historically, the following week after the experiment, University Foundation Anniversary (Pasiklaban) had been celebrated. This was celebrated for the entire week and everybody was prohibited to have a class during this week. Additionally, the next week after the Pasiklaban, final examinations had been administered. Thus, within the week the experiment was conducted, most of the students and teachers, if not all, were in hurry finishing their lessons for the upcoming final examinations. Hence, these all perhaps caused mental fatigue among participants.

But why on the average, there was an increase instead of deterioration of level of spatial intelligence in the experimental group? “Mozart effect” theory said when you listen to Mozart music both hemispheres of the brain will be simultaneously stimulated. This contemporaneous activation of two hemispheres of the brain boosts learning and information intake, thus augment cognitive skills (Maglione, 2005).

The result of this research therefore affirmed the study (first study coining the Mozart music to spatial intelligence) conducted by Shaw et al. (1993). The study demonstrated that after listening to Mozart music (Sonata in D major for Two Pianos, K488) the spatial intelligence of the participants got a relative increase (Anderson, 2000). However, this study contradicted those studies which asserted that Mozart music has no impact on the spatial intelligence.

Most of the studies in this subject used Stanford-Binnet Intelligence Scale in measuring the participants' level of spatial intelligence. However, Stough et al. tried to use the Standard Progressive Matrices (the same test used in this study) to test whether after listening to classical music the increase in spatial intelligence (as Rauscher et al. asserted) can still be measured. This study of Stough et al. failed to measure the increase in spatial intelligence among participants. The study "Classical Music: Its effect on Spatial Intelligence" therefore, also contradict the Stough et al study.

## CONCLUSIONS AND RECOMMENDATIONS

The results of this study are summarized as follows: The mean pre-test scores of the experimental group and the control group have no significant difference. But the mean post-test scores of the experimental group and the control group have a high significant difference. Also the mean pre-test and the mean post-

test of the experimental group have a high significant difference as well as the the mean pre-test and the mean post-test of the control group have a high significant difference. In consideration to these results, the study concluded with respect to the subject being considered that the spatial intelligence test scores of the participants significantly increased through the intervention of classical music. Thus, listening classical music plays a vital role in increasing the spatial intelligence. Hence, it is recommended that since Mozart music (Sonata in D major for Two Pianos, K488) will be used in the GGP (Group Growth Program) for the USM students as one method to increase academic performance since it was already proven that method plays critical role in architecture, engineering, mathematics, arts, games, and other scientific disciplines. The researcher also recommends that athletes may listen to classical music for it may enhance their performance during the game. It is also recommended to replicate the study using different methodology such as screening procedure, sampling procedure and different music like relaxing music, pop music, ballad or rock music whether results will be still consistent.

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# THE EFFECT OF POSITIVE REINFORCEMENT ON CLASS PARTICIPATION AMONG PRESCHOOLERS

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## Abstract

A study was conducted to determine the effect of positive reinforcement on class participation among preschoolers particularly to investigate if positive reinforcement can increase class participation among preschoolers. Forty eight (48) preschoolers of Kabacan Pilot Central School were subjected for the experiment using five evaluation forms namely the Daily log, Instructional Schedule Matrix, Rating scale, Task analysis data collection sheet and lastly Behavior log. Tally sheets for the days of observation were also employed. The evaluation forms were answered by the class teacher and were retrieved right away. Result of the study shows that positive reinforcement has a highly positive significant effect on the class participation of preschoolers and that positive reinforcement regardless of its forms has significantly improved and increased the preschooler's participation in classroom activities.

**Keywords**: class participation, positive reinforcement

# INTRODUCTION

There is a well-known belief that academic performance rely largely on the intellectual capacity of an individual. This belief asserts that only person with high IQ level excel in the academe. This belief discredits other factors relating to academic performance and centers alone on the cognitive aspects of individual. However, this belief has gradually been replaced by research findings mostly provided by the professionals on behavioral sciences. Particularly, Psychology on its part has great and immeasurable contributions when it comes to learning. Observational learning has been credited with a major role in cognitive development and in academic skills learning by a wide range of researchers and theorists (e.g., Bandura, 1977; Bereiter, 1985; Bereiter and Scardamalia, 1985; Palincsar and Brown, 1984; Piaget, 1958). Leading experts in this field have proposed using positive reinforcement to change behavior in children.

Positive Reinforcement was first introduced by Burrhus Frederick Skinner (1971), a behavioral Psychologist who defined the positive reinforcement as a way to highlight behavior by rewarding and likely to increase its occurrence. Negative behavior on the other hand is simply ignored. Positive reinforcement is a very important part of behavioral analysis, which is a field within psychology. Its applications are most often seen in the way specialists recommend to talk and behave with our children as they are growing up. In this study we examine how positive reinforcement affects class participation of students as well as their academic performance.

In recent years, there have been increasing emphasis on the use of 'positive behavior reinforcement' as a way of improving behavioral standards among young learners, in connection with this, many research have been made to validate the effectiveness of positive reinforcement in academic setting but one that is difficult to master is the behavior management in the classroom (Sanders, 2011). Although techniques are often taught in teacher training classes, good behavior management usually comes with time, practice and trial-and-error. The correct use of positive reinforcement in the classroom will work wonders on managing the behavior of students. Different authors has their different opinions of positive reinforcement but all of their opinions focuses on the significance that it brings to people to become motivated in every aspect of life. Studies have been made to provide evidence on how positive reinforcement takes place in academic setting, majority of the studies focuses on how this reinforcement works with children since children are known to have short attention span, it is for this reason that many researchers have worked hard to validate positive reinforcement to help children in terms of academic performance. According to Charlie (2010), positive reinforcement for children is a way of motivating them by appreciating their good qualities.

Positive reinforcement is about Implementing reward system, and implementing such reward can be tricky. According to Bafile (2000), the first step in developing a successful reward system is to determine your goal and be specific about it. If a teacher's goal in giving reward is for children to participate in classroom activities then the reward program should reinforce participation, if it is to increase or maintain good

behavior then the reward should reinforce good behavior. Also, balance is important in implementing reward system.

There are many kinds of reinforcements, it could be verbal reinforcement such as praise or in the form of material rewards. McCarthy (2010), stated that Positive reinforcement can either be a reward for good behavior, or simply positive communication in the form of praise or encouragement. However, Positive reinforcement in the classroom in the form of awards and gifts, can at times, is more effective than the oral ways of appreciation. Sometimes material rewards which the students always wished to possess can increase the enthusiasm and energy in them to perform better at every outing. Nonetheless, this does not indicate that praise is less effective when it comes to motivating children. In fact, Behaviorists believe that children want praise and they will do something more often if they are praised for it. (Sullivan, 2011).

In this study, we attempt to contribute supplementation in the understanding of cognitive attributes of children on how they take action with the positive reinforcement and to investigate how such reinforcement could make a difference on their part. The study also intends to confirm Skinners' conclusion to positive reinforcement and uphold fresh insights that would add to the intellectual enrichment of early childhood pupils around the globe.

The results of the study help parents in the monitoring of their children's school performance and motivate them to do even more in school activities. This also give parents a clearer sight or better comprehension on the areas where they would sustain

their children and not purely rely on the institutions since a growing consensus showed that positive reinforcement is a more effective method of improving children's behavior . Second, the study will lend a hand to teachers as educators in developing strategies to adjust to their pupils' learning difficulties. Preschoolers are known to have short attention span so there is a call for motivation. Thus, teachers will be able to apply innovative techniques when it comes to teaching. They can make the instruction more exciting and constructive. Moreover, this will not only be helpful for teachers but for larger institutions as well. Larger institutions such as Department of Education should be encouraged to build up fresh approaches to toughen the programs in preparing the children for the higher level. Implementing positive reinforcement in classroom setting as one teaching technique will uplift the intellectual capability of early childhood pupils, thus molding them to become the best of what they can be. Thirdly, the study will help promote a healthier understanding of the lessons hence, it will help motivate and boast student confidence in preparing much higher stage of learning.

The study was conducted among preschoolers at the Kabacan Pilot Central School, North Cotabato. The school is situated right at the heart of poblacion. Since July 5, 1938, the school started its multi- grade classes. Led by the teachers, parents, pupils and community, the school continued to grow and now have a land area of four (4) hectares. In 1948, Kabacan became the mother district of the neighboring municipalities such as Kidapawan, M'lang, Matalam and Carmen which was headed by only one district supervisor, the late Ciriaco Gapasin who was holding office in Kidapawan. This earned a place for the school a name in the division. Many years later, the Kabacan

Pilot School has officially changed its name to Kabacan Pilot Central School in 1975. Kabacan Pilot Central School garnered several prestigious awards and had passed the level I and level II Accreditation Program for Public Elementary Schools (APPES)

## METHODOLOGY

This study utilized a true experimental- comparative design, specifically two match group designs. One group (the experimental group) is subjected with positive reinforcement while the other group (control group) is in normal condition.

EG $O_1$ X $O_2$

\- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

CG $O_3$ $O_4$

where:

EG = Experimental group

CG = Control Group

O1 = Observation 1

O2 = Observation 2

O3 = Observation 3

O4 = Observation 4

X = Treatment

**-------**Randomization of the participants to the two groups.

and was conducted among the selected preschool pupils of Kabacan Pilot Central School. The school was selected as favorable road of conduct since it has a large

population of pupils who will prove the validity and reliability of the study. Kabacan Pilot Central School is one of the recognized schools in the place that provides quality of education that is painstaking essential to the Preschool children.

Forty eight (48) participants were divided into two groups by the use of random assignment. Twenty three (23) were placed in the experimental group and twenty five (25) in the control group. The experimental group is the group with positive reinforcement applied during class hours/days and the control group without any positive reinforcement applied.

The study made use of positive reinforcement instrument developed by Burrchus Frederick Skinner and also used evaluation forms, data collection and progress monitoring for preschoolers to examine a class of children in their respected classrooms. The evaluation forms, data collection and progress monitoring consist of 1.) Daily log 2.) Instructional schedule matrix 3.) Rating scale 4.) Task analysis data collection sheet and 5.)Behavioral log.

**Daily log** is a brief description of a day's events, student's performance on activities and tasks, and any incidents that may be important to record may be logged. The **Instructional Matrix** may be used as a data collection form for a child's IEP objectives/goals within daily routine and ongoing activities in the classroom. It also visually summarizes which IEP objectives/goals may be embedded into which daily activities in the classroom. The **Rating scale** is used to record the performance of multiple children. **Task analysis data sheet** is a data collection rating scale. A target behavior may be analyzed and divided into small steps. Data codes may be determined

in a convenient way for users to indicate the child's performance level or the level of support needed. Lastly, the **Behavior log** is a sheet to record data related to a child's challenging behavior. It includes time of occurrence, duration, antecedent condition, and other comments that may guide a hypothesis about the function of the behavior.

These instruments were used to measure the performance of the participants – mainly on how they take participation in activities. The evaluation was developed by kid's first early intervention program, The Kids FIRST Early Intervention Program is an educational program that provides early intervention and educational services for children ages three to five years who have a variety of special needs.

A voice recorder was utilized to document the entire process, and there was also presence of extrinsic rewards such as non-toxic colored pens, notebooks and other school supplies which will be given to pupils who will show participation throughout the conduct of the study.

In the conduct of the study, the researchers were introduced to the preschoolers as assistant teachers. This was done for the purpose of observing how the participants will react with the positive reinforcement without them knowing that they are being observed. The researchers arranged the schedule with the teacher and asked lists of non-participative and participative pupils for the researcher to a have starting point and to complete the number of participants needed. Also, this was done for the researcher

to determine the participant's level of thinking and to consider factors that could interfere with the study.

The researchers have decided to have the observation lasts for one (1) straight week for her to thoroughly observe the changes in the experimental group. For the experimental group, the researchers, as assistants of the teacher will see to it that every good behavior will be rewarded. The researcher gave reward to those pupils who behave, finish their works in time and shows interest. These positive behaviors were listed and a class participation tally sheets were used to score their participation (if there is any). Also, to have the validity and reliability of the study, the researcher recorded the verbal reinforcement that was used during the observation and took pictures of the participants while doing activities to document the actual and exact event. For the control group, the participants in the control group were also under observation in one week period.

The performance of the respondents in the study was determined as to whether they participate after given such positive reinforcement or not at all. The study was designed to find out if positive reinforcement has truly an effect in Preschool children's academic performance- mainly their participation in class activities. This was done by thoroughly observing the participants throughout their class hours. Information's obtained from the study were subjected to data analysis using linear regression to determine the effects of positive reinforcement on the children's academic performance. Further, the comparison between the experimental and control on their performance levels was tested using t-

test for independent samples. Mean, standard deviation, frequency and percentage were also used to describe other information of the study.

## RESULTS AND DISCUSSION

The result of the study is presented and discussed below.

**Table 1. Testing positive reinforcement operation on academic setting**

| Group | reinforcement | n | mean | s.d | t | df | p-value | mean difference |
|---|---|---|---|---|---|---|---|---|
| Expe | Praise (Day 1) | 21 | 1.86 | 1.195 | 3.590 | 27.128 | 0 .001** | 1.017 |
| Control | NONE | 25 | 0.86 | 0.554 | | | | |
| Expe | Material (Day2) | 23 | 2.26 | 1.657 | 3.820 | 28.863 | 0 .001** | 1.421 |
| Control | NONE | 25 | 0.84 | 0.688 | | | | |
| Expe | Praise (Day3) | 23 | 2.00 | 1.732 | 2.325 | 30.199 | 0.027* | 0 .917 |
| Control | NONE | 24 | 1.08 | 0.776 | | | | |
| Expe | Material (Day4) | 21 | 3.00 | 1.449 | 4.555 | 33.014 | 0.000** | 1.682 |
| Control | NONE | 22 | 1.32 | 0.894 | | | | |
| Expe | Praise and Material (Day5) | 22 | 1.95 | 1.174 | 2.294 | 45 | 0.26* | 0 .75 |
| Control | NONE | 25 | 1.20 | 1.080 | | | | |

*significant at 5% level
**significant at 1% level

Referring to Table 1, the result shows that there was a significant difference on participation between the control and experimental group. Hence, this result implied that positive reinforcement as an intervention of the study has an effect on improving class participation of the preschoolers. Variations was also observed as of rewards from day one (1) to day five (5) to identify how participants react with the reinforcement either by praise or materials or a combination on it.

**Table 2. Performance level of the participants of the different variables as rated by the class teacher**

| Rating | Group | mean | n | sd | t | df | p-value |
|---|---|---|---|---|---|---|---|
| Cognitive | experimental | 2.28 | 23 | 0.887 | 0.106 | 46 | 0.916 ns |
| Cognitive | control | 2.80 | 25 | 0.816 | | | |
| Social | experimental | 2.78 | 23 | 0.422 | 1.524 | 43.65 | 0.135 ns |
| Social | control | 2.76 | 25 | 0.583 | | | |
| Self help | experimental | 2.78 | 23 | 0.422 | 0.863 | 38.89 | 0.394 ns |
| Self help | control | 2.64 | 25 | 0.700 | | | |
| Language & com. | experimental | 2.87 | 23 | 0.920 | 1.269 | 46 | 0.211 ns |
| | control | 2.56 | 25 | 0.269 | | | |

ns not significant at 5% level

Table 2 shows that the rating of the class teacher on the performance level of the participants for both the experimental and control group for the different variables on cognitive, social, self-help and language communication. These results revealed that there was no significant difference on the mean rating among the variables on the cognitive, social , self help and language and communication between experimental and control group as rated by the class teacher. These results of no variation among the evaluator in their mean rating between the two groups is perhaps due to the fact that whether there is a praise or reward or nothing, the class teacher have the same perceptions on the different variables to the participants.

**Table 3. Effects of positive reinforcement on class participation**

| Independent Variables | Coefficient | Std.Error | t | p-value | effects |
|---|---|---|---|---|---|
| Praise reward day 1 | 2.288 | 0.345 | 6.622 | 0.000** | highly significant |
| Material reward day2 | 2.811 | 0.354 | 7.941 | 0.000** | highly significant |
| Praise reward day3 | 3.103 | 0.894 | 9.145 | 0.000** | highly significant |
| Material reward day4 | 2.528 | 0.306 | 8.266 | 0.000** | highly significant |
| Material and Praise Reward day 5 | 2.125 | 0.326 | 6.527 | 0.000** | highly significant |

Dependent variable: class participation
** significant at 1% level

Table 3 Shows that both praise and material rewards has a highly significant positive effect on the preschooler's class participation ($p< 0.000$). From day 1 up to day 5, participants displayed consistent interest in classroom activities which resulted to numerous amount of participation. Participation was recorded by counting the number of times a child raises his/her hands and answers questions and engages in fun learning. The above calculation shows that both praise and material rewards as positive reinforcement contribute to preschooler's numbers of participation in the experimental group. However, the data above also reveals that praise alone as a verbal reinforcement can be enough to motivate preschoolers participation in classroom activities.

According to VanBaren (2011), praise from a teacher can be effective or ineffective depending on the nature and quantity of the praise. He added that teacher praise has been highly regarded as a good, necessary part of classroom learning. And that by

using praise appropriately, it usually creates positive effect of modeling others' behaviors. Furthermore, since there is only one teacher in a classroom of 25 or more students, verbal praise is only one piece of the puzzle for motivating students (Sullivan, 2011). The variable praise rewards for day 1 determines around 69.8% to predict class participation. The 30.2% is contributed by other unknown variables. The purpose of having praise as positive reinforcement in the beginning of the observation was to find out if praise alone can motivate preschooler's participation, the result shows that praise solely can increase and improve participation. The preschoolers from the experimental group were praised individually during the five (5) days observation; the praise was more to reinforce class participation. According to Kelly (2011) one effective way of teaching is by providing students with praise, when praise is used correctly, it provides students with positive reinforcement and it also motivates them to learn and participate in class. However, in order for praise to truly be effective, it must be specific. To be specific is to personalize the praise by using the child's name, praise statements, and identify what the child did to merit being praised (Burnett, 2011). The material reward used in the intervention as positive reinforcement during the second day which shows a highly significant positive effect on class participation of preschoolers ($p<0.01$). This shows that material rewards predicted 75% chance to engage in class participation and the other 25% is contributed by other unidentified variables. Preschoolers from the experimental group were given material rewards for their involvement in classroom activities, as expected; the number of participation during day one (1) was closely related to the number of participation on day two (2) observation, which made it

consistent at this point. Positive reinforcement on day one (1) and two (2) both were highly significant.

Material rewards has long been proven to be effective when it comes to motivating children, the act of extending rewards to students helps promotes positive and appropriate behavior among students in a classroom. However, according to Lewis (2011) too much dependency on it can be risky, particularly when one rely regularly in material rewards.

The praise rewards during the third day showed a highly significant positive effect on class participation of the preschoolers ($p<0.01$). The variable praise rewards for day 3 determines around 79.9% chance to predict class participation, the 20.1% is contributed by other unknown variables. The result of the observation for day three (3) is quite similar to the outcome of day one (where both days used only praised rewards) it might be because the first day of intervention for the experimental group was a novel situation for the participants and the praise giving might have appeared to be more exciting to them, whereas for day three (3) expectations were already present since they have already idea about the reinforcing but are sightless of the flow of situation. They have no information about what kind of reinforcement and rewards they can get after participating in classroom activities. Day 1 has a highly significant positive effect while day 3 has also showed significant effect on preschooler's participation.

Further, the material rewards during the fourth day have a highly significant positive effect on class participation of the preschoolers. Like the prior forms of positive

reinforcement, this type got a 78.2% to predict class participation. Material rewards were used during the second day and fourth day and on the fifth day it was used alongside praise of observation, results shows that there is consistency having highly significant positive effect on the three separate days of observation using material reward as reinforcement. The results are quite similar and both have highly significant effect.

Finally for the combination of praise and material rewards during the fifth day (last day of the observation). Results revealed that there was a highly significant positive effect on class participation of the preschoolers. Further, these rewards explained 68.1% prediction of class participation.

Going back to all the observations from day one (1) to day five (5), All results showed that positive intervention have significant effect on class participation perhaps due to the fact that day 1 praise rewards was new to the preschoolers while for the last day there was a combination of both forms of rewards and is again new for them.

We note also the since the participants in the study were preschoolers ages 4-6, which are belong to Stage 3 early childhood and stage 4 elementary and middle school years, Erikson(1950) discussed that children of these age are of possibility to feel initiative versus guilt for the early childhood and competence versus inferiority. In connection, Ho (2009) stressed that parents should provide and show interest in what the child is doing and giving of praise, encouragement and rewards motivates them to further seek success, since preschool is a time for play, the middle years should be

extension for active learning, and so, even a single act of encouragement can do improvements and can bring success (Villar, 2007).

In our study, a significant effect was found of using positive reinforcement, whether praise or material on the class participation with respect to our respondents. These results affirmed Skinner (1971) claimed that when pleasant or desired behavior is rewarded, it will likely to increase its occurrence.

## CONCLUSIONS AND RECOMMENDATION

The study revealed that participants from the experimental group showed interest and engaged in participating in the class after reasonable amount of positive reinforcement. The study showed that positive reinforcement has a highly significant positive effect on class participation of the preschoolers that were selected in this study, whether it is in the form of praise or material both are found to be beneficial to the respondents. Therefore the study concluded that positive reinforcement regardless of its forms has a significantly effect by increasing the preschooler's participation in classroom activities.

A further study is recommended that first, Skinner's schedules of reinforcement should apply three groups and variations of treatments that is, two (2) treatment groups and one (1) control group. In the first group, the reinforcement should be everyday (continuous) regardless of how long the study is to be conducted. In Continuous reinforcement, the desired behavior is reinforced every single time it occurs (Cherry, 2011). Another group should have partial reinforcement wherein response is reinforced

only part of the time. The reason for such recommendation perhaps that it is thought that learned behaviors are acquired more slowly with partial reinforcement, but the response is more resistant to extinction (Cherry, 2011). Hence, the four (4) schedules of partial reinforcement (fixed- ratio schedules, variable-ratio schedules, fixed- interval ratio, and lastly, variable- interval ratio) should also be included, just like most experimental studies, there should be a control group. Secondly in the next study, higher levels participants will be used as respondents and then results should be compared whether the same result yield. Thirdly, the observation of the participants should have least three (3) evaluators that will conduct the assessment. This is for the researcher to compare his/her observations to that of the evaluators work to achieve precise data of the study. Fourth, those variables should be identical in terms of schedule, room, group and teacher that will do the reinforcement and also, the group should be highly controlled. Lastly, since in this study the researchers used three (3) stars for giving one (1) reward per student, perhaps five (5) stars for every one reward should be made so not to limit the rewards so that all participants has chance to have rewards for their participation and have longer time of observation . Lastly, proper orientation be done between parents and teachers about the positive reinforcement and how it is being done inside the four corners of the classroom, this would mean that parents should have awareness of their child's performance and the flow of intervention as well.

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# The Effect of Retention Interval Over a Three Year Period on the Elementary Algebra Performance of High School Students

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**Abstract**

This study provided empirical evidence on the effect of retention interval on the student's performance in elementary algebra mathematics among High School Students. The subjects were the grade 8, grade 9 and grade 10 students of the University Laboratory School, University of Southern Mindanao, Philippines for school year 2012-2013 who took their elementary algebra in their grade 7 in the school under the same instructor. The results of the respondents' grade 7 exam taken prior to the conduct of study was the initial test were used for comparison to their present retest result. Results revealed that there was a highly significant difference between the achievement during and after instruction of the participants according to their year level. Gain in performance across retention interval group were highly significant. Retention interval supplemented enough time for the students to practice what was previously learned as they engaged to new but highly related mathematics courses. Further, exposure to different academic trials facilitated development of expertise and mathematical academic maturity on the students contributed to the increase in gain with increasing retention interval. Thus, retention interval was found to be advantageous in mathematics if practiced and elaborated.

***Keywords:*** achievement; after instruction; during instruction; gain score; test; retest; retention interval.

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## 1. Introduction

Mathematics is a cognitively challenging topic in school. It does not only require analytical skills but one should possess good memory in the concepts, formula and the definition of terms. As a language of Science, mathematics is an important components of the school curriculum in every country. They are seen as integral components of every student's educational program. As the role and impact of technology continue to increase in society and in the workplace, the concepts, processes, and skills of science and mathematics are likely to become even more highly valued and the importance of these subjects in school curricula to become even greater [1]. Through highly abstract symbol operations, students acquire skills such as conceptual understanding, procedural fluency, strategic competence and adaptive reasoning that serve them best in undertaking most career pathways. However, besides all these promising advantages of mathematics, the Philippines and the Filipino students in particular are still in the verge of an appalling academic performance on the subject. [2] Mentioned in their paper an alarming observation among Filipino students revealed that though Filipinos excelled in knowledge acquisition but fare considerably low in lessons that requires higher order thinking skills. Further they said that it cannot be denied that most of the prospective tertiary students are unprepared for learning mathematics with such low understanding level and poor achievements in Mathematics caused a great concern. This observation corroborates poor mathematical performance among Filipino pupils [1]. This poor performance does not only reflect and pose a problem to the teachers and students but to the entire Philippine educational system. Studies have been undertaken to assess the contributory factors to the problem, and such case includes the teacher and his or her mode of instruction; the student and his innate capabilities, self-concept and activities; and the classroom environment and curriculum. However, one cannot get away from the fact that overcoming mathematical difficulties requires good memory and recall on mathematical formula and procedures, thus one can simply assume that one cause of the matter relies upon memory and memory processing. In addition, the studies of [3,4] emphasized and substantiated the researcher's observations that problems in memory retention add to the effect, in which, working memory plays a significant role. More importantly, throughout arithmetic and math, even simple counting and estimation [5], algebra and complex problem solving [6] pervasively rely on good memory which was found to be a major predictor of the students' performance on number-based tasks [7]. These related studies emphasized that retention interval plays a significant role in the performance on some specific topics for basic mathematics. Furthermore, mathematics education in schools of all levels is arranged in such a way that each subject is highly related to the next making previously learned information rehearsed and integrated to new information in each year level. Thus, it might be tempting to assume that students can retain information across longer period of testing delay. Therefore, this study focused on the effect of retention interval to mathematics performance of students across different year levels. If it exists, then mathematics performance is strengthened over time and added experience. This in particular, to know first, if a significant difference exists between the achievement during and after instruction of the subjects according to their year level. Second, if a significant difference exists between the students' mathematics performance gained across different retention intervals. Lastly, to descriptively compare the average gains scores and retest scores over different retention intervals. The results of this study would be helpful in providing teachers a perspective on how students learn and retain information about mathematical concepts and procedures. It may give them ideas on appropriate actions to facilitate better retention of

information. As centers and clients of educational endeavors, results can benefit students to have a better understanding on their mental processes, thereby, giving them ideas to what actions can be done to improve their performance and retain more information. Further, the results of this study may be used by school administrators in their decision-making concerning the arrangement of subjects in the school curriculum which should always be designed for the improvement of human capacity. This study also may provide insights for further research investigations of similar nature and will provide a new perspective and trends in educational researches.

### *1.1 Assumptions, Scope and Limitations of the Study*

The study assumes that the subjects' mathematical performance is normally distributed across different retention intervals so as to satisfy the requirement for the use of it statistical procedures. The study only considers the responses of the retest of the grade 8, grade 9, and grade 10 students in the University Laboratory School of the University of Southern Mindanao, Kabacan, Cotabato, Philippines in the period of the school year 2012-2013 only assuming they have been given the same exam on their pre-test during their grade 7. These heterogeneous groups composed three year levels that each corresponds to the length of retention interval during that time. The subjects studied Advanced Algebra and Geometry during their grade 8, analytical Geometry and Statistics during their grade 9 and Calculus and Trigonometry in their grade 10. The retention interval covered one year, two years and three years. The study limits only on the topics covered in the instruments for the experiment that was obtained in the first and second grading period in their elementary Algebra particularly on the Real Number System, Percentage, Ratio and Proportion, Measurement, Algebraic Expressions, and Polynomials only. Subjects were assumed to have received same level of instruction in their grade 7 was only taught by the same instructor. However, in this study, we do not have control on the exposure to different academic trials facilitated development of expertise and mathematical academic maturity of the students that perhaps might later contribute to the increase of mathematical performance with increasing retention interval. Further, only subjects with complete data on pre-test and retest results were considered in the study. Non-completers are discarded in the analysis. In addition, no transferees were included in this study.

## 2. Materials and methods

### *2.1. Experimental Procedure*

The research method employed a Test-Retest Comparative Design where three year levels represented the different retention intervals. The level treated under one-year retention interval were the grade 8 students who received their test on October 2011; those treated under two years' retention interval were grade 9 students who received their test on October 2010; and lastly those treated under three years' retention interval were grade 10 students who received their test on October 2009, using a teacher made validated test instrument. The same instrument was administered to the same three groups on February 2013. The performance was determined by their scores in the test-retest instrument consisting of 125 item multiple choice exam. Only the retest was carried out since test data were already available and the subjects took the test during their grade seven in high school as part of their quarterly examination. However, it is also customary to describe the events that took place prior to

the retest. In the Philippines school year starts in June and ends in March in the following year but at present some schools are adapting school year from August to June.

### *2.2. Retention Interval*

There were no interventions made by the researcher after the students finished their grade seven in high school. A normal curriculum was followed for their Mathematics. Their only source of rehearsal and elaboration was from discussions and activities conducted during their Mathematics class. The grade 10 students treated with 3-year retention interval, already took Elementary Algebra, Advanced Algebra, Geometry, Analytic Geometry, Statistics, Calculus and Trigonometry. The grade 9 students treated with 2-year retention interval already took Elementary Algebra, Advanced Algebra, and Geometry, Analytic Geometry, and Statistics. The grade 9 students treated with 1-year retention interval already took Elementary Algebra, Advanced Algebra and Geometry.

### *2.3. Present Retest Activities*

With the retention interval, all three sections were all accounted that is to create a heterogeneous representation of each group. On February 2013, the retest was administered using the same material as with test in their grade 7. Papers were checked with anonymity to ensure protection against teacher-student bias. Data in the form of examination scores were gathered. Analyses included comparison between pre-test, retest and gain scores performance of students over different retention intervals.

### *2.4. Statistical Analysis*

The results of the test and retest were compared using t-test for paired samples. To determine the difference on test and retest scores and the mathematics performances gained across retention intervals of the subjects, one-way Analysis of Variance (ANOVA) was used. If significant differences occurred among means, Post hoc mean multiple comparison test is applied.

## 3. Results and Discussion

### *3.1. Comparison of Achievements*

In order to determine how the recall of information can be affected by the length of retention interval and the factors associated with time, three groups of students from University Laboratory Schools were studied. Each group came from different grade levels specifically grade 8 with 118 students, grade 9 with 90 students, and grade 10 with 87 students representing one, two, and three years of retention intervals, respectively.

To establish equivalence of each group in terms of their grade 7 mathematics ability in terms of score performance, their grade 7 first and second grading Mathematics examination results were summed up, used, and compared. Moreover, to determine the effects of retention interval, the same examination was given and compared.

**Table 1:** Test of difference between the total score of math examination during and after instruction. ULS-USM, Kabacan, Cotabato. SY 2012-2013.

| Student Group | Test | n | Mean Score | SD | t | df | p-value | Mean Difference |
|---|---|---|---|---|---|---|---|---|
| **8** | *During* | 118 | 65.04 | 15.377 | 8.763 | 117 | 0.000** | 13.87 |
| | *After* | 118 | 78.92 | 24.044 | | | | |
| **9** | *During* | 90 | 66.61 | 18.384 | 10.179 | 89 | 0.000** | 15.033 |
| | *After* | 90 | 81.64 | 17.472 | | | | |
| **10** | *During* | 87 | 68.89 | 15.067 | 12.89 | 86 | 0.000** | 21.00 |
| | *After* | 87 | 89.89 | 18.976 | | | | |

***–highly significant; p<0.01 (paired t test)*

Table 1 shows the mean scores of each group during instruction. It shows that the present grade 10 students obtained the highest mean score of 68.89 while the current grade 8 students obtained the lowest mean score of 65.04 and grade 9 students ranked in between the two (66.61). Meanwhile, it can also be seen from the table that the scores of the third year students were more dispersed (18.384) than the scores of the grade 8 and grade 10 students (15.377 and 15. 067, respectively). On the other hand, the hierarchy of scores existed between the groups, the scores were not considered to be significantly different due to the relative closeness of the values. This implies that the three groups of students were to be more or less equivalent in terms of their grade 7 mathematics skills and abilities, and were comparable in terms of the knowledge obtained when they took their examination.

After the retention interval specified for each group, the same examination was given to the same students and results were compared. Results revealed that students from each group significantly gained scores even after retention intervals. Comparison of the previous and recent scores, using t-test for paired samples, revealed that the gain of an average of 21 more points was observed for the grade 10 students and was highly significant. Also, there was gain of an average of 15.033 more points on the previous scores of the grade 9 students which was highly significant. Further, for grade 8 there was a gain score 13.87 more points from previous scores and was also highly significant. In the three results, a highly significant difference existed between the achievement during and after instruction of the participants according to their grade year level These results contradicted the studies of [8,9,10,11] which stated that the ability to recall information decreases with increasing retention interval. This decrease is maybe due to memory loss or perhaps have lesser interest to remember or even cause by a disease that affects the brain. However, in this study, it was found out that achievement of students seemed to increase with the retention interval. Based on the result of this study, it was also evident that retention intervals significantly affected the achievement of students in Mathematics for it yielded a significant increase in the scores of students as the length or degree of retention interval increased. However, we do not know yet how this retention interval causes the significant increase of scores, is it due to the students' ability to retained

previous information? Or other exposures that made him more mature on dealing with the given problems to solve. Retention interval may possess in itself a myriad of activities that may enhance and relearn the previously learned material; information and events that may strengthen the bond between prior and recent knowledge and skills; and especially the increased attention span, reasoning skills, and memory storage that come along with maturity. In a similar sense, Reference [12] implicitly revealed some properties of retention interval such as exposure to increasingly multifaceted mathematics courses as students move from one level to the next. Such exposure, according to them build new knowledge that should be integrated with prior knowledge. With the theory of elaboration, basic knowledge is strengthened when it forms a connection with new but highly related ideas, and the more connections are formed, the easier it is for the person to recall information. What might had happened during the retention interval in this study was that as students move from grade 7 to the next level, they encountered new mathematical concepts using the basics learned from their grade 7 Mathematics. In addition, the consistency and frequency of application of the basic mathematics provided the student the chance for rehearsal and elaboration, thereby, strengthening the retention of the basic information.

### *3.2 Comparison of Achievement Across Retention Intervals*

Table 1 illustrated the mean total score of each group during and after retention interval. It did give an implication how the mean score of a group differed from the other in both times of test administration, that is during and after instruction, as well as the difference that existed between the scores obtained during instruction and the scores obtained after the specified retention interval for the group. Though the latter implications were justified by statistical procedures (t-test for paired samples), it did not on the former implication. The former implication required statistical procedures (One-Way Analysis of Variance) in order to validate the following claims that there was no significant difference between the mean score of each group during instruction that is students were equivalent and comparable in terms of their knowledge in mathematics when they took their grade 7 math exam; there existed a significant difference between the mean scores of each group after retention interval that is to establish that different retention intervals can cause variation in student achievement; in terms of the average score gained, each group differed significantly that is to further corroborate the variations brought by different degrees of retention interval. In a concise manner, such justification aimed to reject or accept the second null hypothesis stating that there was no significant difference between the students' mathematics performance gained across different retention intervals.

The scores obtained by the students when they took their grade 7 exam (during instruction) were used to determine if the groups have equivalent mathematical knowledge and skills. Using One-way Analysis of Variance, there was no significant differences ($F=1.398$, p-value=0.249) among the three grade level on their average overall scores obtained during their mathematics examination taken in their grade 7 in high school. These results suggested that in terms of the grade 7 mathematics knowledge and skills, the three groups were said to be equivalent and comparable, the skills and the fact that they were all grade 7 when they took the test, their exposure to the same mathematical experience was facilitated by the same instructor who probably gave the same amount of instruction and contributed to such comparability.

Analysis of the data of the achievement scores for the three groups in the second administration of the same test

yields a highly significant differences in their average scores (F=7.265, p-value=0.001). It would be more evident and substantial, if not customary, if gain in achievement be taken into account.

Scores of each student during and after instruction were compared to obtain the score gained by each. The averages gained scores of each group were then compared shows that there was a highly significant differences on the gain scores among the three retention interval (F =5.603, p-value=0.004).

To summarizes the performances of the students during instruction, after retention interval, and the difference or gain of scores by each group. It can be noted that during instruction, that is when the students took their grade 7 mathematics examination, the current grade 10 students obtained the most average of scores, followed by the grade 9 and then the grade 8 students who got the least average score. Though difference existed between the mean scores, it did not qualify to be significant. This leads to the implication that the students were comparable in terms of their grade 7 mathematics achievement. Each group of student, even if weighed against their mathematics knowledge and ability when they were grade 7 students were compared (see table 2).

**Table 2:** Summary of performance during and after instruction, and the gain in performance. ULS-USM, Kabacan, Cotabato. SY 2012-2013.

| Grade | Retention Interval (years) | During | After | Gain |
|---|---|---|---|---|
| **8** | 1 | 65.04 | 78.92[a] | 13.87[a] *(21.33%)* |
| **9** | 2 | 66.61 | 81.64[a] | 15.03[a] *(22.56 %)* |
| **10** | 3 | 68.89 | 89.89[b] | 21.00[b] *(30.48 %)* |
| **p-value** | | 0.249[ns] | 0.001** | 0.004** |

*ns – not significant at 5% level*

***– Highly Significant (p<0.01)*

*Column mean with similar letter superscripts are not significantly different (Tukey's HSD)*

The examination score during instruction were all considered and assumed having the same mathematical ability, skills and amount of knowledge during their grade 7. Factors about this occurrence perhaps can be attributed to the same amount of exposure to the same mathematical activities (assignment, quizzes, examinations, board-work, and pedagogical methods) as facilitated by the same instructor who probably rendered the same amount of instruction. These results imply that the test-retest and respondent selection

methods used in this study contradicted with studies by [8,11,13,14] in which their results showed that the ability to recall information decreases with increasing interval retention. Intricately, retesting group of students with different age and competency level at present may be as efficient, valid, and reliable as its methodological predecessor, that is retesting similar subjects over different retention intervals. The different groups retested were found to have the same ability and amount of knowledge in Mathematics when their grade 7 exam scores in mathematics were compared. In Table 2, the group performance varied with large differences in mean total score when the effects of retention interval were tested on each group of students considering that such variation in the mean total score resulted from substantial gain in score. It can be observed that the current grade 8 students obtained a mean total score of 65.04 during their first time they took the exam. After one-year retention interval, the results of retake gain of 13.87 points, leading to a mean total score of 78.92. The gain consequently ensued to a test and retest results with high degree of significant difference. Similarly, the present grade 9 students obtained a retest result averaging to 81.64 after gaining an average of 15.03 more points to their first year test score that averages to 66.61, these group retook the exam after two years and resulted a high significant difference in their gain scores. It also showed on the three-year retention interval which gave the present grade 10 students an average gain of 21.00 points to their grade 7 test score that averages to 68.89. Such gain leads the group to a retest mean score of 89.89. It can be noted the performance gain increases with retention interval. The test and retest score on this group proved to have a high significant difference. The comparative analysis of the retest results between each group proved that the variations observed in the mean total score of each group's retest exam generally possessed a high significant difference ($F = 7.265$; p-value = 0.001). Further analyses for the multiple mean total score comparison using Tukey's test showed that the grade 8 and grade 9 groups were not significantly different. Significant differences only occurred when either of the two groups mentioned were compared to the grade 10 group. Additional analysis proved that the discrepancies in the mean total score gained by each group were, in general, significantly different ($F = 5.603$; p-value = 0.004). However, it can be noted that only when the mean score gained by either grade 8 group or grade 9 group was compared with mean score gained by the grade 10 group that significant difference occurred.

### *3.3. Courses and Trends on the Results*

Based on the preceding analyses, two important courses and trends were deduced and were considered for the foundation of the conclusion. The students manifested gain in retest score after the retention intervals and resulted to significantly higher scores. Gains and retest score increased with retention intervals. The foremost goal of this study was to determine the effects of retention interval to the students' performance in Mathematics as manifested by students' scores in an examination conducted when they were grade 7 students and the retake of the same material after a specified length of retention intervals. Under uncertainties, two possibilities were expected at the beginning of the study: First, one can expect that the amount of information recalled decreases after the specified length of retention interval. Such anticipation can be affirmed by mere observation of the everyday activities and through the experiments conducted by [8] when he discovered that the forgetting increases as retention intervals increase; Moreover, Reference [13] found out that as retention interval increase, participants' performance on extensively practiced single-digit multiplication problems deteriorates, and [14] also found that difficulty encountered by participants on complex mathematical problems increased over the long retention intervals however in our study it is not the case since over long retention intervals subject's

mathematical performance increased. . Even though this was the most plausible event that may result from the experiment, this was the least expected for reasons dealt in the succeeding discussions. Lastly and the most expected was that the test and retest scores of each group did not differ significantly. It was highly expected due to the fact the mathematics curriculum in secondary schools is organized and sequenced in which the present subjects – unlike sciences and other course – are interrelated with the former as well as the subsequent subjects to be taken up. This well-known fact provides an avenue for the grade 7 mathematics that is Elementary Algebra to be practiced by the students as they are learning higher or more advanced branches of mathematics. Conversely, what can be noted from Table 1 and Table 2 were far from both expected outcomes. It showed that not only that the students in each group recalled the information required by the exam but, surprisingly, were able to surpass their previous performance. Analyses revealed that such gain resulted to a significantly higher score. Even though the students were now in different ages and year levels, analyses found that their abilities when they were in grade 7 were comparable and equivalent that is, they possessed similar amount of mathematical knowledge and abilities. For this reason, it is customary to consider factors (other than their initial abilities) that may influence the outcome of the experiment. These factors are in terms of the properties of retention interval: exposure to substantial amount of practice or rehearsal, elaboration by related branches of mathematics, and expertise brought by maturity and exposure. To further understand how practice resulted to improved retention of information, consider the study conducted by [14]. Eleven consecutive daily sessions of single digit multiplication problems were given to the two participants. After the training, their retention was measured on regular intervals ranging up to 14 months. After which, they found out that the participants experienced a decrease in difficulty on complex mathematical problems with increased practice. More importantly, it was revealed that when practices were given to respondents, retention of information increased over the long retention intervals. This implies that practice contributes to the durability of retention of information over long periods of time. Such result was consistent with the views of [15,16,17] that explicated the amount of practice acquired through increasing the number of academic avenues led to improved retention of newly learned items. These comments coincided with the results of this study. Practice, as one of the properties of retention interval in this study had been extensively experienced by the students throughout the entire length of retention interval even though the students may not be rehearsing the exact materials as with their grade 8 subject. Apparently, this became possible since elementary algebra was so basic that it was one of the requirements and tools in understanding many advanced branches of mathematics particularly those that followed after the Elementary Algebra in the locale of the study namely, Geometry, Advanced Algebra, Geometry, Analytic Geometry, Trigonometry, and Calculus. As the students undertook new subjects, they encountered new concepts and problems. Thus, in order to understand the concept and solve the problem, the students considered to a much more basic concept. For example, in order to understand the concept of the derivative, a student would recall the concepts of functions, exponents, slope of the line, et cetera. In this case, not only that the new material came into meaning and solved, but also the prior knowledge and skills were reviewed, rehearsed, and activated that is maybe explain to their exposure to much advance topic that provided recall of previous learnings. As this happens frequently, as it always does in mathematics, more and more information is recalled and practiced, thereby, strengthening the retention of the previous material. This explanation corroborated with the idea of [18] that the strength of knowledge and were influenced by regular practice experienced through repeated academic trials. Therefore, home-works, quizzes, examinations, group

discussions and other similar activities can serve as best examples of academic processes since these require the students to practice or re-experience the situation or information over time [18,19]. In addition, higher levels of strength can be achieved when skills, facts, or procedures practiced regularly through similar processes. And more importantly, newly learned materials were retained better when they were associated with prior knowledge [20]. The aforementioned accounts explained why and how information can be recalled in similar situation as in this study. However, it did only a little to explain why retention interval and its underlying properties not only allow the material to be recalled but also to significantly outperform the student's previous records. Forgetting occurs when information underwent a period of inactivity information was neither elaborated nor rehearsed, interference or overlapping of other memories and decay happened when neurochemical memory trace created during encoding disintegrated as time passed [21]. Apparently forgetting occurs most of the time regardless of age. What had happen explained that the gain in scores way back the students' first year (grade 8) in high school and the interval between instruction and test were considered to be relatively small amount of time – one grading period or two months. This limited time may not give enough time for all the students to be exposed to related materials that may strengthen the retention of information can limit the frequency of rehearsal, and may not allow relearning and rehearsing forgotten materials. As compared to the length of time specified for the retention interval, a grading period was relatively not enough for the students to gain an average score obtained after a specified retention interval. Moreover, Reference [12] suggested that new knowledge and skills must build on, and be integrated with, prior knowledge as students move through different levels of school and take increasingly comprehensive math courses. In a concise manner, gains of score occur due to relearning of forgotten materials, consistent rehearsal of information and elaboration of new knowledge to prior knowledge. In this study, perhaps more exposure to different activities of students related to mathematics they have passed since their grade 7 until grade 10, contributed to much recall of previous topics that possibly explained the increased gain in mathematical performance of the students.

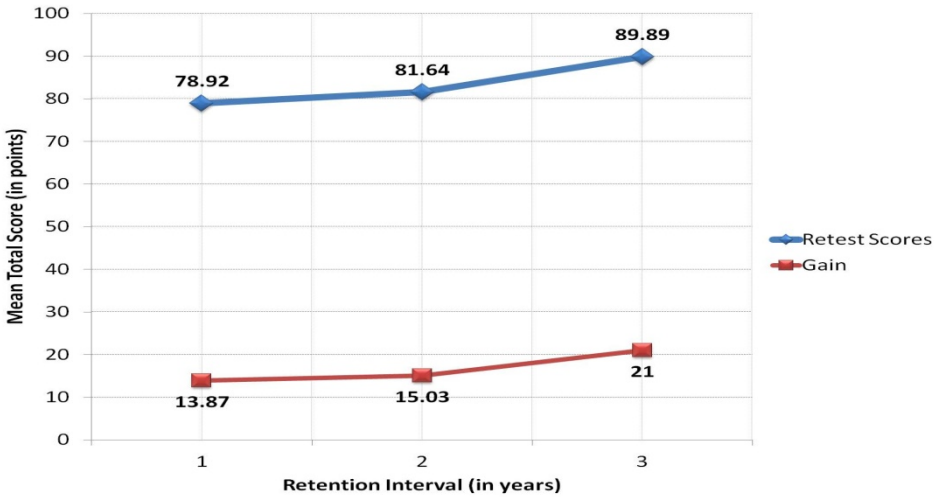



**Figure 1:** Comparison Between Retention Interval and Gain, Retest Score. ULS-USM, Kabacan, Cotabato. SY 2012-2013.

Consistent with the tables 1 and 2 discussed previously, Figure 1 does only show the retest score and the

average gain but also the two results graph against retention interval. Generally speaking, it can be observed that as the retention interval increases, so as the gain of score resulting to a retest score that behaves in similar aspect. Previous analyses revealed that the gains and retest scores were significantly different. It was an atypical result since a less peculiar event would lead to a negative response in every increase in retention interval. As mentioned earlier, the retention intervals in this study possess some properties that would explain this phenomenon. The aforementioned statements left the remaining pages allotted for elucidating the function of expertise that brought by maturity and exposure to material. Reference [22] mentioned that a person's ability to recall a certain material depends heavily on the amount of his or her prior knowledge. This may be possible his exposure to previous knowledge related to Mathematics. Reference [23] also found that amount of prior knowledge was positively correlated to the tendency of the material to be recalled where those who have prior knowledge (experts) had the highest tendency of recalling the material while those who possess the least (novices) behaved otherwise. Experts who tend to detect features and meaningful patterns of information can adapt to new situations, retrieve important aspects of knowledge with little effort and most importantly, accumulate more content knowledge and organize it in a manner that shows understanding of the topic [24]. Reference [21] added that children remember less than adults since they are far less expert in different areas. Apparently experts' knowledge is organized around many ideas and concepts. Moreover, they tend to have deeper understanding of knowledge than novices [25]. Expertise, as implied from the mentioned accounts is a function that involves length of exposure to a subject matter to which an individual can master the subject and the amount of material to which he or she was exposed to. Thus, in relation to this study, one good reason why performance of students increases as retention interval increases is apparently when student stayed longer in school, he or she is much exposed to a more advanced mathematics subject, received longer amount of time to practice, received substantial amount of rehearsals and more importantly learned more new concepts where his or her prior knowledge is linked and in turn strengthens the retention of the basic information. Even though the three groups had equivalent abilities when they were in first year, it was still expected that the current grade 10 students performed better, followed by the grade 9 and grade 8 group, since the said group represented and was retested after the longest retention interval (three years) which entails a longer time to receive enough practice and more information.

This was theoretically and empirically true due to the fact that the grade 10 students attained a certain level of maturity and expertise greater than the other group. It was also true for similar reason as to why the grade 9 group would fall next to the grade 10 group. This idea was supported by [21,26] stating that having many and organized knowledge results to sophisticated system of information, more interconnections and better organization in an area that allows individual to retrieve more information with greater fluency. Moreover, in terms of organization of knowledge, the fourth year group ranked first, followed by the third year and the second year group. This would still hold true since the variation in the length of retention interval before they were retested affected the level and amount of study training they received. Finally, the results are reasonable since the mathematics curriculum is carefully arranged in such a way that it is easier for students to organize the knowledge and skills in a more meaningful way. Concisely, gain in score was a result of longer and frequent practice throughout the retention interval.

## 4. Conclusion

The following conclusions were drawn from these study. Firstly, the retention interval promoted a significant increase in the performance of the same examination. An increase in score significantly differs with varying retention interval were observed and recall of information is strengthened as retention interval increases. Secondly, subjects' exposure to related academic trials such as meaningful and organized knowledge as well as maturity may have contributed to the increase of mathematical performance across retention intervals.

## 5. Recommendations

The authors recommend that first; a similar study shall be conducted to compare mathematical performance that consider using shorter range of retention intervals but with over long periods of time. Perhaps a different result might occur and subject exposure to related academic trials and other organized knowledge might not be a factor for increased performance but rather due to good memory recall of shorter range of retention intervals. Second, this study might be replicated considering different subjects such as elementary pupils or college students as to determine if it yield the same results and not only due to prior knowledge. Lastly, to conduct a similar study which shall consider single group of subjects being taught over 3 years in their grade 8, 9 and 10 and evaluated every year with the same test given in their grade 7.

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