Crocodile Specialist Group, Species Survival Commission

CROCODILES

Proceedings of the 25th Working Meeting of the Crocodile Specialist Group of the Species Survival Commission of IUCN - The International Union for Conservation of Nature - Convened at Santa Fe, Argentina, 7 - 10 May 2018
(Unreviewed)

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Proceedings of the
25th Working Meeting of the Crocodile Specialist Group of the Species Survival Commission of the International Union for Conservation of Nature convened at Santa Fe, Argentina, 7-10 May 2018

(Unedited and Unreviewed)
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Front cover: (top left) "New" technology such as drones is now being used for locating and monitoring *Caiman latirostris* nests (Photograph: Carlos Pina); (top right, bottom left) Local people (gauchos) are key beneficiaries of the Argentine ranching program for *Caiman latirostris* and *Caiman yacare* (Photograph: Pablo Siroski); (bottom right) Linking livelihoods of local people to healthy wild populations allows conservation goals to be achieved (Photograph: Pablo Siroski).

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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, management and sustainable use of crocodilians (crocodiles, alligators, caimans and gharials). The CSG is supported financially through the International Association of Crocodile Specialists Inc. (IACS), and operates under the auspices of the Species Survival Commission (SSC) of the International Union for Conservation of Nature (IUCN). The CSG members in their own right are an international network of experts with the skills needed 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 policies. The CSG itself keeps its members updated on international events with crocodilians, conducts reviews of country programs, and tries to track and prioritise issues in forums such as CITES that encourage legal trade and discourage illegal trade. CSG Working Meetings are generally held every two years.

Foreword

Binennial CSG Working Meetings are remarkable forums, highly valued by CSG members, who fund their own travel and expenses to attend. The 25th Working Meeting (7-10 May 2018) was no different. It was attended by 236 delegates from 30 countries, and maintained the very high standard of formal proceedings ... and a vibrant social agenda!

The meeting was organised by CSG Deputy Chair Alejandro Larriera, with the Proyecto Yacaré team. It was held at the Universidad Nacional del Litoral Campus, Santa Fe, Argentina, and hosted by State and Local Governments and a range of other sponsors. I extend our gratitude to everyone concerned on behalf of all CSG members.

The general theme for the meeting was *"Thirty years later ... a problem becomes a livelihood"*. Sub-themes included: biology and general research; conservation and sustainable use; zoos and education; and, human-crocodilian conflict. Once again a Veterinary Workshop (5 May) with 38 participants preceded the Working Meeting. The CSG Steering Committee meeting (6 May) gave all CSG members the opportunity to contribute to CSG core business and directions. We were delighted that the SSC Chair Dr. Jon Paul Rodriguez could attend.

The working meeting (7-10 May) saw a wide range of research subjects and review articles presented, by young and old researchers. There was a major poster session, with additional material and various thematic and working group meetings - Veterinary Science, IUCN Red List Authority, Zoos, Taxonomy, Future Leaders, Human-Crocodile Conflict, and the erosion of conservation benefits linked to overproduction on farms and changed marketing strategies within the fashion industry.

Many of these results are contained in the Proceedings of the 25th Working Meeting, which is arguably the most important output of Working Meetings. Our thanks to authors of papers and members of Proyecto Yacaré for assembling and compiling the Proceedings.

Professor Grahame Webb, *CSG Chair*

Summary of the Meeting

The 25th CSG Working Meeting was held at the Faculty of Biology Conference Centre at the Universidad Nacional del Litoral Campus, Santa Fe, Argentina, on 7-10 May 2018. The meeting was attended by 236 delegates from 30 countries (Argentina, Australia, Benin, Belize, Bolivia, Brazil, Colombia, Costa Rica, Cuba, Denmark, France, Germany, Hong Kong, India, Ireland, Italy, Japan, Malaysia, Mexico, Norway, Panama, Philippines, Slovakia, South Africa, Spain, United Arab Emirates, United Kingdom, United States of America, Venezuela, Zambia). The meeting was hosted by the State Government Ministry of Environment (represented by Jacinto Speranza) and Local Government (represented by Pablo Tabares as Director of the Agency for International Cooperation, Investments and Foreign Trade of the Government of the City of Santa Fe), and many additional sponsors provided financial support. The principal non-government sponsors were: Mutual de la Unión Personal Civil de la Nación (MUPCN); Yacaré Porá (farm from Corrientes Province); Trachter Genuine Reptile Skins; and, Asociación Biológica Santa Fe (BIOS).

The theme for the meeting was "*Thirty years later ... a problem becomes a livelihood*", and included a strong emphasis on programs looking at strategies to generate incentives for conservation and livelihoods. Presentations covered: biology and general research; conservation and sustainable use; zoos and education; and, human-crocodilian conflict. The Organising Committee (Alejandro Larriera, Alba Imhof, Lucía Fernandez, Carlos Piña, Guillermo Príncipe, Melina Simoncini, María Virginia Parachú Marcó, Gisela Poletta, Pablo Siroski, and the whole Proyecto Yacaré team) ensured that the venue, program, sponsors, entertainment, etc., were in place. The Organizing Committee also assisted with the complex logistic arrangements required by delegates from around the world. A Veterinary Workshop preceded the working meeting on 5 May 2018 at the Proyecto Yacaré facilities (Santa Fe). Unfortunately, participation had to be capped at 38 participants. Enormous thanks to the local team (María Virginia Parachú Marcó, Gisela Poletta, Patricia Amavet, Pablo Siroski and volunteers) for coordinating the event, and to Paolo Martelli, Cathy Shilton and Marisa Tellez, who presented the various veterinary activities.

Figure 1. Veterinary Workshop. Photograph: Pablo Siroski.

Following the CSG Steering Committee meeting on 6 May 2018, a cocktail reception was organized on a boat that made its way along the river surrounding Santa Fe City and natural environments characteristic of the Parana River Islands. The official opening of the working meeting on 7 May 2018 included addresses by CSG Deputy Chair Alejandro Larriera, CSG Chair Grahame Webb, Jacinto Speranza (Minister of Environment of Santa Fe Province), Jon Paul Rodriguez (Chair, IUCN Species Survival Commission), Laura Tarabella (Head, Faculty of Humanities and Sciences, UNL) and Pablo Tabares (Government of the City of Santa Fe).

Figure 2. Official opening. From left: Alejandro Larriera, Grahame Webb, Laura Tarabella, Jacinto Speranza, Pablo Tabares, Jon Paul Rodriguez. Photograph: Alvaro Velasco.

For some presenters, this was their first time participating in a CSG working meeting, and simultaneous translation for presentations was very helpful to both English- and Spanish-speaking participants. To assist translators, the schedule of presentations was coordinated on the basis of themes and the language of presenters. At the beginning of each of the four days of the meeting, a plenary lecture was delivered: Alejandro Larriera (Argentine crocodile conservation programs assessment: Which is the baseline?); Jon Paul Rodriguez (The role of the IUCN Species Survival Commission in saving the world's species); Grahame Webb (Learning as we go); and, Perran Ross (Dynamic change in crocodylian conservation: An historic perspective on CSG's role and work).

The first day of the meeting was dedicated to "Local issues", beginning with presentations on research and history of Proyecto Yacaré, followed presentations on conservation and sustainable use within management programs in Bolivia (*Ca. yacare*), Colombia (*C. intermedius*), Brazil (*Ca. latirostris*) and Mexico (*C. acutus*). Later in the day, presentations covered a range of topics, including body condition score for crocodilians, sexual identification of *Ca. latirostris* hatchlings by cloacal inspection, and microorganisms in oral, blood and cloacal samples from crocodilians, etc. Before dinner, delegates enjoyed a tango music show. The second day was dedicated to a biology and general research, and covered diverse topics ranging from morphometry, embryology, nesting behavior and predation, influence of climatic variables on different physiological systems, general behavior and the impact of pollution.

The poster session followed the afternoon break, where participants enjoyed a beverage and spoke with poster authors.

Figure 3. Poster session. Photograph: Alvaro Velasco.

On the third day, the session on biology and general research continued, and presentations explored environmental health, chemical pollution and ecotoxicology effects on different species with particular focus on genes expressions and biochemistry. Also, a update on humane slaughter methods for crocodilians was highlighted. The rest of the day included presentations on conservation and sustainable use, with focus on population ecology, status, and novel tools (eg drones, apps.) to improve the work in crocodilian habitats for conservation and management purposes. During the dinner, a local music band presented a dance show with traditional dancers dressed as "gauchos". The last day covered conservation and sustainable use, as well as HCC, zoos and education themes, and included new ranching programs in Mexico, interesting studies on ecology and education on crocodilian conservation in northeastern Brazil and the Philippines, telemetry studies, and updates on conservation of the Critically Endangered *Mecistops cataphractus* and Gharial, the "European Croc Network" and a current perspective on HCC and some mechanisms to mitigate them, amongst others. In addition to the oral presentations and the poster session, various thematic and working groups met as side-meetings, including; Veterinary Science (Dr. Paolo Martelli, Dr. Cathy Shilton), IUCN Red List Authority (Dr. Perran Ross, Dr. Sally Isberg, Dr. Sergio Balaguera-Reina), Zoos (Dr. Kent Vliet), Taxonomy (Dr. Kent Vliet), Future Leaders (Dr. Matt Shirley, Dr. Marisa Tellez). Participants interested in human-crocodile conflict (Dr. Simon Pooley) also met, as did Regional Chairs from Latin America and the Caribbean (Alfonso Llobet, Alvaro Velasco, Dr. Pablo Siroski).

The Argentinean Banquet on the final night was a highlight of the social agenda, with a typical music show called "gauchos night". After dinner, CSG Chair Grahame Webb presented various prizes and awards:

- The Castillo Award for Crocodilian Conservation was presented to Alejandro Larriera on behalf of Projecto Yacaré. Although the Castillo Award is typically awarded to an individual, the contribution of Proyecto Yacaré to crocodilian

research, management and conservation over a long period of time is considered exemplary.

- The Chair's Encouragement Award was shared by Pablo Siroski ($US250) and Sergio Balaguera-Reina ($US250).
- Prizes for student oral presentations were awarded to Joseph Brown ($US250), Sergio Balaguera-Reina ($US150) and Rob Gandola ($US50).
- Prizes for student posters were awarded to Sierra McLinn ($250), Todd Bell ($150), America Jaimes ($50) and Karin Ebey ($50).

We thank the judges who devoted their time and effort to assessing more than 60 student oral presentations and posters.

Figure 4. Projecto Yacaré team. Photograph: Alvaro Velasco.

Figure 5. Student prize winners flanked by CSG Chair Grahame Webb (right) and CSG Deputy Chair Alejandro Larriera (left). Photograph: Alvaro Velasco.

For the first time in CSG history, the meeting website provided an option for participants to donate money for student travel grants through the registration process. This resulted in $US2250 being raised, which allowed financial assistance for 9 students ($US250 each; Sierra McLinn, Etiam Perez Fleitas, Joseph Brown, André Felipe Barreto Lima, Fabiola Mejías Rodriguez, André Costa Pereira, Stephany Michelle Del Rosario Rivera, Grégoire Boussens-Dumon, Felipe Parra). A donation of $US2000 from the Ebey family was matched by the CSG, thus providing $US4000 to support the attendance of 8 students from Central and South America ($US500 each; Edgar Marina, Juan Carlos Morales Franco, Verónica Arias Perez, Nidia Farfán Ardila, Paulo Braga Mascarenhas Júnior, Yairen Yairen Alonso Jiménez, Gustavo Soza Rodríguez, America María Jaimes Ortiz). We thank the Ebey family, the CSG, and participants who contributed funds that allowed the participation of so many students to the working meeting.

On Tuesday night, the traditional auction took place, and it was once again skillfully choreographed by Carlos Piña and Shawn Heflick. A variety of items were donated, some of which were offered through a silent auction. The silent and open auctions raised $US9216, and Dr. Rich Fergusson ($US500) and Dr. Jeff Lang ($US300) kindly added to the proceeds, bringing the total to $US10,016. These funds will be used to develop a project to update the status of *C. latirostris* populations in Bolivia, Paraguay and Uruguay.

Figure 6. Auctioneers Carlos Piña and Shawn Heflick in action. Photograph: Alvaro Velasco.

A raffle with prizes of *Ca. latirostris* leather products raised $US830, which will be used to evaluate a system of socioeconomic indicators that provide the necessary information for the promotion of socio-environmental policies. These policies must be oriented towards the conservation of resources on the basis of the recognition generated by the integration of livelihoods, an essential asset in the development of the region (Santa Fe and Corrientes Provinces).

Also for the first time in the CSG's history, the group photograph was taken using a drone. Due to heavy rain, the planned field trips on 11 May had to be cancelled. However, a trip was organised to The Archaeological Park, The Old City of Santa Fe, at Cayasta. We thank Carlos Piña who acted as translator for the tour guide, and who added his unique humorous spin to the translation. Lunch at the Vuelta del Pirata Restaurant allowed participants to sample a variety of the region's fish dishes.

Pablo Siroski, *member of the 25th Working Meeting Organising Committee (cocokaima@hotmail.com)*

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Evaluation of the social, Economic and Cultural Impact of *Proyecto Yacare*: Key Issues of a Desired Sustainability

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The sustainable use of wild fauna of commercial interest is an alternative that assesses our ecosystems, provided that its profits benefits may act as the stimulus for its preservation. *Proyecto Yacaré* is a clear example of this. It is oriented towards the management of caiman under Ranching, favoring and involving local communities. There are three projects in Argentina that include more than 1000 people related to the annual harvest. The use of natural resources is an essential activity for rural inhabitants, because it allows them to generate an important economic income. Although the variable regarding the income of the activity is already recognized, with the realization of this study, variables that increase our knowledge about the producer are being analyzed, such as theirs families, theirs community and the environment where they live. Therefore, they are not considered anymore as a link in the productive system, but they are considered as a special population of interest to be studied. For this, the development and evaluation of a system of indicators is needed in order to evaluate the situation of the societies that live in rural areas. The methodology for the evaluation of socioeconomic, cultural, and environmental impact implies fulfilling basic requirements of validity and reliability in order to give legitimacy to our results. In this sense, instruments for gathering information were developed as qualitative interviews and structured surveys, promoting the gathering of information where the dialogue between the interviewer and the interviewee assumes a preponderant role. Currently, the first surveys corresponding to the reality of these people are being carried out. They have a very good predisposition when interacting with the different interviewers considering that part of the team are old members of *Proyecto Yacaré*. The tools used are in permanent change; although they serve to collect the variables of our interest. We must recognize that as we move forward in this study, new tools emerge that serve us for a future. This allows us to raise, project and carry out ideas that benefit to the protagonist arguments of the annual harvests, transforming their socio-economic and cultural weaknesses in strength.

Keywords: local communities, caimans, sustainable use, Proyecto Yacaré

Caimans in Argentina, Some History and Environmental Education

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The man and the caimans have lived together in our area for thousands of years. The ancient cultures of used the meat and leather of the caimans, as well as their fat for medicinal purposes. The Jesuit Florian Paucke arrived in Santa Fe in 1752 and left among his drawings, images of the caiman in delicate watercolors that are still preserved in Austria today. The "El último malón", a silent Argentinean film filmed in San Javier and premiered in 1918, includes images of Mocoví Indians hunting broad snouted caimans with spears. Margarito Tereré was a popular character of Argentine television and the cinema that began in the 1970s and represented a Yacare caiman from Corrientes dressed as a gaucho. The Yacaré Project has been operating in the city of Santa Fe for almost 30 years, however, the relationship with the society in which its activities are carried out remained within the academic scope and the necessary relations of a Ranching program with the local people, work that is widely documented. But it is only in 2015 that non-formal Environmental Education activities planned at the time of the hatching season begun to be carried out. Through social networks and media, the community of Santa Fe and surrounding areas were invited to visit the Project facilities on certain days of the week, after confirmation that the hatching is occurring. There they are received by researchers and interns of the same who give a small talk about the activities, a video is projected and children and adults are encouraged to collaborate in the births and eggs management, after a brief talk about the correct way to do it. More than 1000 people have participated on these activities up to now during the last four seasons and the number is going up, on the other hand, the interaction with the community by means of questionaries and interviews with them, allows us to evaluate the benefits of such interactions on the social understanding of the sustainable use programs as a tool for ecosystem conservation.

Keywords: caimans, environmental education

Sustainable Use Programs of *Caiman yacare* Wild Eggs by Indigenous Communities of Beni, Bolivia

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For more than 10 years, Crocoland Farm has specialized in the management, conservation, and sustainable use of *Caiman yacare* based on a mixed system of ranching and farming. Their success inspired multiple propositions since 2014 to increase the number of communal caiman farms in Bolivia to help develop sustainable use programs in rural areas and support the private sector economies. In the last three years, two experimental community-owned caiman farms were constructed in the northeastern Bolivian department of Beni, one in San Lorenzo and another in Beremos. Training workshops were organized to educate each community on the methodologies of population monitoring and wild egg harvesting. Required infrastructure such as egg incubators and hatchling maturation pools were also installed at each farm. Crocoland Farm guarantees to purchase all products from the communal farms with the understanding that 10% of the animals raised must be reintroduced to the wild. The San Lorenzo farm commercialized 4210 juveniles in 2017 and reintroduced 467 juveniles to the wild, while the Beremos farm retailed 750 juveniles and reintroduced 83 individuals to the wild. Both sustainable communal farms continue operations to this day. To propound their success, new *Caiman yacare* farms are projected for construction in the coming years in other territories. From the beginning, Crocoland Farm has financed the construction of communal caiman farms in conjunction with indigenous institutions, environmental authorities, and universities. The aim is to generate economic alternatives for rural communities through the revalorization of natural resources contributing to the knowledge of the species' natural history, conservation and sustainable management of *Caiman yacare* and its habitat.

Keywords: farms, communities, sustainable use, conservation

Management, Transportation and Liberation Strategies of the Caimán Llanero or Orinoco Crocodile (*Crocodylus intermedius*) in Colombia

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Introduction: One way of action in the National Program to conservate the Orinoco Crocodile (*Crocodylus intermedius*) in Colombia, is the reintroduction of the specimens to wildlife; The National University of Colombia has extensive experience in handle this animals in captivity, their transportation and their liberation. That was the reason why in 2015, the University associated with the Regional Environmental Corporation (Cormacarena) made the first liberation of 4 animals and in 2017 were liberated another 15 specimens. To that process were designed strategies, procedures and protocols to apply during the operation; that included the animal selection, veterinary medical examination and the way to capture the animals which would be transported by car, plane and boat and liberated in a specific selected place. **General objective:** Provide guidelines to manipulate, transportation and liberation of the Orinoco Crocodile (*Crocodylus intermedius*) for future liberations in Colombia. **Materials and methods:** In 2015 were selected 4 specimens of *Crocodylus intermedius*, which were liberated in Guayabero River located in Meta department. After that were selected in 2017 another 15 animals which were liberates in Manacacias River located in Meta department too. For that activity were designed different procedures to select, enroll and capture the animals, others protocols to guarantee the safe transportation by land, air and maritime tracks and a guide to stablish strategies of liberation in the selected place. In 2017 were adjusted the protocols, procediments and guides to the liberation of the animals in the new conditions of the Manacacias River. **Results and conclusions:** Results and conclusions: In the first liberation (2015) were selected 2 male and 2 female animals with different genotypic characteristics. A veterinary clinical evaluation was made, blood and cloacal swab samples were taken also the animals were isolated in quarantine. During that procedure, it was planificated the route and hour of transportation, it was designed a special model of kennels which were build to animal restraint and movilization. Groups of animals were selected to be liberated in 2 different sites of the Guayabero River. In 2017, for the second liberation the procedures were adjusted according to the specific conditions of the Manacacias River. The planification and stablish of contingency plans strengthen the crocodile liberation programs in Colombia and permit generate guidelines to be applied in future liberations.

Keywords: conservation, management, transportation, liberation, *Crocodylus intermedius*

The Broad-snouted Caiman (*Caiman latirostris*) Farming Program in Brazil

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From 1998 to 2009 the "Captive Breeding Program of the Broad-Snouted Caiman (*Caiman latirostris*)" at the University of São Paulo, in Piracicaba, State of São Paulo in Brazil, has distributed a bit more than a thousand animals to a dozen farmers. Currently, the captive population of the species in São Paulo, Rio de Janeiro and Minas Gerais States reached approximately 7000 individuals with an annual production of 2000 hatchling per year. São Paulo State has a human population like Argentina in an area like the Britain, with 1/3 of the Brazilian GDP, with a well-developed agroindustry, including a strong chicken production. The use of chicken production dejects might allow the establishment of 10 to 15 thousand farmers in the future 20 years, based on a conversion rate of 4:1, with an expected annual production of 300 thousand animals. São Paulo has a huge consumption potential for both caiman meat and leather due to its strong internal market. The Farming Program of the Broad-snouted Caiman (FPBNC) is being currently established based on the following directions: a) Standardized pen-design and management; b) Water and sewage full treatment; c) Full trackability of animals and their products; and, d) The resume of the Regional Studbook of the species. In parallel, an active research program in action for economic-financial evaluation analyses and marketing to assure economic viability. The current program has the support of local farmers and the São Paulo State Research Foundation (FAPESP).

The Bita River: the Next Target to Recover the Orinoco Crocodile in Colombia

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The Orinoco crocodile was overexploited during the first half of the twentieth century and for this reason today is considered as the crocodile most threatened of extinction in Neotropic. Recent population assessments estimated less than 250 adult individuals in the wild. To prevent the extinction of the species, Colombia established a National Program for the Conservation of the Orinoco crocodile focused on reintroduction as conservation strategy. Following the guidelines of the national conservation program, the goals of our research were to assess the populations of crocodilians, the availability and quality of habitat and the human pressures along the Bita River in Vichada state of Colombia with the aim to determine the adequate sites in the river to perform reintroduction of populations in the near future. Between 2016 and 2017 three fieldwork trips was performed through the rainy, dry and transitional seasons. We surveyed crocodilian populations using nocturnal spotlight and diurnal counts. We quantified habitat attributes related to refuge, reproduction and feeding needs of the Orinoco crocodile. We mapped the anthropogenic pressures along the river and additionally we surveyed people along the river in order to describe the human perception about the crocodilians. We recorded only two species of crocodilians, *Caiman crocodilus* and *Paleosuchus palpebrosus* with mean abundances of 3.1 and 0.5 individuals/km respectively. The population structure of *C. crocodilus* was represented by all size classes while population structure of *P. palpebrosus* was dominated by the adult class. The assessment of habitat indicates that habitats elements related with crocodile refuge requirements were common along river but the elements related with nesting needs were low (0.83 beaches/km). Riparian forests showed good conservation conditions, in addition the prey offer (fishes, birds and mammals) were high. We detected that the human pressures is relatively low along river but it increase in dry season and especially at the river mouth. Mainly anthropogenic pressures were water sports, commerce and subsistence fishing activities. The Bita river arises as a key area to recover the Orinoco crocodile due to its good habitat conditions, low human pressures and low abundance of other competitors crocodilians. Our data indicates that the best conditions occur at the middle of the river and we predict that reintroduction processes performed surely will be successful.

Keywords: species reintroduction, habitat assessment, human pressures assesment, crocodilians assessment

Lagoon Lizards: a Metapopulation of the Royal Lizard (*Crocodylus acutus*) in the Biosphere Reserve "La Encrucijada", Chiapas, México

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Introduction. The Biosphere Reserve "La Encrucijada" is one of the areas that can guarantee the survival of crocodilians and shelter the best populations of *Crocodylus acutus* from the coast of Chiapas. Information received by local people says there is a lagoon with more than 300 royal lizards in the center of a massif of mangrove island, which could have been closed after a meteorological phenomenon in 2005. **General objective**. Locate and evaluate the real lizard metapopulation. **Material and methods**. Prospective visits were made to locate the metapopulation of the royal lizard. The visits were in 2013, 2014, 2015 and 2017. For the transfer to the site, a boat with outboard motor of 25hp was used, walking tours were made to the lagoon and in the periphery of it for data collection. The coordinates of the site, surface, types of vegetation, number of specimens observed, were recorded with photographic and video. For each observation of specimens was estimated the total length of the organism to classify them by categories. For this, the equipment used were GPS, field notebooks, camera, strings and tapes to capture some specimens. **Results**. The lagoon has a perimeter of 1230 m and an approximate area of 5 hectares. In April 2013, 140 crocodiles were counted, in February 2014= 20, in April 2015= 20 and in February 2017= 30 crocodiles. All these years without the presence of offspring. The highest encounter rate was 28 crocodiles per ha or 116 crocodiles per km, a report never documented in the REBIEN and possibly nationwide. On 22 March 2017, illegal looting and exploitation of royal lizard occurred on the site, 17 dead individuals were found tied hands, legs and jaws and 19 specimens with the same condition but alive. Live specimens were unleashed and released to the same environment. During the verification and rescue of specimens an egg was observed, but there was no presence of neonates or offspring in the lagoon. **Conclusion.** The metapopulation was localized and there is a possibility that similar sites exist in the REBIEN, however, it is observed that these isolated populations are being directly affected, therefore, it is a priority to continue with a monitoring established annually to know their dynamics, protection and surveillance, evaluate their recovery to promote their preservation and conservation with the inclusion of community groups for future actions of sustainable use.

Keywords: La Encrucijada, Lizard Lagoon, metapopulation, royal lizard

A Body Condition Score for Crocodilians

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The corporal condition (cc) it's an important tool in ecological studies indicates the fitness of the animals. Traditionally in crocodiles the Fulton index (K) is the most used; however, it presents shortcomings because is based on the relationship between weight and mass under the assumption of isometric growth. Their interpretation is complicated and in many cases requires a secondary analysis for a biological meaning. Because of these shortcomings, in this study the body condition score (BCS) was adapted to crocodilians, based on the morphological subjective evaluation of the energy reserves located at specific anatomical points obtain values ranging from 1 (starvation) to 5 (obesity), 3 as optimal; 2 and 4 represent intermediate states between starvation and obesity. We captured 26 crocodiles of the classes I, II, III and V, length and weight were measured. K was calculated, which averaged 3.0 ± 0.1 and based on four anatomical points the BCS was calculated (average 2.84 ± 0.1). Class I presented a K= 2.2 ± 0.06 and a BCS= 3 ± 0, class II a K= 2.1 ± 0.1 and a BCS= 1.6 ± 0.3, class III obtained a K= 3.6 ± 0 and a BCS= 3 ± 0, and class V presented a K= 5.5 ± 0.4 and a BCS= 3 ± 0.2. The values obtained were related through linear regressions. The correlation between weight, length and K was R^2= 0.75 and R^2= 0.81 respectively. Weight, length and BCS was R^2= 0.028 and R^2= 0.001 respectively; the relationship between K and BCS was R^2= 0.05.These correlations show us that the design of the index is fundamentally different (K conditions the cc to the weight and length, BCS determines the energy reserves, which do not necessarily depend of weight or length) that can generate interpretive biases. The cc of K increases as the individuals are larger, due to the isometric basis of the calculation, while the cc of BCS is independent of size. To try to solve this bias in K, secondary mathematical analyzes are required, which could lead more interpretive problems if not corroborated with direct methods. Using BSC allows us compare the cc of each size class, as well as individuals of the same class but of different sizes. This study proposes the simultaneous use of BCS to corroborate and increase the information and the ecological interpretations of the cc.

Keywords: corporal condition, nutrition, anatomical points

Nationwide Survey of Orinoco Crocodile (*Crocodylus intermedius*) in Venezuela: 1st Report

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Abstract

The Orinoco crocodile (*Crocodylus intermedius*) is one of the most endangered crocodile species on the planet, found only in the Orinoco river basin and its tributaries in Colombia and Venezuela. The first population studies within its historical distribution in Venezuela were conducted by Godshalk and Sosa (1978) and Thorbjarnarson and Hernandez (1992) and over the years other studies have been conducted in specific and limited locations. The National Census of the Orinoco Crocodile in Venezuela seeks to determine and update the current status of the species, through population censuses in localities in which the presence is known, or by reports in the past, areas where specimens have been released from the captive breeding program and new locations where there are reports of its presence, but which has not been corroborated recently. It is planned to visit 27 locations throughout the country, in order to perform day and night monitoring that will determine the existence, abundance and size structure of the population, as well as a general characterization of the habitat where the specimens are observed. The study began at the end of 2016 and will continue until the end of the dry season of 2018, having visited 15 locations so far. Two important discoveries have been carried out, the first are the establishment of a third reproductive population in Estero de Camaguán Guárico state, generated with Cayman liberated in 2008-2009 and the second the increase number of nest in Capanaparo River. The main problems for the development of fieldwork have been the increase in food costs, little or no availability of four-wheel drive vehicles for rent and public insecurity in some areas to visit. These aspects have affected or impeded the realization of visits or censuses in tributaries of main rivers such as Capanaparo and Arauca. This study has been made possible thanks to the support of the Crocodile Specialists Group (CSG) of the World Union for the Conservation of Nature (IUCN), CrocFest and Río Verde, as well as of all the local inhabitants, guides and boat drivers who have supported the field work.

Keywords: crocodiles, Orinoco crocodile, *Crocodylus intermedus*, Venezuela, survey

Resumen

El caimán del Orinoco (*Crocodylus intermedius*) es una de las especies de cocodrilos más amenazadas de extinción en el planeta, encontrándose sólo en la cuenca del río Orinoco y sus afluentes en Colombia y Venezuela. Los primeros estudios poblacionales dentro de su área de distribución histórica en Venezuela fueron realizados por Godshalk y Sosa (1978) y Thorbjarnarson y Hernandez (1992) y a lolargo de los años otros estudios se han realizado en localidades específicas y puntuales. El Censo Nacional de Caimán del Orinoco en Venezuela busca determinar y actualizar el status actual de la especie, a través de censos poblacionales en localidades en las cuales se conoce la

presencia, bien por reportes en el pasado, áreas donde se han liberado ejemplares provenientes del programa de cría en cautiverio y localidades nuevas donde se tienen reportes de su presencia, pero la cual no ha sido corroborada recientemente. Se tiene previsto visitar 29 localidades en todo el país, a fin de realizar censos diurnos y nocturnos que determinarán la existencia, abundancia y estructura de tamaños de la población, así como una caracterización general del hábitat donde se observen los ejemplares. El estudio comenzó a finales del 2016 y se prolongará hasta la finalización de la temporada seca del 2018, habiéndose visitado hasta el momento 15 localidades. Dos importantes descubrimientos se han realizados, el primero es el establecimiento de una tercera población reproductiva en el Estero de Camaguán estado Guárico, generada con ejemplares liberados en el año 2008-2009 y la segunda el aumento en la nidificación en el río Capanaparo. Los principales problemas para el desarrollo del trabajo de campo han sido la poca y costosa disponibilidad de alimentos, poca o ninguna disponibilidad de alquiler de vehículos de doble tracción, fuertes limitaciones para obtener dinero en efectivo para pagar servicios en campo, y la inseguridad pública en algunas áreas a visitar. Estos aspectos han afectado o impedido la realización de visitas o censos en afluentes de ríos principales como el Capanaparo y el Arauca. Este estudio se ha podido adelantar gracias al apoyo del Grupo de Especialistas en Cocodrilos (CSG) de la Unión Mundial para la Conservación de la Naturaleza (IUCN), CrocFest y Río Verde, así como de todos los pobladores locales, baquianos y motoristas que nos han apoyado en el trabajo de campo.

Palabras claves: cocodrilos, Caimán del Orinoco, *Crocodylus intermedius*, Venezuela, censos

Introduction

The Orinoco Cayman (*Crocodylus intermedius*) is one of the biggest crocodiles species in the world and originally it was distributed in the basin of Orinoco River in Colombia and Venezuela. It is classified by the International Union for the Conservation of Nature (IUCN) like in Critical Danger (IUCN 2007); the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) places it in the Appendix I (CITES, 2017) protecting the species of the international trade. In Venezuela it is listed in the List of Species in Danger of Extinction (Decreto 1.486 of the 09/11/1996), in the Official List of Animals Preserves (Decreto 1.485 of the 09/11/1996) and qualified as in danger of extinction in the Red Book of the Venezuelan Fauna (Rodríguez and Rojas 2008).

The first national census of Orinoco Cayman was published by Godshalk and Sosa (1978) a work carried out with the support of Fudena. This study determined the status of the wild populations in 9 differentiated regions, equivalent to 68 rivers or creeks, covering an approximate 10,790 lineal km of rivers or creeks, and reporting an abundance of 273 Orinoco Cayman. In 1987, Ayarzagüena carries out air censuses covering 390 km observing 595 crocs. Thorbjarnanson and Hernández (1992), 12 places traveled with a surface of 1911 km^2 and they reported a population of 73 crocs.

Other 12 studies have been published that evaluated the status of the different subpopulations in wildlife in punctual places as: the hydric system Cojedes River, National Park Santos Luzardo Capanaparo-Cinaruco, Wildlife Refuge Caño Guaritico, among others (Ramo and Busto 1984; Franz *et al.* 1985; Seijas and Meza 1994; Seijas and Chávez 2000; Chávez 2000; Llobet and Seijas 2002; Llobet and Seijas 2003; Antelo 2008; Ávila 2008; Mena *et al*. 2010; Espinoza and Seijas 2012; Moreno 2012).

The first Action Plan was product of a workshop organized by Fudena, "Action Plan: Survival of the Orinoco Cayman in Venezuela 1994-1999" (Fudena 1993), which contained and developed the guidelines and actions to take for the conservation of *Crocodylus intermedius* in Venezuela. In 1994, the Autonomous Service of Fauna (Profauna) of the Ministry of the Environment and the Resources Natural Renewable (MARNR) publishes the Strategic Plan: "Survival of the Orinoco Cayman in Venezuela" (Profauna1994), document that designed the strategies to fulfill the Action Plan, in a 15 year-old period. In the 2003 was carried out an evaluation of the implemented actions described in the previously mentioned documents and the Plan of Action is upgraded (ONDB 2003), which includes as additional strategy the international cooperation.

The Conservation Program of Orinoco Cayman in Venezuela begins in the decade of the 70 (Quero *et al*. 1995) consisted in captivity of gathered hatchlings from wild and born in the farms for their later liberation or reintroduction to the natural habitat (Ayarzagüena 1990).

In 28 years it was liberated 10,164 Orinoco Cayman (GECV, pers. comm.). Babarro (2014) carries out a balance of these liberations and concluding that this action has been successful, but that however should be carried out a bigger emphasis in the wild populations surveillance and management. The successes of the Conservation Program, is generation a reproductive population in the Wildlife Refuge Caño Guaritico-El Frío ranch, Apure state, (Velasco 1999; Velasco *et al*. 2008). Additionally the same situation has been observed in the Cedral ranch Apure state with the establishment a small reproductive population with 7 confirmed females, product of animals liberated there.

Balaguera-Reina and col. (2017) carried out an analysis of the status of the conservation and priorities of the habitat for the Orinoco Cayman in Venezuela and Colombia. For Venezuela, the authors identify 17 places with conservation priority with better conditions to maintain populations.

Objectives

The general objective of this project is to carry out a national census of *Crocodylus intermedius*, in order to establish the current state of its wild populations in locations high-priority, included geographical places where the possible presence of this species has been informed, but there are not recent available data.

The parameters to be determinate are abundance, density and sizes class structure, estimate of abundance of nests and of being possible to evaluate the reproductive annual success inside each subpopulation.

Study Area

It was carried out a bibliography revision that reports populations in Venezuela, elaborating a database in which was for each author, the census locality, total individuals and size class reported, and traveled longitude. The principal result was identifying 51 localities reported and only in 37 was observed *Crocodylus intermedius*. Based on this revision, we select 29 localities to visit, that include 14 with historical and 10 suggested by Balaguera-Reina *et al.* (2017) and 14 new places (Table 1; Fig. 1).

Localities to be visit	Survey	Localities to be visit	Survey
Guaritico Creeks	X	Guamacho Lagoon (Estero de Camaguan)	X
La Ramera-Macanillal system	X	Cuchivero River	X
Manapire River	X	Garza Creek (Hato Garza)	X
Cojedes River system	X	Parguasa River	
Capanaparo River	X	Suapure River	
Anaro and Suripa Rivers		Wildlife Refuge tortuga Arrau (Orinoco river)	
Arauca River	X	Caribe Creek	
La Colorada Creek	X	Riecito River	
Zuata River	X	Guarico River	
Orituco River		El Caballo Creek	
Cinaruco River		Aceite River	
Portuguesa River		Caris River	X
Tucupido River and Ban		Pao River	X
Quitaparo Creek		Iguez Creek	
Matiyure Creek (Hato El Cedral)	X		

Table. 1. Localities to be visit and survey (X ready).

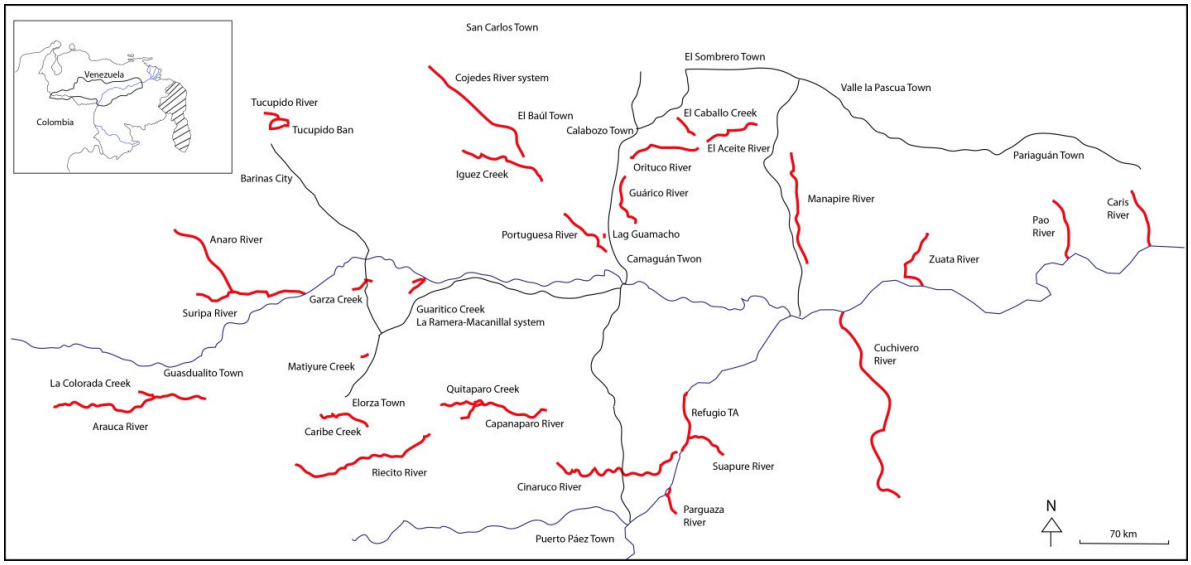

Figure 1. Localities to be surveyed.

Methods

Surveys were made from aluminums boats of 16 feet and motor 40 hp or 12 feet and motor 15 hp, through nocturnal counts using a spotlight 3,000,000 candle power for observed the reflected eye. When are located each crocodile and confirmed the specie, all individuals will be classified in defined size classes by Seijas and Chávez (2000), it is not possible we reported like only eyes. All surveys routs will be georeferenced using a GPS Garmin Map 76 CSX Datum REGVEN (WGS -84). Of being possible, each located individual and identified it will also be georeferenced.

We evaluated also the reproductive success during the collecting hatchling and counting the nest in areas were the conservation program traditionally work (Cojedes and Capanaparo Rivers) and estimate how many females are there.

Results

There are visited 15 localities (Table 2), which include Cojedes River system (Espinoza-Blanco *et al*. 2017), Guaritico Creek and La Ramera Lagoon-Macanillal system (these places are part of the Thesis to obtain the PhD degree of Ariel Espinoza-Blanco), for this reason we had only a preliminary data of the situation of the Orinoco Cayman in Venezuela. We hoped to culminate the fieldwork by the middle of the one 2018.

Localities	km covered	*Crocodylus intermedius*	# times visited
Zuata River	153.00	Yes	3
Caris River	21.1	No	1
Pao River	21.6	No	1
Cuchivero River	60.5	No	1
Guamache Lagoon Estero de Camaguan	16.36	Yes	2
Garza Creek (Hato Garza)	0.36	Yes	1
Matiyure Creek (Hato El Cedral)	16.0	Yes	2
Capanaparo River	97.5	Yes	1
Manapire River	5.5	Yes	1
Arauca River and La Colorada Creek	80.6	Yes	1
Cojedes River system	24.60	Yes	1
Guaritico Creek	48.2	Yes	1
La Ramera-Macanillal system	17.0	Yes	1
Total	562.32		

Table 2. Localities visited and surveyed (modified from Velasco *et al*. 2017).

Some localities have been visited in three opportunities, due to the necessity of preliminary exploratory visit and logistics, to determine the feasibility of carrying out the survey and navigable extension, location of camping areas, and identification of local collaborators.

Two important discoveries are observed during the surveys, first is the establishment the third reproductive population in the Reserve of Fauna Silvestre Estero of Camaguán, created with animals liberated in 2007- 2008, supported by a young Cayman of 70 cm TL observed (Velasco *et al*. 2017). The second is the increase the number of nest in Capanaparo River (Hernandez *et al*. 2017) between 1992, 2013 to 2017, product to the reintroduction 16 young females born in Dallas World Aquarium in 2009.

It is necessary to emphasize the diverse problems with what we have met for the realization the fieldwork, which have been those following:

1. The costs for the acquisition foods increase in each fieldtrip in function of the country inflation, that that joined to the little offer of the same products.

2. Difficulties to obtain cash for small expenses like gasoline, oil, payment the local guides and improvise.

3. The repair of vehicles and motors outside of overboard has become difficult for the shortage of parts, as for example appropriate tires, smaller pieces, etc.

4. The small offer of vehicles 4x4 traction for rent and their excessive cost.

5. Some localities to visit present security public problems, what has not allowed us to carry out the night work or to cover the distances drifted initially, like Garza Creek

or Arauca River and their tributaries (binational sector with Colombia).

6. Difficulties to carrying out surveys in tributary creeks around the big rivers for the impossibility of navigating them.

Acknowledgments
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Sexual Identification of *Caiman latirostris* Hatchlings by Cloacal Inspection

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Sexual identification of crocodilians is important in population studies. This information is also useful when preparing or proposing conservation and management plans, as well as monitoring these populations over time. In nature, it is possible to differentiate males and females of *Caiman latirostris*, by cloacal palpation or eversion of the penis in individuals larger than 75cm in total length; but smaller animals show a hardly differentiable cliteropene. In neonates, sex determination methods involve surgical examinations, necropsies or analysis of cranial dimorphism, which are not applicable to field sampling conditions. In this work, we classified hatchlings of *C. latirostris*, observing the color and shape of their genitals. We were able to characterize penis as a milky white organ with a rounded shape at the tip and a purple hue at the end; while clitoris is shorter, whitish and with a pointed end. The procedure was tested on hatchlings from 3 nests, half of the eggs of each nest were incubated at a constant temperature of 31°C (producer of females) and the other half at 33°C (producer of males). After the observation and classification into males or females, the sex of the animals was verified by direct gonads observation and histology. According to our observations, in the first days of life we correctly assigned the sex of 80% of the individuals of *C. latirostris*, but it was observed that misclassification decreased as animal size increased. It should be mentioned that the number of correct identifications was slightly lower for males than for females. We correctly classified the sex in 100% of the cases when the caiman reached a total length of 36 cm. This technique would be a useful tool for field studies, for making it possible to estimate the sex of small offspring in situ.

Keywords: Broad-snouted caiman, sex, cliteropenis, sex ratio

Reptilario Cipactli: 18 years of Conservation and Management of *Crocodylus acutus*

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Banderas Bay, located western Mexico, has growth in tourism infrastructure and public services, resulting in the loss of natural environments. The above, triggered the University of Guadalajara to create the Unit for the Conservation, Management and Sustainable Wildlife (UMA) "University Center of the Costa Reptilario Cipactli" which gets its registration by the Secretary of Environment and Natural Resources key: INE/ CITES/DGVS-CR-iN-0610-JAL/00, on 24 January 2000. It has an area of 0.32 hectares where 10 crocodile shelters are distributed. Its objectives include research, conservation, preservation, and the rescue of crocodiles. In 18 years, 152 students have made social service, internships, and theses. It has developed research projects in captivity and wildlife with *Crocodylus acutus* culminating in 17 theses, 1 MSc and 3 PhDs. Research has generated several published notes and 31 articles on *C. acutus*; 21 with national editorial committees and 10 international. To solve the human-crocodile troublesome in the states of Nayarit, Jalisco, Colima, Michoacán, Guerrero, Chiapas, and Tabasco, were given 42 workshop courses in biology, management and conservation of crocodiles, training 1085 people, highlighting firefighting personnel and civil protection. In addition, 32 local, state, and national meetings with officials, business groups, and civil society have been performed. To evaluate places with diverse populations, Cipactli has been working with UMA Ejido La Manzanilla, Centro Ecológico de Cuyutlán El Tortugario, Natural Protected Area Estero El Salado, Ecology Department of Puerto Vallarta. The environmental education program has attended 63,080 people, in which biology, ecology, distribution and social, cultural and environmental importance of crocodiles in Mexico and the world is mentioned. 16 *C.acutus* nests were incubated with the birth of 408 offspring. 596 crocodiles of all ages have been received from the authorities, all of which have been provided with clinical services that were from surgery to physical inspections. 795 crocodiles, of which 318 were offspring born in captivity, and 477 rehabilitated bodies of all ages were released. Indicators of teaching, research, resource management, biological management and dissemination, shows that the Reptilario Cipactli has accomplish the objective of the conservation of *C. acutus* on the west coast of Mexico.

Keywords: management, conservation, crocodiles, México

Analysis of Antibiotic Sensitivity Profiles in *Escherichia coli* Isolates Obtained from Cloacal American Crocodile (*Crocodylus acutus*) of Animals Captured in Tilapia Production Ponds in Cañas Guanacaste, Costa Rica

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In the last years, not only has observed re-emergent illnesses affect to the human being and to the animals, but it has seen that the cepa bacteria have done resistant to a lot of drugs, inducing to a new problematic call: antimicrobic resistance. Where the agricultural industries, of animal production and aquaculture, are one of the main causes of a said problem, since the immoderate use of antibiotics is very evident and finish freeing so much in aquatic means like terrestrial. Therefore the American crocodile when being in zones very near to these sources, can turn it into a vulnerable species. Because of such situation, the aim of said investigation is to analyse the profiles of sensitivity to the antibiotics in isolations of Escherichia coli obtained from the cloacas of the American Crocodile (*Crocodylus acutus*), for the identification of the degree of resistance to antibiotics used in the treatment of gram-negative bacteria in human beings and aquaculture. The study carried out in the company AquaCorporación in Cañas, Guanacaste Costa Rica and Laboratories of the University National of Costa Rica (UNA). Where took samples of crocodiles captured in the lakes of tilapia and of the reciento of maintenance. Next it realised a hisopado of the cloaca of each animal and inoculated in situ in two means of agar MacConkey and Levine with antibiotic and without him. An incubation the samples to obtain pure isolations of E.coli, that later realize them a profile of sensitivity to the antibiotics with the team Vitek 2, that includes 15 antibiotics for human use. Incidentally, the ones of veterinary use evaluated them by the technique of diffusion of disk Kirby Bauer and inhibitory minimum concentration (MIC). Once obtained the analysis of the profiles, will be related to the variables: time of estadía, size, sex, place of origin to effect correlations and analysis of main components. Therefore this work will be provided notable information of the state of the crocodiles and like these animals could consider like bioindicators of the environmental health and public health.

Keywords: antibiotic resistance, *Escherichia coli*, *Crocodylus acutus*

Analysis of the Interactions Between Humans and Crocodilians in Costa Rica

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In Costa Rica, the habitat of crocodilians (*Crocodylus acutus*) and caimans (*Caiman crocodilus*) has been subjected to pressure due to human development and expansion, which has considerably reduced the space available for those species. This, added to the growth of the crocodilian populations, increases the probability of interactions between both, leading to conflicts. Little research has been done about human-crocodilian interactions in the country, and there is no official interactions database, but this information is vital to be able to establish management measures. It is not possible to know the magnitude of the interactions between humans and crocodilians without a systematized database and constant monitoring. Therefore, the objective of the work was to systematize and evaluate the interactions between crocodilians and humans in Costa Rica. Information on interactions was collected from the following sources: attacks and encounters reported by the national press, complaints reported on the "Integrated System for Processing Environmental Complaints of the Ministry of Environment and Energy" (SITADA-MINAE), and reports of the situations attended by the Fire Department of Costa Rica. Historical data found was considered, except for the case of Fire Department reports that include only the year 2017, so they were analyzed separately. A database was created including: date, species, location, size and sex of the crocodile, age and sex of the person, type of interaction and activity at the time of the interaction. The interactions were divided into: sightings, encounters, non-fatal attacks and fatal attacks. 99 records were found from 1990 to 2017 from the press and SITADA. Fire Department recorded 123 events in 2017. 100% of the records done by Fire Department correspond to encounters or sightings, although they are reported as emergencies. From the records extracted from the press and SITADA, 35.4% were nonfatal attacks, 27.3% fatal, 21.2% encounters and sightings and 16.1% other situations. Most of the interactions occurred during the day, when people were inside body waters or on the shore. In most cases there is no information about the management of the crocodiles. The Central Pacific is the region of the country where most interactions occur, followed by the Caribbean, the South Pacific, the North and the North Pacific. The information available is brief and insufficient to establish management measures. The country needs to create a national registry of interactions that, together with information on the status of crocodile populations (the country also needs a national monitoring program), allows for appropriate decisions to be made regarding the management of crocodilians and their relation to humans.

Human-Crocodile Interaction and the Problem of Illegal Use of Crocodile Populations in Biosphere Reserve "La Encrucijada", Chiapas, Mexico

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Introduction. The Biosphere Reserve "La Encrucijada" is located south of the State of Chiapas, has an area of 144, 864 hectares, with two core zones that comprise 6 municipalities. It shelters wild populations of the "lagarto real" (*Crocodylus acutus*) and "pululos" (*Caiman crocodilus chiapasius*) which have interacted with human populations in previous years, with minor incidents, however, these have increased due to the increase in human population, habitat reduction of the lagarto real and pululos, traditional diving fishing, poor handling of domestic fauna, indirect feeding to the crocodilians, sedimentation of bodies of water, among others. **General objective**. Raise awareness among the inhabitants about the importance of crocodiles and alligators in wetlands and minimize and avoid incidents, achieving a harmonious coexistence. **Material and methods**. Reporting incidents are validated by telephone calls and from visits in the interaction zone, all cases are officially reported to the environmental authorities. Depending on the type of interaction, the planning and programming of activities with the attention group are made, including: Diagnosis of the case, monitoring actions, capture, management and transfer of risk specimens, data collection, photographic records and environmental education. For this purpose, it is used automobiles, boats, outboard motors, nets, ropes, traps, insulation tapes, GPS, field book and camera. **Results**. From 2011 to 2017, there have been 35 reports of interactions: sighting of specimens to risk, predation of domestic fauna and incidents that are non-fatal and fatal to humans. From these cases, a community group has been formed to deal with contingencies and monitoring of trained crocodilians interested in the conservation of the species. Attention and prevention strategies have been designed, as well as Environmental Education actions in at least 10 communities. to protect the Crocodilians and minimize these interactions. **Conclusions**. It is important to document the causes of the increase of the incidents and design strategies that mitigate the illegal use of the resource for both species; generate and design an environmental education program with focus on the Crocodylia of Mexico; form more community groups trained and qualified for this actions and continue monitoring activities of wild populations to generate alternative management and sustainable use at the community level.

Keywords: "La Encrucijada", Human-Crocodile Interaction, illegal use

Are We Ready for Successful Apex Predator Conservation in Colombia? Human-Crocodilian Interactions as a Study Case

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We reviewed and assessed the human-crocodylian interactions in Colombia based on published information in scientific and non-scientific sources as well as reports coming from community and Environmental Regional Agencies. We found 151 crocodylian interaction reported cases between 1992 and 2017 with American and Orinoco crocodiles and Spectacled and Black caimans. Illegal trade and conservation were the main sources of reports (23% and 20%, respectively), followed by crocodylian translocation (19%), and crocodylian killed by locals (11%). We found reported cases in 26 out of 32 departments of Colombia, covering all 5 regions. The department of Magdalena had the highest number of reports (23%), followed by Atlántico (10%) and Bolívar (7%). To date, four fatal attacks have been reported, two in Magdalena, one in Norte de Santander, and one in Vaupes. In contrast, eight non-fatal attacks have been reported in Magdalena, Córdoba, Guajira, Atlántico, and Boyacá. Strive for a coexistence model rather than a separation model is likely the way to face crocodylian conservation in Colombia due to the idiosyncrasy and Colombians livelihood. However, all actors that direct or indirectly affect conservation must follow a common north to prevent divulgate confusing and contrasting information to the general public, that affects the conservation goal.

Aerobic Microbiota in Oral and Cloacal Swabs from Orinoco Crocodile (*Crocodrilus intermedius*) from Wild Populations with High and Low Human Intervention

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The Orinoco crocodile (*Crocodylus intermedius*) is an endangered species and knowledge about the bacterial community of its digestive tract or how habitat destruction can be affecting these microorganisms is poorly documented. The objective of this research was to characterize the mesophilic aerobic bacterial community present in oral and cloacal swabs from Orinoco crocodile from two wild populations with high (Cojedes River System, CRS; 9,365 Lat; -68,709 Lon) and low (Capanaparo River, CAP; 6,993 Lat; -67,294 Lon) human intervention. Oral and cloacal swabs from two individuals (Size II: TL= 0.6 to <1.2 m) in each locality were used to perform culture and isolation of different aerobic bacteria at 37°C. The bacterial isolates were identified by amplification *16S rRNA* gene using PCR and sequencing. A total of 119 isolates were obtained: 61 (29 oral and 32 cloacal) from the CRS and 57 (32 oral and 26 cloacal) from the CAP. The oral cavity bacterial composition in the CRS was highly dominated by genus *Bacillus* sp. (65%; 19/29) and in the CAP were dominated by *Bacillus* sp. (40,6%;13/32) and *Morganella* sp. (18,8%; 6/32). Other genera were identified in lower proportion like *Enterobacter* sp., *Fictibacillus* sp., *Morganella* sp., *Staphylococcus* sp., and unidentified Gram negative coccobacilli in both localities; however, *Erwinia* sp., *Lysinibacillus* sp. and *Ralstonia* sp. were found only in CRS, and *Flavobacterium* sp., *Gordonia* sp., *Stenotrophomonas* sp., and *Providencia sp.* only in CAP. The cloacal cavity was highly dominated by *Bacillus* sp. (53,1%; 17/32) in CRS, whereas in the CAP were dominated by *Providencia sp.* (32%; 8/25), *Bacillus* sp. (20%; 5/25) and *Stenotrophomonas* sp. (16%; 4/25). Other genus found in lower proportion in both localities were *Acinetobacter* sp., *Morganella* sp. and *Streptococcus* sp., but *Fictibacillus* sp., *Lysinibacillus* sp*., Enterobacter* sp., *Brevundimonas* sp., *Arthrobacter* sp. and *Ralstonia* sp., were found just in CRS, and *Gordonia* sp., *Orchrobactrum* sp., *Salmonella* sp. and *Staphylococcus* sp. just in CAP. In CRS, the *Bacillus* genus was highly dominated in both cavities whereas in the CAP there is not dominance of an only one genus. In conclusion, our results show differences in the microbiota diversity comparing both localities.

Keywords: bacteria, microbiology, Orinoco crocodile, Venezuela

Presence of *Leptospiras* spp. in Populations of *Caiman latirostris* (Crocodylia, Alligatoridae)

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Leptospirosis is a disease caused by pathogenic spirochetes of the genus *Leptospiras*, transmitted by wild and domestic animals. To know the variables related to the eventual acquisition of infection in animals have great importance for the design of sanitary policies. Our objective was to assess the prevalence of antibodies *anti-leptospira* and the presence of *Leptospiras* spp. in *Caiman latirostris* in captivity and in the wild, in the north of Santa Fe province. Blood samples were taken from 45 individuals (20 wild animals and 25 in captivity). Before the extraction, we cleaned the neck of caimans, in order to prevent samples contamination. Anticoagulant blood with heparin was cultivated in two specials media, EMJH and Fletcher. From serum, the presence of antibodies was determined by microscopic agglutination test (MAT) and polymerase chain reaction (PCR) to detect DNA of the bacteria. In addition we determined pH of the urine in captivity animals. pH was 8 (slightly alkaline pH). We excluded 9 of the 45 samples analyzed by MAT, because 5 had lipemic serum and 4 were contaminated. In 15 of 19 captivity samples and 11 of 17 wild samples, *anti-leptospira* antibodies were detected by MAT. In 69.2% (18 of 26) of these samples the presumably infecting serogroup was Pyrogenes, presenting coagglutinations with Icterohaemorrhagiae (23.1%) and with Icterohaemorrhagiae and Grippothyphosa (3.8%). Other serogroups detected were: Canicola, Autumnalis, Copenhageni, Grippothyphosa, Tarassovi, Wolffi and Javanica. On the samples analyzed by PCR, one of a captivity animal was positive; from this sample we could not isolate leptospires because of agar contamination. Blood agar media were negative, but 17.8% (8 of 45) of them were contaminated. This work allows to determine the presence of *Leptospiras* spp. in one caiman, and the high prevalence of antibodies, both in captivity and nature. In this way, it concludes that caiman are in frequent contact with the bacteria at some point in their lives, and that they could present an acute infection without any obvious symptoms. According to the pH obtained, to the seroprevalence found and that the caimans spend time taking in the margins of the bodies of water, where the water is very shallow and the temperature is high, there would be adequate conditions for the transmission of this disease in the studied area and in places where this species frequently lives.

Keywords: zoonosis, *Leptospira*, reptiles, Broad-snouted caiman

Isolation and Bacterial Sensitivity of the Aerobic Cloacal and Oral Microbiote with Zoonotic Potential in Free Living Broad-snouted Caiman, *Caiman latirostris* (Daudin, 1802) in Peri-urban Environment in the Atlantic Forest, Brazil

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The habitat loss and the advance of the cities over the natural environment has been changing the distribution of *Caiman latirostris* in Brazil. Under a scenery of broad poaching and the advance of the agricultural frontier, the caimans are searching the cities as refuges. The knowledge of the zoonotic potential of the microbiote of *Caiman latisotris* is important to access the health risk which the caimans are subjected in athopized environments and also to people in touch with these animals. Thus, the present work aimed to describe the bacterial isolate of cloacal and oral cavities of free living *Caiman latirostris* in anthropic environment and the resistance of these bacteria to antibiotics. Microbiological samples from the mouth and cloacae of 30 healthy animals were collected in a peri-urban environment in Espirito Santo State, Brazil. After collection the samples were inoculated in blood and McConkey agar, incubated at 35ºC for 48 hours and the colonies were identified by macroscopic, microscopic, biochemical and colorimetric tests. Samples of 8 specimens were tested for sensitivity to 17 antibiotics by disk diffusion test. There was bacterial growth in 87% of samples. The following species were identified: *Escherichia coli* (23%), *Proteus mirabilis* (3.3%), *Morganella morganii* (3.3%), *Klebsiella pneumoniae* (3.3%), *Salmonella* spp. (3.3%), *Pseudomonas aeruginosa* (16.6%), *Enterobacter cloacae* (6.6%), *Serratia marcescens* (3.3%), *Pantoae* spp. (3.3%). Only one sample (*Salmonella* sp.) did not show resistance to antibiotics. Isolated microorganisms have zoonotic potential. The capacity of disease development is related to the host's health and immunological status. These bacterias are responsible for serious infections and may lead to death in humans and animals. The results indicate that the presence of the caimans in urban environments, besides being an ecological problem, is also a challenge for public health once people consume poached caiman meat. Bacterial resistance to antibiotics is a phenomenon of worldwide concern. It is a problem that has serious impacts on ecosystem health and, as a major public health challenge that demands multidisciplinary approaches aiming at environmental health and species conservation.

Keywords: microbiology, sentinel species, conservation medicine, One Health, conservation biology

Morphogenesis of the External Genitalia in Broad-snouted Caiman

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In the most animals, an intromittent organ denominated phallus facilitated reproduction by internal fertilization. Considerable variation in phallus morphology among different amniote lineages has contributed to the debate about their structural homology. However, comparatively little is known about the development of reptilian external genital and how evolved in males and females. Here we described morphology development of the penis and clitoris in the Broad-snouted caiman, *Caiman latirostris*. Consequently, we set embryos incubated at specific sex-determine temperatures: at 31°C (100% female) and 33°C (100% males), and hatchlings coming from these temperatures. The embryonic precursor of the penis and clitoris is the genital tubercle, which appear -at stages 4- as paired swellings of mesenchyme. By stage 8, a single cylindrical genital tubercle is forms. Outgrowth of the tubercle continues, and by stage 18, it has an oval appearance and the indentation of the phallic sulcus running along its dorsal surface. At stage 20 (onset of the sexual differentiation), the tubercle is regionalized into the shaft, phallic ridge and glans. We used histology to examine the internal differentiation during the thermosensitive period. Up to 24 stage of development the genital tubercle at both temperatures comprise a sulcus lined with a bilaminar epithelium and bordered by the dense mesenchyme. Signs of corpora differentiation are first detected at stage 26 under male-producing temperatures, when a ring of vascular elements outlined the anlage of the corpora cavernosa from the corpus spongiosum. At feminizing temperatures such structures appeared later. At hatching, males and females exhibit corpora of the network of collagen fibers loosely organized. Our analysis suggests that initial external genital outgrowth occurs prior to temperature-sex determination, and penis and clitoris development have similar trajectories.

Keywords: embryos, genital tubercle, corpora, phallic sulcus

Use of Habitat of the *Caiman crocodilus fuscus* in the Hidroprado Dam in the Department of Tolima

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HIDROPRADO is the most important wetland of the department of Tolima, has a size of 4300 ha, an average depth of 45 m, the reservoir presents consolidations rocky outcrops, patches of tropical dry forest, crops, housing and hotel infrastructure. In order to know the habitat use of *C. c. fuscus* in the dam at the time of capture the microhabitat characteristics were recorded where the individuals were found. Made maps of coverage and land use of the transects at scales of 1: 10,000 1: 40,000 in QGIS. The microhabitat was categorized and analyzed based on the position of the individuals in relation to the edge of the reservoir under a Correspondence analysis where the sizes of individuals and microhabitat categories were taken into account. The *C. c. fuscus* are distributed by all the reservoir of an aggregate although there are isolated individuals. The females with offsprings were found in the bays or mouths of the streams. These areas have less slope, aquatic vegetation and flooded trunks or palisades, which favors the feeding and shelter of the breedings. Class II associated with aquatic vegetation, rocks, caves and palisades. Class III, show preference for rocks, palisades and open water. However, some individuals were observed in the aquatic vegetation and floating trunks. Class IV was found more frequently in open water and flooded vegetation. It was observed that in Hidroprado the habitat availability for *C. c. fuscus* is related to the level of water which is restricted in a large part of its area by the slopes of the reservoir as well as the water dynamic that is conditioned to the 14 m of operation of the dam for the production of energy and supply of the irrigation system. When the water level is high, the *C. c. fuscus* prefer areas with flooded riparian vegetation, the bays and mouths of streams where aquatic vegetation and floating logs accumulate. When the water level is low the areas with emergent vegetation, rocks, caves, palisades and bare soil stand out and *C. c. fuscus* also uses these zones reflecting that the individuals adapt themselves in a convenient way to the different elements of the landscape resulting at the different levels of the water.

Keywords: babilla, Brown caimán, *Caiman crocodilus*, habitat use, Hidroprado, Tolima

Variability in the Shape of the Skull in *Caiman yacare* Hatchlings in Argentina

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The crocodilian skull is a structure that has evolved through millions of years and nowadays is easily distinguishable among the 23 living species. Geometric morphometric studies have great importance since they provide sustenance to other disciplines such as ecology, evolution and systematics. Through the tools provided by geometric morphometry it is possible to correlate differences in the shape of the studied specimens with different environmental variables. This study aims to analyze the shape skull variability among different populations of *Caiman yacare* from Argentina. The skull forms of the individuals were compared taking into account their place of origin and environmental variables. 68 hatchlings of *C. yacare* from three different regions were photographed. In each photo were registered 17 landmarks using the tpsDig2 software; all subsequent analyzes were performed using MorphoJ software. The landmarks coordinates of all the specimens were superimposed using the GPA (Generalized Procrustes Analysis) method. To analyze the influence of size on the shape (allometry) among animals of different sizes, a regression was made between the variables of form and the value of the centroid. From the alignment of the coordinates of the landmarks the variation of form was studied by means of a PCA (Principal Component Analysis) and a CVA (Canonical Variate Analysis). The correlation between the variables of the form with the environmental and geographical variables was carried out by means of the PLS (Partial Least Squares) method. In the regression analysis, non-significant values were obtained indicating that there is no allometry (p= 0.6282). Through the PCA two variations of shape were observed for the set of the 6 sampled sites, where the greatest variation occurs in the length of the snout and in the length and width of the cranial cavity. The Mahalanobis distances in the CVA were all statistically significant (p= 0.0001), which indicates that there is morphological variability among populations. The PLS method showed a statistically significant correlation (p<0.0001) between the shape of the skull and the environmental and geographical variables. The temperature and latitude would be the variables that most influence the form, since they explain 87% and 88% respectively of the observed covariation. From these data we can infer that the shape of the skull in the studied populations is very strongly influenced by the geographical location and the ecosystem in which they live, in addition, the two variations of the form found would correspond to sexual dimorphism.

Keywords: *Caiman yacare*, cephalic region, geometric morphometry

The Art of Growth: Body Growth Models for Wild and Reintroduced Broad-snouted Caimans (*Caiman latirostris*)

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Body growth curves are extremely useful tools to assign ages and to compare the environmental pressures that can condition growth. In ectothermic animals, growth depends on the temperature of the environment and the food resources. Here we describe for the first time functions that best adjust the body growth curves of Broad-snouted Caiman (*Caiman latirostris*), comparing those of wild individuals vs. reintroduced individuals. Snout-vent-length (SVL, cm) and age (years) of juvenile and adult females belonging to two different experimental groups were recorded. On the one hand, we worked with specimens (Py) that were born from eggs collected from wild populations, which were tagged and reared on a farm in their first year, and then reintroduced to nature, where they were recaptured. On the other hand, we worked with wild caimans (Wy) of Class I (<25 cm SVL), whose ages were estimated using an analysis of the size frequencies and corrected by Discriminant Analysis. To estimate the asymptotic SVL of both curves, we included the same set of data registered from reproductive females recaptured in the wild at Class III, (70 cm<SVL<90 cm), from 7 to 10 years old, which had been reintroduced by the Proyecto Yacaré. To describe body growth, we adjusted five models by non-linear regression (Rstudio, package: nls): Logistic, Logistic with constant (LC), Gompertz, Gompertz with constant, and Von Bertalanffy. We analyzed each group separately (Py and Wy), selecting the most parsimonious model based on the Akaike´s information criterion, the residuals, and the significance of each parameter ($P<0.05$). For Py, the best model was the Logistic one, while for Wy it was LC. The mean asymptotic SVL ranged from 74.8 to 77.4 cm, which was attained through different curve shapes. Once reintroduced into the wild, the estimated growth was higher in Py than in Wy of similar age; this difference was noticeable during the first three years of age. Py reduced their growth the fourth year, while Wy increased it, reaching their maximum growth when they reached five to six years old. The bigger size and faster growth of Py animals, allow them to reach Class II at an earlier age after releasing than Wy ones. Considering that mortality is size-dependent, the fast-initial growth of PY animals could increase their survival chances.

Keywords: nonlinear regression, growth models, reintroduced animals, wild animals

Nesting Behavior of the Broad-snouted Caiman (*Caiman latirostris*) during Two Reproductive Seasons

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Crocodiles show behaviors associated with environmental temperature, including nesting and parental care. To evaluate nest behavior of the broad-snouted caiman females, a video camera was installed in an enclosure with similar conditions to the natural habitat, at Granja La Esmeralda Experimental Breeding Station in Santa Fe, Argentina. Activities in a nest were recorded 24 hours a day during two reproductive seasons, between December and March 2014-2015 and 2015-2016 for a total of 4320 hours. An ethogram was made and the frequency of each activity was analyzed. The effect of environmental variables, time of the day and weekly period on the presence of females in the nests were analyzed. The observed behaviors were laying, maintenance (digging nest, adding material to the nest, compaction of material), vigilance (around and over the nest), aggressive social behaviors (confrontations, persecutions, aggressions) and collaboration in the nesting site. Females attended the nest more frequently during early morning and night, which corresponds to the lower temperatures of the day. Visits to the nest were more frequent before the posture and the week following the event. The appropriate periods by the local people involved in the harvest of eggs corresponds to the period between 11 and 16 h. during the two weeks after clutch. The described behaviors corresponded with what was observed by other authors for other species in nature, and also collaborative social behaviors were identified in the construction of the nest.

Keywords: reproduction, nesting, video cameras

Effect of Extreme Environmental Conditions on Corticosterone Levels and Immunological Indices in Juvenile Broad-snouted Caimans

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The effect of stress in wild vertebrates has been little investigated, especially in crocodilians. In the wild, crocodilians face numerous stressors such as climatic factors, toxicant exposure, and infection. Exposure to stressors may affect the physiological processes of crocodilians, with consequences on its fitness and survival. We experimentally investigated the effect of a 4-week exposure to environmental challenges (food restriction, water restriction and high temperature) on growth, immunological investment and corticosterone levels of juvenile *Caiman latirostris*. White blood cells counts, natural antibody (NAb) levels and complement system activity were evaluated to characterize the influence of those treatments on the immune system. We found deprivation of food resulted in reduction in growth and body condition, whereas high temperature had a beneficial effect on the variables measured. Individuals exposed to heat treatment (37 1°C) grew more, and obtained better body condition, higher NAb levels, and significantly lower corticosterone levels relative to caimans in all other groups. Food restricted caimans also illustrated higher NAb levels. Our findings suggest that *C. latirostris* juveniles are able to tolerate environmental stressors and they are even favored by high temperatures.

Keywords: immunology, corticosterone, caimans, temperature

Relationship Between Predators and Parental Care of *Caiman latirostris*

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Interaction of predation and parental care determine the mayor part of *Caiman latirostris* eggs survival, and play an important role in population dynamic. Environmental structure influences the presence or absence of predatory species and parental care, affecting the ability to mitigate the damage produced by predators. The relationship of these phenomena is poorly understood due to the difficulty of its observation, in this study we pretend to know the patterns of predation and its relationship with paternal care. In the nesting season 2016/2017, camera-traps were placed in 13 nests, they were programmed to take a photo every 5 minutes and when movement was detected. A total of 210,638 photos were obtained, but only 6,741 correspond to usable events, of these 11% (737) were possible predators and 63% (4233) of *Caiman latirostris*, the rest correspond to animals that do not represent risk to eggs (mainly birds). Nine species of possible predators were identified, only three of this were detected predating: *Salvator merianae* was present in 4 nests but only one was predated, *Galictis cuja* and *Sus scrofa* were present in one nest, which suggests, that predators have different degrees of efficiency. Depredated nests were visited by higher richness of species potentially dangerous, they were located in forest environments and had lower presence of the female; on the other hand nests with higher female presence showed less visits of potential predators. These data suggest that female presence limits the actions of predators and also indicates that not all species prey the nest even finding it.

Keywords: Tegu lizard, wild boar, ferret, parental care

Influence of Climatic Variables on Corporal Attributes of *Caiman latirostris* Females and their Relationship with Reproduction

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Although the reproductive ecology of *C. latirostris* has been studied in several aspects, it is still unknown the influences of climatic variables on female's conditions and their reproduction. Studies in reptiles recorded that energy invested in reproduction is conditioned by the availability of preys, which is affected by local and regional rainfall. The aim of this study is to evaluate influence of rainfall and temperature over corporal attributes of *C. latirostris* females (body and physiological condition) and their reproductive performance (clutch size and hatching success [hatchlings/number of eggs]) from wild populations in Santa Fe Province. Body condition was calculated by the Scaled Mass Index (SMI), physiological condition was characterized by fatty acid profiles (FA) in plasma and muscle of reproductive female. Through principal component analysis was evaluated the effect of climatic variables (temperature, local rainfall, and rainfall in the headwaters of the rivers prior to oviposition) on the female's corporal attributes and reproductive performance. No relationship was found between female's body condition and climatic variables; this could indicate that only females that have managed to overcome the minimum required body condition can reproduce. Although for the moment it is unknown what are the causes that would allow a female to reproduce and it is possible that this threshold is flexible. A relationship was found between female body condition and hatching success of their nests, females with better body condition have greater hatching success. Is possible that there is a minimum energy threshold to reproduce, but once the threshold is overtaken, the excess energy could be invested to improve progeny quality. In addition, it was observed a negative association between clutch size and rainfall in the headwaters of rivers in March-April. According to the relationship between body size, clutch size and energy cost of reproduction, it is possible that in years with lower rainfall, only larger females would reproduce. Respect to the physiological condition, muscle FA showed that essential fatty acids such as linoleic (C18:2) has a strong association with minimum temperatures of September, which shows that temperatures of first warm months (spring), determines the availability of food items that are in relation to values of essential fatty acids (EFA). Also, muscle FA (C18:1, C18:2, C22:6 and total polyunsaturated) showed association with hatching success, which in turn are indirectly related to a higher body condition. In plasma EFA and total polyunsaturated was associated with rainfalls in headwaters of rivers in December, showing that precipitation would affect plasma FA through diet. Our results suggest that body condition allows to evaluate reproductive performance of the species; and females physiological condition is affected by precipitation and temperature.

Keywords: Broad-snouted caiman, body condition, physiological condition, hatching success, clutch size, fatty acids

Food Habits and Ontogenetic Dietary Partitioning of American Crocodiles in a Tropical Pacific Island in Central America

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Food habits studies are fundamental to understanding the ecology of a species and its interactions with the community to which it belongs. Among crocodylians, diet affects a variety of biological, physiological, and behavioral characteristics. However, despite having one of the largest distributions across the Americas, some aspects of *Crocodylus acutus*' natural history remain poorly studied, particularly in insular areas. We characterized American crocodiles' food habits in Coiba Island, Panama, assessing ontogenetic dietary variation and dietary niche overlap by age group and size. We captured and collected stomach content samples from 49 individuals from four transects from March to December 2013. Form these samples we could taxonomically identified three phyla, four subphyla, eight classes, 11 orders, 17 families, 14 genera, and 12 species as prey items. However, not all samples could be identified to the lowest taxon (species), having most them identified only to family. Large juveniles had the largest proportion of prey items and subadults the largest proportion of gastroliths and vegetal content. Percent occurrence per major categories (insects, arachnids, crustaceans, fish, reptiles, birds, and mammals) showed crustaceans and insects as the most prominent groups of prey items on this island. Overlapping group analysis showed a reduction in the consumption at invertebrates (crustaceans and insects) as individuals aged. However, these items were the most common throughout all American crocodiles sampled. Dietary niche overlap showed a clear ontogenetic dietary partitioning with high overlap (>60%) between small and large juveniles and low overlap (<30%) among small juveniles, subadults, and adults. To date, 69 species have been reported as prey items for American crocodiles. However, relying on prey items identified only to genus, we had at least 95 prey items. Thus, *C. acutus* can be defined as generalist with a broad spectrum of prey inhabiting all types of habitats and having all types of consumption classifications. Overall, American crocodiles inhabiting coastal areas present some differences in both dietary composition and structure with those dwelling inland and freshwater habitats as well as an inter-individual diet variation, which reflects the plasticity and adaptability of *C. acutus* to a variety of conditions.

Acoustic Characteristic of the Distress Call of the Morelet's Crocodile (*Crocodylus moreletii*) in Acoustically Contrasting Environments

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Introduction. The communication system of crocodrilians is compoused wide of acoustic signals. Although short and monosyllabic, their vocalizations constitutes wide repertoire that is adapted to amphibian habits of their lifestyle. This repertoire is essential for the establishment of specific and complex social interactions: reproduction and survival of individuals in the wild. For the crocodilians the distress call is key because it increases the group and individual survival of the offspring, since it is intimately involved with in the establishment of care and protection provided by the adults. This study propose to characterize the distress call of neonates, yearlings and juveniles of Morelet's crocodile (*Crocodylus moreletii*) with origins wilds and of rearing; from two localities in southeast Mexico. With the systematic comparison of the recordings, I prove the existence of variation of the distress call between acoustically differentiated localities. **Materials and method.** In the management unit for the Conservation of Wildlife (UMA) of the Universidad Juarez Autónoma de Tabasco and Nicte-Há, Campeche, were registrered and captured *C. moreletii*, during the hatching season and after it. Distress calls were recorded with a NTG-2 microphone connected to a Marantz PMD-660 digital Sound Recorder, in WAV format. The Raven Pro 1.5 CornellLab software was used obtaining spectrograms and performance the acustic analysis. For the statistical analysis the R studio was used. **Results.** Nocturnal acoustics is equivalent in both locaties but the entire day's acoustic atmosphere was different betwen each one. Relative homogeneity was observed in the intra-population comparison yearlings' and neotates' distress call, except on the juveniles. Whereas in the interpopulation comparison on yearlings, significant differences were found in at least one quantified acoustic attribute. When comparing the distress call between age categories, significant difference were found in at least one acoustic attribute, indicating that the distress call is in function of the size. **Conclusions** With some reservation, these preliminary results are perhaps congruent with the acoustic adjustments that have been found in bioacoustic investigations carried out in other vertebrates; in response to the pressure of environmental noise (anthropogenic and experimental) to which they were exposed, for example: birds oscillates and one species of gecko. But there are other possible factors that could influence these results, such as: diet, water quality or degree of hybridization (with *C. acutus*). Thus, the present study contributes to the establishment of empirical bases to reach the understanding of possible factors that influence the evolution of acoustic signals in the communication system of Mexican crocodiles.

Keywords: distress call, bioacoustic, Morelet's crocodile, antropic noise

Gene Expression Pattern of Antioxidant Enzymes and its Relation to Oxidative Damage and Genotoxicity in *Caiman latirostris* Hatchlings Exposed to Pesticide Formulations During Development

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The loss and fragmentation of natural habitat due to agricultural expansion has caused that wild populations of *Caiman latirostris* in Argentina become threatened by the constant exposure to pesticides. In previous studies, we demonstrated genotoxicity, oxidative damage and imbalances in antioxidant (AO) enzymes after exposure to different pesticide formulation by topication on the eggshell and through nest material. The aim of the present study was to analyze if alteration observed in the AO enzyme activity involve a modification of their gene expression patterns and its relation with oxidative damage to lipids, DNA and genotoxicity. Two identical experiments (E1 and E2) were carried out in consecutive years, where eggs of *C. latirostris* coming from different clutches were distributed in 6 groups of 12 and 15 eggs, respectively. The treatments were as follows: negative control (NC) treated with distilled water; vehicle control (VC) treated with ethanol; three groups exposed to Cypermethrin (CYP -Atanor ®, 0.12%), Chlorpyrifos (CPF -Lorsban*, 0.8%) and Glyphosate (RU-Roundup ® Full II, 2%) formulations, and one last group exposed to the ternary mixture of the three formulations (M- 0.12% + 0.8% + 2%, respectively). The concentrations applied for each pesticide formulation corresponded to that recommend for field application in soy crops. After hatching, blood samples were taken from each animal and the following biomarkers were applied: activity of the antioxidant enzymes Catalase (CAT) and Superoxide dismutase (SOD) and the expression of the corresponding genes; lipoperoxidation by Thiobarbituric acid reactive substances (TBARS), DNA damage and specific base oxidation through the standard and modified Comet assay (CA); and chromosome damage by the micronucleus test (MN). Results showed a statistically significant increase in oxidative DNA damage, DNA damage index and FMN for RU, CYP, CPF and M respect to their corresponding controls ($p<0.05$). *Cat* and *sod* expression levels were obtained from animals of all treatments and correlated to the activity of the enzymes. This study proved that the use of pesticide formulation at low concentrations recommended for field application, generate adverse genetic effects and imbalances in the oxidative state of this species. This situation could be worst considering repeated applications done in environments near crops.

Keywords: catalase, superoxide dismutase, lipoperoxidation, DNA damage

American Crocodile (*Crocodylus acutus*) Food Web in Two Insular Ecosystems: Monte Cabaniguán, Cuba and Coiba Island, Panama

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Important mediators of the complex relationships in biological communities are trophic interactions. To provide knowledge on how biodiversity affects the functioning of coastal marine ecosystems, we established studies on the trophic network of *Crocodylus acutus*, in two insular ecosystems: Monte Cabaniguán, Cuba and Coiba Island, Panama. We hypothesized that the natural history of the species that are part of the marine coastal communities affected biomass and diversity of resources available for the top predator (American crocodile) and, the presence of crocodiles maintain the diversity of environmental niches controlling the population density of the prey. Our study aims to answer the following questions: 1) what do crocodiles eat in each area per station / sex / age? 2) Are crocodiles generalist predators? 3) Is there correlation between abiotic characteristic and diversity of species eaten by crocodiles in each environment? 4) Crocodiles population size imbalance could cause trophic cascades? 5) Could we use crocodiles as indicators of the status biodiversity (species richness, productivity and stability)? The proposed methodology includes a morphological and molecular characterization (Barcode new generation sequencing of cytochrome b and cytochrome oxidase subunit I) of the crocodiles stomach contents at each ecosystem; ecological characterization of marine and coastal environments and design of trophic models using Ecopath-Ecosim-Ecospace software. This project began in January 2018. Here in, we present Panamanian preliminary results. In Coiba Island, we selected five sites to measure alpha, beta and gamma taxonomic diversity and crocodile prey diversity: Quebrada Sin Nombre, Playa Blanca, Playa El María, San Juan River and La Producción River. Crocodile feeding areas are found in areas with a tropical vegetation profile highlighted by mangrove forests of *Rhizophora mangle*, *Avicennia germinans*, *Pelliciera rhizophorae*, *Conocarpus erectus*, *Cocos nucifera*, *Terminalia catappa*, *Hippomane mancinella*, and *Prosopis julifora*. Playa Blanca and El Maria showed inverted mangrove forest structure where *Aviciennia* is on the front followed by *Rhizophora mangle*. We have obtained 150 stomach content; 80% of the items found belong to the Decapoda order. The 70% of the Decapod items belong to four families: Grapsidae, Gecarcinidae, Portunidae and Ocypodidae. We have also collected at the sites, manually or using expandable traps, 33 individual belonging to those four families. We have extracted DNA and successfully amplified and sequenced (Sanger sequencing) 560 base pairs of the mitochondrial gene Cytochrome oxidase 1 from 20 individual out of the 33 collected. Only one individual was identified at species level when sequences were compared against GenBank Data Base. Our next step will be to prepare and sequence COI new generation amplicon libraries; estimate biodiversity indexes, available biomass and energy and design food webs.

Accumulation of Organochlorine Pesticides in Fat Tissue of Wild Nile Crocodiles (*Crocodylus niloticus*) from iSimangaliso Wetland Park, South Africa

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Nile crocodiles (*Crocodylus niloticus*) are important apex predators in many tropical and subtropical aquatic habitats throughout much of sub-Saharan Africa. In South Africa, large crocodile populations inhabit lakes and wetlands that are impacted by organochlorine pesticides (OCPs). Despite the continued use of these compounds and their potential adverse effects on key wildlife populations in southern Africa, limited ecotoxicoloigcal data exist. In this study, we examined the accumulation of OCPs in fat tissues of live, wild Nile crocodiles from iSimangaliso Wetland Park, a region of significant biological importance. All samples (n= 15) contained multiple contaminants in highly elevated concentrations, with total residue burdens varying between 3600 and 8000 $ng.g^{-1}$ ww. DDT and its metabolites were the dominant compounds detected in most samples, with PDDT concentrations ranging between 520 and 3100 $ng.g^{-1}$ ww. Elevated levels of other OCPs were also detected, including lindane (67-410 $ng.g^{-1}$ ww), aldrin (150-620 $ng.g^{-1}$ ww) and heptachlor (170-860 $ng.g^{-1}$ ww). Our findings show that crocodiles are exposed to OCPs throughout their range within iSimangaliso Wetland Park and contain some of the highest concentrations ever recorded in crocodilian tissue. Results indicate the need for a greater understanding of the impacts of OCP exposure and toxicological responses in crocodiles from iSimangaliso, and in Nile crocodile populations in general. The novel surgical technique described in this study provides an effective method for assessing relationships between contaminant body burdens and their potential reproductive and developmental consequences in crocodilians.

Keywords*:* Nile crocodile, organochlorine pesticides, DDT, ecotoxicology

Green Ponds, Fat Fish and Dead Crocs: A Testable Hypothesis of Crocodylian Mass Mortality

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Observations of mass die offs of crocodylians in three different parts of the world demonstrate similar features. In Florida, between 1998 and 2004 300+Alligators in central Florida died after demonstrating obesity and neurological symptoms associated with Thiamine deficiency. In India a mass die off of Gharial in the Chambal River 2007-2008 was associated with gout and kidney damage of unknown origin. In the Oliphants River, South Africa, mass mortality of Nile crocodiles in 2008-2009 also reported steatitis and gout. In all three cases, while toxicity from environmental contaminants was a suspected factor, extensive testing failed to establish this cause. Further examination of the three cases indicate some factors common to all three. They all involved disrupted eutrophic aquatic systems that supported periodic massive blooms of blue green algae (cyanobacteria). In all three cases crocodylians were shown to have fed extensively on hyper-abundant filter feeding fish; Gizzard Shad (*Dorosoma cepedianum*) in Florida, *Tilapia* sp. in India and Silver carp (*Hypophthalmichthys molitrix*) in South Africa. Work by David Huchzermeyer in the Oliphants River has shown that the linkage between bluegreen alga, filter feeding fish and steatitis and gout in their predators is mediated through the metabolism of Polyunsaturated Fatty Acids. These are produced by Blue green algae and bioaccumulated up the food chain causing cyclic oxidation of fats and associated effects on antioxidant vitamins. These in turn lead to the observed physiological symptoms and mortality. Interactions among these factors and other drivers such as seasonal climate, plankton dynamics, fish behavior and predator feeding create a complex 'cascade' of events linked through multipath ecological pathways that obscure simple cause and effect analysis. For example, warm water temperature leads to anoxia causing fish die-offs that encourage binge feeding by crocodiles, overloading their fat oxidation controls. I propose here a general hypothesis linking these factors that should be applied and tested on future crocodylian mass mortality events.

Analysis and Detection of the Autophagy Pathway in the American Alligator (*Alligator mississippiensis*)

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In response to environmental temperature depression in the fall and winter American alligators brumate, which is characterized by lethargy, fasting, decreased metabolism, and decreased body temperature. During brumation, alligators will periodically emerge for basking or other encounters when appropriate. This sporadic activity may place strain on nutrient reserves. Nutrient scarcity, at the cellular and/or organismal level, promotes autophagy, a well-conserved sub-cellular catabolic process used to maintain energy homeostasis during periods of metabolic or hypoxic stress. It is our working hypothesis that alligators upregulate the autophagy pathway during their winter dormancy. Using published genomic data, we have determined that the autophagy pathway is highly conserved, and alligator amino acid sequences exhibit high identity with human homologues. Transcriptome analysis conducted on liver tissue confirmed expression of one or more isoforms of 32 autophagy related genes. Five autophagy pathway related proteins (ATG5, ATG9A, BECN1, ATG16L, and LC3) with functions spanning the pathway, have been detected in multiple tissue types by western blot analysis. Additionally, ATG5 was detected in alligator liver tissue by immunohistochemistry. Future studies will be focused on the differences in expression between tissues, and seasonally driven difference using longitudinal blood sampling.

Keywords: autophagy, brumation, bioinformatics, crocodilian

Movements of Adult Female American Alligators within a Barrier Island Population at the Kennedy Space Center, Florida

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From 2006 to present a multi-disciplinary study ranging from ecotoxicology to basic life history of an estuarine population of the American alligator (*Alligator mississippiensis*) has been conducted on the Kennedy Space Center (KSC), Merritt Island National Wildlife Refuge (MINWR). The study area includes 56,655 ha (140,000 acres) of estuarine habitat including brackish water surrounding the main land mass from two water systems. Freshwater lakes and ditches are inter woven within this system bordered by the Atlantic ocean to the East. To determine the movement patterns of the adult female alligators (189-250 cm) within this rocket launching facility we attached ten Telonics 4310-3 satellite transmitters (± 10 m accuracy) in April of 2017 as described by Brien *et al*. Transmitter installation was under the nuchal scutes on the alligators using stainless steel cable with crimps, then covered with a two part marine epoxy putty to hold the cables and crimps together, providing a barrier from entanglement and the effects of salt water. To provide a "natural" look the transmitters were then painted black to help camouflage the transmitter while it was on the alligator. All ten alligators were captured in five designated geographically different areas on KSC within a 16-day time period. Transmitters were programed to take positions every four hours and uplink to the Argos satellite for downloads every four days from spring to fall. During fall and winter the schedule changed to satellite uplinks every seven days. The data is preliminary showing nesting site fidelity and also lessons learned.

Activity Budget and Behavioral Patterns of American Crocodiles (*Crocodylus acutus*) in the Coastal Zone of Belize

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American Crocodiles (*Crocodylus acutus*) are broadly distributed throughout coastal and lowland wetlands in the Americas. Much of their habitat is threatened due to habitat destruction as a result of increased residential and tourism based growth in coastal areas, and current management is limited in understanding the impact habitat destruction or anthropogenic disturbance has on crocodile behavior. Our study describes diurnal time-activity budgets for American Crocodiles in Belize, and evaluated observed behavioral patterns in relation to a gradient of anthropogenic impact determined by wetland impact assessments that quantified levels of anthropogenic disturbance from low to high. American Crocodiles allotted the greatest amount of time to performing maintenance activities fulfilling basic biological needs, such as basking for thermoregulation, foraging, and loafing at the surface. Time allotment for agonistic and social behaviors was significantly greater at the site with higher anthropogenic disturbance versus the two sites with moderate to low disturbance ($P<0.05$). Results indicate human disturbance as a potential driver for altered activity patterns. Our research demonstrates that anthropogenic disturbance likely affects the behavior of American Crocodiles, and observed patterns of behavior indicate deleterious shifts towards unproductive and damaging activities. In particular, crocodiles at sites of high human disturbance were observed competing for and ingesting non-food items such as plastic bags, no established social hierarchy, and more frequent aggressive interactions among crocodiles resulting in physical injuries. These behaviors caused negative impact to the overall health and function of individuals as these non-productive behaviors compromised physical condition of individuals and crocodiles spent less time performing activities necessary for daily function.

Keywords*:* conservation, crocodilian, ecology, Mesoamerica, time-activity budget

Intraspecific Variation and Spatial-Temporal Differences in the Isotopic Niche of *Caiman crocodilus* (Spectacled Caiman) in an Agricultural Landscape

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Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of different body tissues can provide information about variation or similarity in the trophic niche at different spatial-temporal scales. We assessed the effects of sex, ontogeny, and habitat use on the trophic niche of *Caiman crocodilus* using $\delta^{13}C$ and $\delta^{15}N$ from five tissues with different turnover rates (plasma, muscle, red blood cell, nail, and scute). We sampled 42 *C. crocodilus* (22 females and 20 males) in an agricultural landscape in the Araguaia floodplain, Lagoa da Confusão, Tocantins, Brazil. We used Bayesian Model Averaging to assess models of isotopic composition and estimated niche width and overlap with Bayesian standard ellipses. $\delta^{13}C$ increased according to isotopic incorporation time, but there was high overlap among different tissues. $\delta^{13}C$ and $\delta^{15}N$ varied significantly between habitats, with *C. crocodilus* from pond and lake having higher variability and significantly larger niche widths than those in ditch and river. Females had higher variability in $\delta^{13}C$ and $\delta^{15}N$ and larger niche width than males, independently of tissue. Females in the pond and ditch had larger niche width than males, although higher overlap between sexes was evident in river and pond. $\delta^{13}C$ decreased with snout-vent length (SVL) and slopes differed between sexes, whereas $\delta^{15}N$ increased with SVL in males, but decreased with SVL in females. In the Araguaia floodplain, *C. crocodilus* has a diverse and relatively invariable diet over time, as inferred from stable isotope ratios. Yet, between-habitat variation in stable isotope ratios suggests a trophic dynamics resulting from movement patterns across interconnected habitats, or human influences on the ecosystem. Presumably, differences in foraging and habitat use patterns lead to wider niches in females, despite both sexes feeding in similar trophic levels.

Keywords: Araguaia floodplain, diet, sexual niche variation, temporal isotopic specialists

Comparing the Male Copulatory Anatomy of American Alligator and Nile Crocodile: Differing Forms, Differing Functions?

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The specific shape of a male copulatory organ determines its functional interactions with female reproductive anatomy and, in turn, can have profound impacts on an individual's reproductive success. But the shapes of these organs are morphologically diverse, even among closely related taxa. The penis of crocodylians present a distal glans that inflates during copulation into a complex cup-like shape. While it is hypothesized that a primary function of glans inflation is to act as plug of the female cloaca uroproctodeal junction to exclude water from the urodeum, the site of insemination, this hypothesis does not address the reason(s) for differing glans shapes among crocodylians. To that end, we compared the gross glans morphology, underlying tissue types, and cellular anatomy of the phallic glans of two species: American alligator (*Alligator mississipensis*) and Nile crocodile (*Crocodylus niloticus*). By examining both flaccid and artificially inflated glans, we characterized structural similarities and morphological novelties that may better inform our understanding of conserved or divergent functional specializations. The glans of both species contains similar tissue types: a medio-ventral sulcus spermaticus flanked by smooth muscle fiber bundles, dorso-lateral regions of inflatable tissues, elastin fiber-rich areas underlying the lateral epithelial, and a glandular stratified epithelium. While tissue types are similar between species, the substantial difference is in the organization of these tissues resulting in species-specific shapes. The inflated alligator glans becomes a hollow conical structure with a circular opening to a significant luminal space at the distal aspect. Further, a ventral, elongated, filiform tip extends the sulcus significantly beyond the termination of the conical glans. In contrast, while the inflated crocodile glans has a conical proximal shape and lumen, the ventral aspect of the inflated crocodile glans protrudes farther then the dorsal aspect and displays on each lateral face a prominent notch between these structures. Further, the glans tip curves dorsally after the termination of the main glans and the sulcus terminates in a blunt, upturned, and bifid structure. While both species have proximal glans structures that could act as a cloacal plug, the distal glans shapes and especially the termination of the sperm-conducting sulci suggests different mechanical interactions within the female cloaca and variation in how and where ejaculate is transferred into the urodeum and/or oviducts. Therefore, further study of the corresponding female cloacal anatomies and how the inflated glans may interface with these tissues is clearly required.

Keywords: genitalia, copulation, phallus, reproduction

New Findings in the Immune System of Crocodilians

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In crocodilians, it is common to see the loss of limbs and other serious injuries as a consequence of strongly marked social behaviors; these include the disputes between conspecifics at the reproduction season, for food, nesting sites selection, or others such as those caused by exposure to stressors, high densities, animal handling, presence of predators, antropic actions, among others. Also, it is known that these animals inhabit environments that contain a high concentration of pathogenic microorganisms, both in natural systems and in captivity. In general, this combination of factors predisposes to the development of localized or systemic infections that can even cause death. The frequent question is, how can these animals survive with significant injuries and in environments with a high exposure to pathogenic microorganisms, without showing signs of disease. There is evidence to suggest that these animals are resistant to some infections by potentially pathogenic microorganisms because they would have immunological mechanisms developed to avoid them. From some important findings, the study of the defense mechanisms of the crocodilians, has taken a significant impulse the identification and the characterization of components of the innate immune system (IBS) in greater proportion than the adaptive, under the argument that the vertebrate ectotherms have a SII more diverse than that of higher vertebrates; mainly, because they recognize a wider range of antigens. However, the close relationship between both systems makes the deepening of adaptive knowledge imminent. In recent years, the activity of mechanisms, molecules and a large number of genes encoding proteins with immunological functions in ectothermic vertebrates has been identified, and most tend to be isolated, cloned and sometimes expressed as recombinant proteins. In some cases they are subjected to different evaluations where they try to reproduce the same functions in other species and assess the therapeutic potential. Therefore, a detailed analysis on the identification and characterization of the components is very useful to understand the functioning of the system involved in the defense of these species within an individual, ecological and evolutionary context and, in addition, to plan the utility as bioindicators of individual and environmental health.

Scaling Arterial Pressures with Body Mass in *Alligator mississippiensis*

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Crocodilians exhibit increases in body mass (BM) that can reach 10,000 fold during their life span. This tissue mass addition may impair blood flow as the increase in blood vessel length could increase vascular resistance. Therefore, we predicted that blood pressure should increase in order to maintain an adequate blood perfusion with increasing BM. Furthermore, the completely separated ventricle of crocodilians, unlike most reptiles, would allow mammalian-like blood pressures, which is characterized as lower mean arterial pulmonary pressures (P_{pul}) than mean arterial systemic pressures (P_{sys}). Thus, we also wanted to compare how P_{sys} and P_{pul} change with BM. To test that, we studied 14 American alligators, *Alligator mississippiensis*, from a large range of body masses (118 to 6804 g), representing a mass increment of about 60-fold. Animals were intubated and kept under anesthesia with isoflurane (2%), after which we performed a non-occlusive cannulation of the left aortic arch and the left pulmonary artery. Heart rate (HR) was derived from the pulsatile blood pressure signal. While P_{sys} showed a significant increase with body mass ($P_{sis}= 2.15BM^{0.21}$; $R^2= 0.73$), P_{pul} remained unchanged while heart rate decreased ($HR= 29.79BM^{-0.11}$; $R^2= 0.43$). In the snapping turtle (*Chelydra serpentina*), a reptile without complete intraventricular separation, both P_{sis} and P_{pul} increase with body size. These findings suggest perfusion pressure is greater in larger individuals which may facilitate tissue perfusion. Conversely P_{pul} constant indicating a tight regulation of perfusion pressure to the lungs in crocodilians. The decreased heart rate suggests a lower specific rate of oxygen consumption of bigger animals, such as observed in mammals. Increased blood pressures lead to elevated arterial wall tensions, and further studies will determine the morphological adaptations of those vessels to sustain higher loads in the vasculature of massive crocodilians.

DNA Binding Activity of Transcription Factors in Infected and Uninfected Alligators (*Alligator mississippiensis*)

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A transcription factor binding assay was used to determine the differences 48 different transcription factors in the nuclei of hepatocytes isolated from uninfected alligators and those that had been infected with a mixture of Gram-negative and Gram-positive bacteria. Noticeable increases in nuclear factor kB (NF-kB), a transcription factor is activated shortly after the onset of infection, were noted in infected alligators. Nuclear factor kB also bound DNA specifically in band shift assays. Nuclear extracts from the hepatocytes of infected alligator also stimulated the expression of a luciferase in HeLa cells which contained a stable construct under the control of the consensus NF-kB promoter. Infection of alligators with a mixture of different bacteria also promoted the DNA-binding capacity of other important proteins that control the inflammatory response, including Activator Protein-1 (AP-1) and CCAAT enhancer-binding protein (CEBP).

Keywords: crocodylian, innate immunity, gene expression

Why are there Skinny Alligators? Preliminary Blood Chemistry Results for Alligators in the Florida Everglades, USA

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The American alligator (*Alligator mississippiensis*) is a keystone indicator species occurring in water bodies throughout Florida, USA. The presence of alligators in the Greater Everglades ecosystem of Florida plays a critical role in maintaining ecosystem structure, function, and productivity. We initiated a program in 2004 to monitor alligators in Everglades National Park (ENP), Everglades and Francis S. Taylor Wildlife Management Area, and Arthur R. Marshall Loxahatchee National Wildlife Refuge (LOX). We used body condition to track responses of alligators to environmental stressors and changes resulting from Everglades ecosystem restoration. In response to a declining trend in alligator body condition and an increase in reports of underweight alligators, we collected blood samples from 20 alligators in ENP and 15 from LOX in October and November 2017. We investigated biochemical responses associated with different body condition indices by evaluating hematological parameters related to stress and health. Blood panels suggest alligators in ENP are of poorer health than alligators in LOX or in captivity. Hematological analyses can be a useful tool to assess how alligators respond to environmental conditions in Everglades ecosystems.

Genotoxic Effect of Complex Mixtures of Pesticide Formulations on Broad-snouted caiman (*Caiman latirostris*) Exposed Under Controlled, Semi-natural and Environmental Conditions

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In world agricultural production pesticide formulations used for pest control are commonly applied in complex mixtures or "cocktails", in order to increase pesticide action and decrease costs of application, but the effects on non-target organisms could be really worst. In previous studies, we demonstrated genotoxic effects of the pesticides formulations mostly applied: the herbicide Glyphosate (GLY), along with several insecticides such as Endosulfan, Cypermethrin (CYP), and Chlorpyrifos (CPF). The aim of this study was to evaluate the genotoxic effects of binary and ternary complex mixtures of the commercial formulations of pesticides previously analyzed separately: CYP Atanor® (25% active ingredient: a.i.), CPF Lorsban 48E® (48% a.i.) and GLY Roundup® Full II (66.2% a.i.) on *Caiman latirostris* under controlled laboratory condition and semi-natural condition, as well as in environmental exposure in natural populations. We made the following experiments: 1) embryonic exposure under controlled conditions by spraying through incubation material; 2) *in vivo* exposure in yearlings: performed by semi-natural *ex situ* exposure through pesticide spraying; 3) finally, these results were compared with those observed in hatchlings from nests environmentally exposed during all incubation period in natural populations Biomarkers of genotoxicity evaluated were the frequency of micronucleus (MN) and other nuclear abnormalities (NAs): buds, notched nucleus (NN), binuclei erythrocytes (BiN), eccentric nuclei (EN), and total nuclear abnormalities (TNA), and also growth was considered in experimental conditions. Interactions between pesticides complex mixtures were considered as additive, synergistic or antagonistic. A significant increase in the FMN and BiN were observed in all experimental conditions tested compared to the control ($p<0.05$, in all cases); but respect to other NAs, we observed different effects depending on the evaluated condition. Some of these data coincide with previously results reported in neonates exposed under controlled conditions to binary and ternary mixtures of pesticides, as well as to single compounds. Outstandingly, it was shown that the greatest alterations in FMN, buds, NN and EN were observed in environmentally exposed individuals compared with the controls ($p<0.05$), and with all exposure conditions (single compounds, bi and ternary mixtures) due to a possible additive interaction of all the compounds applied in the environment and to repeated exposure. A greater growth was only observed in the individuals exposed to semi-natural condition to the ternary mixture, respect to the control ($p<0.05$) and it could be explained as a possible obesogenic effect for this case. This study is considered an important contribution for assessing the action of pesticides complex mixtures widely applied in the environment, on genetic material of *C. latirostris* and is an approximation to the real situation of wild populations exposed also to other potential stressors.

Keywords: crocodilians, nuclear abnormalities, xenobiotics

Expression and Molecular Characterization of *mRNA* Melanocortin Receptor 2 in Broad-snouted Caiman (*Caiman latirostris*) Gonads

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Crocodile farming management response would induce stress and immunosuppression that can affect acting reproduction. Melanocortin-2 receptor (MC2R), also known as ACTH receptor, is a member of the G protein-coupled receptor family. Interactions between melanocortins and reproduction have been previously established, associating them with various processes related to stress, metabolism, immunity, and neuroendocrine signaling pathways. This study was designed to localize the expression of MC2R in neonatal and juveniles gonads. In order to identify the mRNA coding *C. latirostris* MC2R, a set of primers were designed from alignments of sequences of phylogenetically related species obtained from NCBI. To assessment them, a set of 30 animals of different ages were utilized to determine MC2R mRNA expression in the transcriptome of gonads cells. MC2R mRNA expression was detected in *C. latirostris* gonads at different ages of both sexes by reverse transcription polymerase chain reaction (RT-PCR) and real time PCR. These findings are the first evidence for ACTH receptor in crocodile specie gonads. Moreover, ACTH is recognized to have a strong effect on reproduction among all of the proopiomelanocortin (POMC) peptides. In this way, the ACTH-specific receptor MC2R detection could be important in the physiological response to stressor in *Caiman latirostris* reproduction management.

Keywords: melanocortin, reproduction, ACTH

Transcriptomic of Blood and Liver Tissues of *Caiman latirostris* through Next Generation Sequencing after Acute Exposure to Glyphosate-base Formulation Roundup®

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A major challenge in the emerging field of toxicogenomics is to define the relationships between chemically induced changes in gene expression and alterations in conventional toxicologic parameters. Fundamental assumptions are that all toxicological relevant effects are accompanied by gene expression changes. The tremendous progress in the development of new technologies in molecular biology and bioinformatics enables interrogation of cellular responses to toxicant treatment at a global molecular level, allowing the evaluation of toxic effects in the context of molecular pathways. The aim of this study was to analyze gene expression profile of blood and liver samples obtained from juvenile caiman after exposure to the widely used glyphosate-based herbicide formulation Roundup®. Eight juvenile males of *C. latirostris* were distributed in two experimental groups: a negative control and a treated group exposed to Roundup® Full II. Animals were maintained under controlled conditions of temperature (30 ± 2 ºC) into plastics containers and the exposure was performed by voluntary immersion in water, at a concentration of 8 mg/l of RU during one week. After exposure, blood and liver samples were taken to all animals and immediately frozen in liquid nitrogen and storage at -80°C until processing. TRIzol reagent was used for RNA extraction following protocols previously adapted for the specie. All liver samples showed good RNA integrity numbers (RIN) but RNA from blood was purified with the *Zymo Research* column to obtain suitable values for the library construction. We used the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina* for libraries preparation (DNA 0.1-5.0 μg) and then DNA was sequenced using *Illumina* sequencing Technology (NGS). Bioinformatics analysis showed that from those genes identified in blood, 201 have significantly different expression levels between exposed and control caiman. From them, 106 (52.74%) were downregulated and 95 (47.26 %) were upregulated in exposed animals. In the case of liver, from the identified genes, 605 demonstrated a significant different expression level and from them, 478 (79.00 %) were downregulated while 127 (21.00 %) were upregulated in exposed caiman respect to controls. Then, we performed a Functional Annotation analysis using *DAVID Bioinformatic database* in order to identified Gene enrichment clusters among deregulated genes. All these data contribute to understand the relationship between temporal changes in gene expression and conventional toxicology endpoints, and facilitate the phenotypic anchoring of toxicogenomic data. Besides, biomarker genes or signatures of certain toxic effects may be derived from this study.

Keywords: crocodilians, toxicogenomics, deregulated genes, enrichment analysis

Animal Care Implications of the Structural and Functional Differences in the Penile Tendons of the American Alligator (*Alligator mississippiensis*)

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Normal phallic eversion and retraction in male American alligators (*Alligator mississippiensis*) are dependent on two collagenous structures attached to the proximal end of the phallus, the ventral penile tendon (VPT) and the ligamentum rami (LR). Both structures connect the phallus to the ischium, but they have different functional roles. The VPT anchors the phallus in the cloaca and acts as a fixed point around which the phallus rotates during eversion and retraction; the paired LR are thought to act as a spring that returns the phallus to its resting orientation after eversion. Tensile tests of both tissues indicate that although mechanical behavior in both the VPT and the LR is described by a J-shaped stress-strain curve characteristic of collagenous tissues, the LR are more extensible than the VPT and have a Young's modulus that is approximately an order of magnitude greater. Histological examination reveals that both the VPT and LR are primarily made up of Type I collagen fibers but the arrangement of collagen inside the tissue differs. Collagen fibers in the LR are long and arranged parallel to the long axis of the structure, while collagen fibers in the VPT are shorter and arranged in an interconnected three-dimensional network. The differences in the mechanical behavior of the VPT and LR during penile eversion and retraction may therefore be the result of differences in collagen fiber arrangement within each tissue rather than differences in material composition. We suggest that tendon damage or inflammation could be one cause of penile prolapse in crocodilians, and the functional differences between these two tissues could affect the presentation and treatment of the condition.

Keywords: genitalia, copulation, phallus, reproduction

Evaluating the Effects of Three Preservation Methods on DNA Quality and Morphology of Museum Specimens of the American Alligator (*Alligator mississippiensis*)

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Museums specimens are preserved in a way that provides a unique perspective of gathering information that can date from the present to millions of years ago. This information allows scientists to have access to an exceptional resource of species that would otherwise prove difficult to obtain. The purpose of this study is to examine two size groups (juveniles and adults, n= 9 per age group) of the American alligator (*Alligator mississippiensis*) to: (1) compare the efficacy of three methods (dermestid beetles, burial, cold water maceration) of cleaning and preserving full body skeletons and give a taphonomic ranking system to the skeletons; (2) evaluate which method yields the highest quantity of DNA by comparing DNA concentrations between three types of bones (articular, tooth cavity, and femur) while also examining two different processes (completely destructive vs. minimally destructive). With the extraction method of choice and from a simple two-sample t-test, there was no significant difference between the "minimally destructive" and the "completely destructive" process of the bones from all preservation methods. However, for the dermestid beetle colony analysis, there was a significant difference for the femur between juveniles and adults (p-value= 0.02984); and between femur and articular regardless of size groups (p= 0.0005971). These results allow for recommendations to museum Texas Tech University, curators, scientific researchers, who may be interested in working with/studying crocodylian museum specimens. The next step we are currently investigating is how the extraction techniques different between the ancient DNA extraction and a modern extraction technique differ in bone extraction success.

Keywords: DNA extraction, *Alligator mississippiensis*, preservation, taphonomy

Nile Crocodile Mortality in an Intensive Housing System due to Systemic *Paecilomyces lilacinus* Infections

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Systemic mycotic infections are known to affect crocodilians. A commercial Nile crocodile farm with a fungal problem, in one of its houses, was investigated over a 2-year period. Significant animal losses were experienced, especially during the cold winter months. Numerous necropsies were performed and a diagnosis of a systemic *Paecilomyces lilacinus* (also known as *Purpureocillium lilacinum*) infection was confirmed. Obvious skin pathology was not seen. Typical granulomatous lesions were observed in most of the internal organs, especially in the livers and/or lungs. Mortality was always associated with a specific crocodile house. This was the oldest house (warm, moist and dark) on the farm. This building is completed covered with corrugated iron - no sunlight coming into the house. *Paecilomyces lilacinus* was successfully isolated, over the two-year period, from crocodile carcasses, as well as the inside surfaces of this "sick building". This fungus was most prevalent in the cracks (creating ideal micro-environments) between the floor tiles. We were also able to isolate the fungus, collected in sterile sample bottles, from the water in the communal pens. The internal surfaces of this specific building were treated with different fungicides without any long-term success in eradicating the fungal infection. The efficacy of different fungicides was also evaluated in the laboratory. This outbreak will be discussed in detail with reference to predisposing factors, macro pathology, histology, follow-up laboratory investigations and control.

Keywords: Nile crocodile, systemic mycotic infection, *Paecilomyces lilacinus*, granulomas

Humane Slaughter of Crocodilians, an Update

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The humane killing of crocodilians and indeed all reptiles has been the object of much scrutiny by NGOs, the public and specialists. This paper will present an update of what the various expert groups such as the OIE, other groups and country legislations have concluded and recommended. The practical application of some of the slaughter techniques will be discussed. We also discuss the broader context of slaughter of crocodilians.

Keywords*:* humane slaughter, welfare

Sexual Dimorphic Pattern that Characterize the External Genitalia in Juvenile *Caiman latirostris* is Modified in Sex Reversed Females

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Caiman latirostris (*C. latirostris*), the Broad-snouted caiman, is a crocodilian species that inhabit South American wetlands. In *C. latirostris*, as in other reptiles, the incubation temperature of eggs during a critical thermo-sensitive window (TSW) of embryo development determines the hatchlings sex. This mechanism of sex determination is called temperature-dependent sex determination (TSD). In *C. latirostris*, as in many TSD species, the administration of 17-β-estradiol (E_2) during the TSW overrides the effect of male-producing temperature, producing phenotypic females. This E_2-mediated effect has been defined as hormone-induced sex determination (HSD). Previously, we have reported that endocrine-sensitive organs, such as ovaries, from females born to eggs incubated at male TºC and exposed either to E_2 or to the xenoestrogen BPA during the TSW differ from the ovaries of TSD-females. Ovaries of neonatal to juvenile HSD-females show altered follicle dynamics and exhibit high incidence of polyovular follicles, changes preceded by abnormal expression patterns of molecules associated with ovarian development and function at embryonic stages. External genitalia, called phallus (or clitero-penis structure), is a sexual dimorphic hormone-sensitive organ. In male crocodiles, its principal function is intromission and fertilization during the copula. In females, aside from gross anatomy, neither data of phallus histoarchitecture nor data related to their functions are found in the literature. The aims of this study were to establish the temporal pattern of phallus growth, evaluate phallus histomorphological features and hormone dependence in male, TSD-females and HSD-females phalli and to establish differences and similarities among pre-pubertal juvenile males, TSD-females and HSD-females phalli. Archived paraffin embedded samples from juvenile caimans incubated at male or female producing temperatures prior to hatching were used. The groups were as follows: TSD-male, TSD-female and HSD-female (incubated at male producing TºC and exposed *in ovo* to 1.4 ppm of 17β-Estradiol). Our results show that the gross anatomy and the general histoarchitecture of the phallus of pre-pubertal juvenile male and female caimans are quite similar, whereas the phallus growth dynamics, many histomorphological features and hormodependence biomarkers are sexually dimorphic. In the phallus of HSD-females, many male characteristics remain, leading to a loss of sexual dimorphism. Loss of dimorphism in histofunctional parameters of the phallus could impair normal female phallus function and, subsequently, could affect *C. latirostris* population dynamics. Our results raise concern about xenoestrogen exposure during TSW and suggest that caution must be taken on using HSD as a tool to recover wild populations of reptiles.

Keywords: reptile, phallus, sex determination, sexual dimorphism

Assessing Habitat Use by the American Crocodile (*Crocodylus acutus*) in the Lower Florida Keys

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The American Crocodile (*Crocodylus acutus*) is a neotropical species whose northern range reaches the United States. It is restricted to southern Florida, where it is found primarily in coastal estuarine habitat, including the Florida Keys and Florida Bay. Due to habitat loss, the species was listed as endangered by the U.S. Fish and Wildlife Service in 1975. In 2007, the species was downlisted to threatened after intensive habitat protection resulted in an increase in number and locations of successful nests. While historically present in the Lower Florida Keys, *C. acutus* is now rare in the region. Recent increased sightings of crocodiles at the Naval Air Station Key West led us to survey the base and surrounding areas from 2013-2016. We conducted spotlight surveys in most accessible waterways and recorded GPS locations for crocodiles. We observed 34 crocodiles over the study period, all juveniles and subadults (>0.65 and <2.25m). We performed a habitat suitability analysis for crocodiles in the Lower Keys by comparing presence and background points. We divided the study area into raster grid cells. For each grid cell, we calculated the distance to each of several important habitat types and assigned these distance values to the crocodile observations and background points. Habitats included mangroves, estuaries, artificial waterways such as canals and marinas, and artificial impoundments, among others. We then fit GLMs to estimate relative selection for each habitat layer and a null model, and used model selection to choose the top model. Finally, we used the top model to predict patches of highly suitable habitat throughout the Lower Keys. The analysis found that distance to estuaries, mangroves, and artificial lacustrine habitat such as impoundments are the best individual predictors of *C. acutus* presence. Estuaries were the single best individual predictor. However, the addition of other habitat types, for example artificial lacustrine habitat and salt marshes, improved the fit of the model. These results indicate that a combination of habitat types is important to sustaining the *C. acutus* population in the region, including estuaries and lacustrine habitat. It also indicates that crocodiles may selectively avoid artificial waterways with steep concrete seawalls such as canals and marinas. Because crocodiles are recolonizing a portion of their historic range that is now heavily developed, continued investigation of habitat selection by crocodiles in the Lower Keys will help identify configurations of habitats that should be protected to ensure the crocodile's continued recovery.

Keywords: American crocodile, Florida, ecology

Ancestral Hybridization, Species Boundaries and Phylogeographic Patterns in Crocodiles from Mexico

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Interspecific hybridization has been recognized as a biological and evolutionary process associated with the generation of diversity via speciation, which may result in the extinction of species or, conversely, favor speciation. However, the general species concept leaves out intermediate hybrids since species boundaries in hybrid zones are genetically and morphologically fuzzy. The main goal of our work was to characterize the hybridization process across the entire distribution of *Crocodylus* along both Pacific and Atlantic coasts in Mexico. We also aimed to explain the historical, demographic, and genomic features that characterize this unique hybridization system. Three types of molecular markers were used, microsatellites (374 samples), mitochondrial DNA sequences (271 samples) and genomic SNP´s (172 samples), as well as morphological and geographic data. Our results demonstrate that this hybridization system challenges the definition of species boundaries. We show that the hybrid zone between *C. acutus* and *C. moreletii* extends from north to south, encompassing practically the entire Gulf of Mexico and the Caribbean. We identified two evolutionary distinct hybrids lineages, which are genetically discernible from the parental species. Hybridization was promoted from secondary contact, where the last parental populations of *C. moreletii* are isolated in continental islands on northwestern Mexico. Morphological characters showed a complex mosaic that does not allow to soundly identify hybrid individuals without a genetic analysis. Hybrid individuals were also identified for the Pacific, despite this is not a natural area of distribution for *C. moreletii*; results suggest that these populations with hybrid individuals are remnants of ancestral genetic flow between both sides of the continent. Phylogenetic analyses, phylogeographic patterns and divergence times showed an ancient genetic exchange between individuals from the Gulf of Mexico and the Pacific, which began about 2.5 million years ago; also that hybridization began on the Gulf of Mexico, near Campeche (a historical sympatric zone), which later extended throughout the Gulf to the north and the Caribbean on the south. Establishing species boundaries is rather difficult in a system with such a complex hybridization system, hindering its evaluation under a strict bifurcation model between species. *C. moreletii* might be potentially facing extinction, although from another perspective, it may actually be evolving under a hybridization process.

Population Structure and Habitat of the Morelet's Crocodile (*Crocodylus moreletii*) in the Calakmul Eco-region, Campeche, Mexico

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Although our knowledge on the population biology of the Morelet's crocodile (*Crocodylus moreletii*) has been rapidly increasing over the last decades, only rudimentary information is currently available about populations from the Selva Maya. In Calakmul (Campeche, Mexico) waterbodies occur in the form of semi-temporary natural ponds (*aguadas*) sustained by rainfall during the majority of the year, possibly leading to a spatial population structure different from other studied *C. moreletii* populations within the Yucatan Peninsula. The importance of this region is further underlined by the fact that over the last 50 years Calakmul experienced significant reduction in annual rainfall, unbalancing water prevalence over time in *aguadas* and threatening aquatic habitat availability. Furthermore, there is the possible existence of populations free from introgression from *Crocodylus acutus*, which threatens *C. moreletii* over large parts of its Mexican range. We conducted exploratory surveys in Calakmul Biosphere Reserve and Balam-Ku State Reserve in 2016 and 2017, identifying 96 waterbodies based on information from CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad), satellite imagery and knowledge from local communities and authorities. Information on general structure, aquatic fauna, habitat quality (water parameters and presence of contaminants), and floristic composition and land cover in the surrounding areas was collected to provide the first available description for the area. Spotlight surveys were conducted to estimate *C. moreletii* abundance and size structure, and to perform captures for marking and scute tissue collection for DNA analyses; further information on presence/absence of crocodiles was collected through interviewing locals. About 25% of surveyed waterbodies held at least one crocodile in 2017, and 47% of waterbodies had crocodile sightings reported in 2016 or 2017. The number of crocodiles per surveyed waterbody ranged from 1 to 22, with crocodiles generally being more abundant in floodable lowlands with minimal human disturbance. The existing outputs of this ongoing project represent the first layer of knowledge for *C. moreletii* populations in *aguada* environments such as Calakmul. Ongoing field studies in 2018, involving telemetry, ecotoxicology, and investigations into body condition and diet, will be followed by genetic investigations to reveal the spatial genetic structure as determined by the environment, patterns of individual relatedness, as well as levels of introgression with *C. acutus.* Taken together, the findings will be essential for management actions towards the conservation of *C. moreletii* populations in *aguadas*, generally serving as an umbrella species for the biodiversity that occurs in them.

Scute Patterns as an Individual Identification Tool in an American Crocodile (*Crocodylus acutus*) Population on Coiba Island, Panama

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Identification of individuals based on morphological patterns is a strategy used primarily in human forensics that has also been applied successfully in several wildlife scenarios. To date, no study has evaluated the potential of these techniques on American crocodiles (*Crocodylus acutus*). We assessed whether the dorsal scute number and pattern of 110 American Crocodiles captured from the wild on Coiba Island, Panama could be used for individual recognition. We estimated scute variation using the number and position of scutes, testing both a binary and a coded assessment for scute presence and pattern, respectively. We analyzed scute patterns using 21 transverse scute lines (TSL) including the three most prominent scutes present on each side of the vertebral column axis. We found significant differences in the number of scutes per TSL and longitudinal scute lines (LSL) by individual. Based on both the binary and coded analyses, we identified all American crocodiles assessed at the individual level, using only the first 13 and 10 TSL, respectively, in an anterior–posterior direction. This gave us a minimum probability of 0.0003 based on the coded analysis and 2.02 x 10ˆ-5 based on the binary analysis to find pattern repetition (one out of 3333 and one out of 49,504 American crocodiles have the most-common scute pattern, respectively). Because the *C. acutus* total population of Coiba Island has been estimated as no more than 1,000 individuals, we could use this individual identification pattern recognition method (IIPR) to identify every American crocodile inhabiting this island.

Keywords*:* conservation, crocodylian anatomy, crocodylian biology, individual identification pattern recognition

Confronting the Challenges to Recover the Populations of the Orinoco Crocodile in Colombia

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The Orinoco crocodile is the crocodylian with most extinction threatened in Colombia mainly to its overexploitation since the middle XX. In order to recover its wild populations, a crocodile reproduction in captivity program was initiated in early 70´ by *Universidad Nacional de Colombia*, later in middle 90´ several Colombian institutions started with the conservation and management actions mainly focused on the reintroduction strategy. With the aim to contribute the country´s initiatives to reintroduce populations of Orinoco crocodile, we developed specific tasks to perform reintroductions in the Meta state of Colombia. We describe our approach to address of the challenges to reintroductions as the choice and characterization of reintroduction site, size and structure of population to release or identification and management of triggers of human-crocodile conflict. We show the particular methodologies and the main results obtained through a framework based on three main issues 1) Basic ecology of species 2) Habitat assessment 3) Human crocodile interactions. Basic ecology data from our most recent crocodylian census indicates that Orinoco crocodile populations reach abundances of 0.22 individuals/km whereas abundances of Spectacled Caiman and Cuvier's Dwarf Caiman reach 6.4 and 7.2 individuals/km respectively. Data from crocodiles tracked with satellite telemetry indicate us that adult crocodiles can travel long distances in short time (>20 km in a week) and the distance of their movements can sum more 90 km in a month. Home ranges rank from 0.3 to 34 km^2, wide areas come from male crocodiles in the dry season when the water level is low however the annual area used by an individual can sum 100 km^2. Habitat data indicate a moderate transformation of riverside forests to pastures for cattle and an increase of boat traffic and fishing with gears that is harmful to crocodylians. Data from people surveyed showed a poorer knowledge of natural history, a lower number of sightings and, a more negative perception of crocodiles than caimans, suggesting that interactions between human and crocodiles have become weak or absent; however we also show how experiential workshops are an useful tool to circumvent this issues and generate awareness in children and adults about the importance of crocodiles for ecosystems and humans. Finally we introduce our approach to deal with data from the main issues to perform a crocodile reintroductions using the best available information but also we highlight that reintroductions cannot be the only conservation strategy to save the Orinoco crocodile.

Keywords: crocodiles, methods, reintroductions, Rapid Ecological Assessment

Population Status of the American Crocodile (*Crocodylus acutus*, Cuvier 1807) Localized in the Turiamo Bay, Aragua State, Venezuela

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The American crocodile (*Crocodylus acutus*) is one of the five species of crocodiles present in Venezuela. As result of different anthropogenic activities, numerous human populations of the Venezuelan coastal region are diminished or extinct. Its vulnerability is considered "Endangered" by official decree and considered as "Threatened" species by IUCN, CITES and the Red Book of Wildlife of Venezuela. The existing population in the bay of Turiamo, Aragua state, located in the center of the northern coastal region of Venezuela (10° 27'17.55"N, 67° 50'55.22"W) is reduced, making it susceptible to stochastic and anthropogenic events that modify it. For these reasons, studies aimed at the conservation of *C. acutus* are of paramount importance. The research was developed with the objective of determining the population status of the American crocodile situates in the Turiamo bay. Between August 2015 and November 2016, nocturnal censuses were carried out by direct counting, during the new moon phase, on footpaths along riverbanks, and on boats along the west coast and brackish lagoon, 25 caimans were observed in this environment and the presence of at least 38, being 11 of these individuals in class I, 14 class II, 6 class III, 1 class IV and 6 Without Classification, this being its population age. This information was compared with past censuses and a downward population trend is observed in the numbers of individuals present in the Turiamo Bay in the last 15 years. To conclude that the principals age of stages in the population are the I and II. The population of American crocodile are relegate to the brackish lagoon on account of the constant war practices and survivor exercises. Although to the tendency indicate an establishment of the population of crocodiles, this are decreasing, fact support to the decline of their individuals along the last 13 years. Under the species conservation, future actions are proposed that raise its population numbers, considering the shelter and the environmental impact that implies to be in an area of restricted access and of military use.

Keywords: American crocodile, population number, conservation of biodiversity

Reproductive Behavior of *Crocodylus rhombifer* Females at Zapata Breeding Farm

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This study has been carried out at the Cuban crocodile breeding center located at Zapata Swamp, Cuba. The crocodile population living in captivity at this center keeps an annual average of 4000 animals, distributed by different categories of age. The general goal was to characterize temporarily the reproductive behaviour of *Crocodylus rhombifer* females living in captivity. The quantity of nests in a retreat of the breeding center during every reproductive period of the adult population was registered monthly from 1981 to 2013. Starting from 2002 and up to 2014, a total of 1875 newborn crocodiles, born in the second half of August, were sampled and their total length and weight were measured. The results indicate that manipulation conditions, as feeding, water availability, vital space and sexual coefficient have an influence on the reproductive indicators. Reproductive frequency increases when female's age goes up and, in the first 22 years of incorporation to reproduction, they show the greatest reproductive frequency, whereas females incorporated to reproduction with a size bigger than two meters show a greater reproductive frequency through the years. Likewise, the size of the clutch of eggs increases as the females get old and the eggs become rounder. The pattern noticed in the tendency of the total length of the newborn crocodiles in relation to the age of the females was irregular, what doesn't fit in with the pattern found in other kinds of crocodiles and suggests that bigger and older females produce longer young.

Distress Call of Cuban Crocodile and American Crocodile at Western Region of Cuba

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The comparison of the acoustic structure of the distress calls between *Crocodylus rhombifer* and *Crocodylus acutus* individuals was the goal of this study. *Crocodylus rhombifer* distress calls were recorded at the Cuban crocodiles farm of Zapata. On the other side *Crocodylus acutus* acoustic signal were recorded in the Sabanalamar farm. We selected 50 individuals from each species, under 1-year- old, and 50 *Crocodylus rhombifer* older than 1-year. Seventeen acoustic parameters were compared between species and age categories within *C. rhombifer*. A multilayer perceptron neural network was used to identify the acoustic signals between species. In *C. rhombifer* these signals had a longer descent time and a higher minimum frequency at -10 dB than in *C. acutus*. However, the number of harmonic at 40 ms from the signal start, the bandwidth at -10 dB, the maximum frequency at -20 dB and bandwidth at -20 dB were lower in *C. rhombifer* than in *C. acutus*. Finally, the descent slope was smoother in *C. rhombifer* than in *C. acutus*. The associations between size and weight with the number of harmonic at the signal's center, the peak frequency and the maximum frequency at -10 dB, were significant. In all cases, up to 45% of the acoustic parameters variation was explained by size or weight. The acoustic parameters of the distress calls encode information related with the age and size-weight of the sender. The distress calls had similar design between species, but differences in some of the acoustic parameters allow the interspecific recognition in the surveyed species.

Model of Potential Distribution of *Melanosuchus niger* (Spix, 1825) (Alligatoridae), in the Amazon Forest Biome, Brazil

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Abstract
Melanosuchus niger (Alligatoridae) has a wide distribution in seven countries of South America, and mainly in the Amazon Basin in Brazil. It occupies a wide diversity of flood areas, large rivers and marginal lagoons, 'igapós', in addition to seasonal flooded savannahs in transition areas with the 'Cerrado' biome. The main objective of this study was to model the potential geographical distribution of *M. niger* in Amazon Basin, in order to identify areas that may be important for species' conservation. We used georeferenciated points from literature, environment data from the WorldClim database, and the Maxent software to create and to analyze the potential model of distribution of *M. niger*. According to the results, the middle-west of the Amazon basin is important for the occurrence and maintenance of *M. niger*, suggesting that the environments in this region should be better preserved, and thus, to serve as future strategic area for the conservation of this species

Keywords: Amazon Basin, Black caiman, modeling, niche ecology

Introduction

Brazil presents six species of alligators, all belonging to Alligatoridae Family (Haddad, 2008). *Melanosuchus niger* (Spix 1825) is found exclusively in the Amazon Basin, but it is widely distributed in South America, occurring in seven countries: Bolivia, Brazil, Colombia, Ecuador, Guyana, French Guiana and Peru (Thorbjarnarson, 2010). The Black caiman is expressively distributed in the Amazon basin, more than 70% of the distribution area of the species is found in Brazil, occurring in the northern states (AC, AP, AM, PA, RO, RR and TO) and part of the Central-West states (GO and MT) (Thorbjarnarson 2010; Marioni *et al*. 2013), bordering the 'Cerrado' biome, in transitional areas (i.e., ecotones). Specimens were recorded in the Juruá, Purus, Madeira, Tapajós, Xingu, Araguaia, Tocantins, Negro, Mapuera, Pará and Amazonas rivers, including in islands at the mouth of the Amazon river (Vasquez, 1991), occupying a wide diversity of floodplains, such large rivers and marginal lagoons and 'igapós', as well as in seasonal savannas (Marioni *et al*. 2013).

The risk of extinction of the species was assessed according to the criteria of the Union for Conservation of Nature (Ross 2000; IUCN 2003). Although it may be affected by loss of habitat and hunting, the species has not yet suffered a significant population reduction, being categorized as Less Worrying (Marioni *et al*. 2013). The species was evaluated in the same category in the workshop for the elaboration of the National List of Brazilian Fauna Threatened with Extinction in 2002, and thus, not included in Normative Instruction no. 03/2003 (MMA 2003). The main goal was to model the potential distribution of *M. niger* in Brazilian Amazon biome. The secondary goals were to create a potential distribution map for the species in this biome; to identify the main

environmental variables that explain their distribution; and to identify the principal areas of the distribution which may be strategic for plans of conservation.

Methods

Maxent software (Phillips *et al*. 2006) was used to explain the potential geographic distribution of *M. niger* in the Brazilian Amazon. To indicate the most important environmental variables in the species distribution modelling (SDM), the relative percentage contribution and the importance of the permutation of the model were considered. Thus, 24 points of occurrence of *M. niger* (i.e., latitude; longitude) were extracted from the literature and the SpeciesLink metadata site. Based in these occurrence points, climatic and geographic environmental variables were obtained from the WorldClim database (Hijmans *et al*. 2005) in the web, for the last 50 years. The resolution of the layers on the environmental data was 2.5 arc minutes. Previous models were made and the variables with less than 1% of contribution were discarded of the model. Based on the environmental data, 10 potential distribution models were generated, using the average of these to create a final model, considering the random selection of 30% of the points recorded for the tests. The map of distribution was made based on the SMD and the ArcGiz software was used to group the distribution of intervals of probabilities (%) by reclassify tool.

Results

In resume, the main environmental variables for the model were: Temperature of Seasonality (Bio 4) with 33.3 percent contribution (PC) and permutation importance (PI)= 44%, Altitude (m) (alt) with 28.1% (PC) and 37.2% (PI), and Precipitation of Driest Month (Bio 14) with 20.8% (PC) and 8.2% (PI). About the data of SDM the average test AUC for the replicate runs was 0.939 (standard deviation= 0.034). This means that 93.9% was explained by the model, being an excellent performance from the occurrence points of *M. niger*, in the Brazilian Amazon.

Discussion

Just the three variables together explained 82.2% of all the model for Black caiman in the Brazilian Amazon. In general, the seasonality, altitude and precipitation were fundamental variables in the potential distribution of the species. On the map of potential distribution of *M. niger*, the probabilities of occurrence were mainly in rivers to the middle-west of the Amazon basin, and secondarily in rivers to the east, as Xingu and Tapajós (Amazon basin), and in the Pará basin. Finally, we intend to continue this study incorporating data of other areas (e.g., Brazilian Amazon and ecotone areas with 'Cerrado'), in order to expand the SDM and to verify if other environmental variables are determinant for the model of this species.

Conclusions

In final analysis, central and lower areas of the middle-west Amazon basin were important for the distribution of *M. niger* (i.e., western rivers of the Brazilian Amazon biome). Finally, the data suggests that the environment in the middle-west of the Amazon Basin should be better preserved, serving as strategic areas for the conservation of *M. niger*.

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A New Method for Measuring the Length of the Head by Using a Laser Rangefinder and Useful for Estimating the Total Length of *Crocodylus acutus*

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Total length (TL) in crocodilians is important for the classification of size classes, some methods to obtain it are: the use of a linear regression from any other morphometric measure (such as the length of the head -HL-) or capture and measure individuals with a flexible tape measure (FTM). In this work we present, for an *ex situ* population of *Crocodylus acutus*, a new methodology for obtaining HL at distance and using a linear regression we estimate the equation to find TL. First, we measured the HL and TL with FTM in 499 individuals and did a linear regression analysis type II under a Reduced Major Axis model between HL and TL, obtaining an R^2= 0.983 (P= 0.001). Subsequently, with 75 individuals, we sought to validate a new technique called Angular Laser Technique (ATL). For this we made the estimation of the repeatability and we obtained that there is an standard error (SD) between measures of 0.029 m; we did a correlation analysis between the HL and the Bias, resulting in a negative (Global value P= 0.908); Later we did a T test with paired samples between HL with the FTM and HL with ATL, obtaining that there is a bias associated with the distance to the objective. Finally, we used the linear equation and the HL with ATL to estimate TL, as a result the global average difference estimated TL and the real one is 0.05 m. Although the validation results of the ATL show that there is a difference between ATL and FTM, we consider that this is negligible since the safety of the personnel manipulating individuals and the animal is guaranteed and therefore the capture is not justified if the ATL can be applied. We consider the new technique and device proposed in the present study are a viable alternative to avoid the unnecessary manipulation of crocodilians and present an interesting opportunity and a wide application for the study of crocodiles, especially in breeding places for commercial or conservation purposes.

Keywords: *ex-situ* population, linear regression, Angular Laser Technique, morphometry

Rustic Incubation of Nesting *Crocodylus acutus* with Conservation Purposes in Bahia Portete, Guajira, Colombia

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Between 2007 to 2008 it carried out the survey of population of *C. acutus* in Portete bay; during this time were identificated the factors that cause the lost of nesting and the hatching success, therefore, it was designed an *in situ* incubation as a conservation strategy for increase size of population. In order to design of rustic incubation, it had keep in mind the following approach: i) the difficulty to use a control and isolated system from enviromental variables; ii) the disadvantage of high consume of energy by the use of tools of high precisión; iii) the disadvantage to produce energy in a remote area, and iv) to avoid large distances of movements for the traslade of nesting. Objetive: To compare the efectivity between rustic and natural incubation of *C. acutus* in Portete bay, as mechanism of management in order to decrease the factors that cause the lost nest. Metodology: during mating season, 2009 to 2011, were placed record data tools for temperatura in two nesting each year, the following way: a record data tool within a natural nesting located in a colonial nesting place and another one in a rustic nesting located in a incubation rustic área. In 2009 and 2010 were used Alla France 91000-016/S readers and PCE-MSR145W. The fertility, success hatching and deformity in the new born were assessed. No matter of the record data tool were located, it carried out the monitoring of general group of the nesting by the soil termic persistence in some deep. Results: 54 nesting were incubated during three years; in 2009 were incubated: 13 natural nesting and 4 rustic nesting; in 2010, were incubated 5 natural nesting and 8 rustic nesting; and in 2011, were incubated 5 natural nesting and 19 rustic nesting, as well. Corresponding to 1579 eggs. Results: The natural inubation presented the higher temperature (32.24 ± 0.91, 31.47 ± 1.22, 30.94 ± 1.00) than rustic incubation (30.86 ± 0.58, 30.86 ± 1.22, 30.78 ± 1.15). The hatching success of the rustica incubation was higher (79.64%, 83.25%, 61.61%) than natural incubation (44.91%, 62.28%, 51.17%). In this study were 10 deformity new born (9 individuals in natural nesting and 11 individuals and rustic nesting). Conclusion: the rustic nesting decrease the radation effects on the nesting and assist in the recovery of population therefore is viable a nesting conservation program in order to increase the size of population of *C. acutus*. The source of deformity in the individuals of *C. acutus* was indeterminated for that reason it recomend a genetic research.

Crocodile Populations Survey in Arauca River between Colombia and Venezuela

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Abstract

The Arauca River has an extension of 1050 km, of which about 300 km are border between Colombia and Venezuela. About 250 km were traveled in the binational part to determine which species of crocodiles they are present and tributary or creeks in both countries. The species with more abundance was the *Caiman crocodilus crocodilus*, with 1.5 ind/km. The sizes class structure is dominate of adults, demonstrating that it is not bean subjected population crops. A juvenile of *Crocodylus intermedius* was also observed, besides a hatchling captured by fishermen. The Arauca River and their tributary or creeks show a good habitat for the different species crocodiles, however it is recommended to carry out another survey in summer to confirm that observed.

Keywords: crocodiles, Colombia, Venezuela, Arauca River, survey

Resumen

El río Arauca tiene una extensión de 1050 km, de los cuales unos 300 km son frontera entre Colombia y Venezuela. Se recorrieron unos 250 km en la parte binacional para determinar cuáles especies de cocodrilos se encuentran presentes en el río Arauca y caños tributarios en ambos países. La especie con mayor abundancia fue el *Caiman crocodilus crocodilus*, con una abundancia de 1.5 ind/km. La estructura de tamaños presenta una dominancia de ejemplares adultos demostrando que no está la población sometida a cosechas. También se observó un ejemplar juvenil de *Crocodylus intermedius*, además de una cría capturada por pescadores. El río Arauca y sus caños tributarios muestran un buen hábitat para las diferentes especies de cocodrilos, sin embargo se recomienda realizar otro censo en verano para confirmar lo observado.

Palabras claves: Cocodrilos, Colombia, Venezuela, Río Arauca, Censos

Introduction

The crocodile species present in Colombia are *Crocodylus acutus*, *Crocodylus intermedius*, *Melanosuchus niger*, *Paleosuchus trigonatus*, *Paleosuchus palpebrosus*, *Caiman crocodilus* with three sub species *C. c. cocodrilus*, *C. c. fuscus* and *C. c. apaporensis*. In Venezuela the crocodile species present are *Crocodylus acutus*, *Crocodylus intermedius*, *Paleosuchus trigonatus*, *Paleosuchus palpebrosus* and *Caiman crocodilus* with two species *C. c. crocodilus* and *C. c. fuscus*.

In 2000 the Ministerio del Medio Ambiente (Ministry of Environment) of Colombia published the Status and Distribution of crocodiles in Colombia (MMA, 2000), few

other studies was develop covering the population status, principal with *Crocodylus intermedius* (Rodriguez M. 2002; Ríos and Trujillo, 2009; Merchan *et al.* 2012). In Arauca Department Colombia, Ardila *et al.* (2002), Anzola and Antelo (2015) and Anzola (2017) evaluate the wild population in some rivers but not cover the Arauca River and tributaries.

The crocodile population surveys in Venezuela are completely different that Colombia, principal because the Venezuela Management Authority implement a *Caiman crocodilus* annual harvest program over wild populations based on the wildlife status (Velasco and Ayarzaguena 1995; Colomine *et al.* 1996, 2000; Villaroel *et al*. 2002). For *Crocodylus intermedius* punctual studies about the population status (Espinosa-Blanco and Seijas 2012; Seijas *et al*. 2010; Antelo. R. 2008; Llobet *et al*. 2002; Moreno *et al.* 2017; Espinoza-Blanco *et al*. 2017; Velasco *et al.* 2017). Godshalk (1978) through interviews report presence of *Crocodylus intermedius* in Brazo Guárico that is a part of the main river inside Venezuela.

The Arauca River is a boundary between Colombia and Venezuela, covering an extension of 300 km. The river continued inside Venezuela and its end in Orinoco River, with a longitude of 750 km more. The crocodile population never was evaluated in a binational section.

The main goal of this study is determinate which crocodile's species are present on the Arauca River and tributaries, determinate the abundance and size structure of the population's species detected.

Study Area

The Arauca River is a boundary between Colombia and Venezuela in 300 km. The expedition began in Las Trincheras (30 September 2017) port in Colombia and finalize in Caracol town (Colombia) on 6 October 2017, covering around 250 km (Fig. 1).

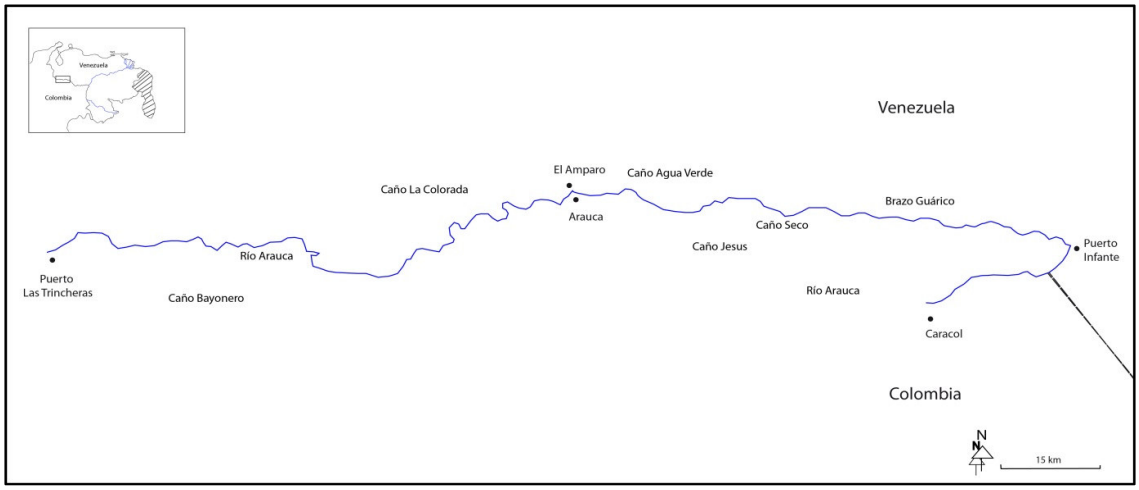

Figure 1. Arauca River.

Methodology

In Arauca River the survey was doing it during the day when the boat navigates, the crocodiles are observed through binocular and identify which specie is. This methodology decided because the river presented high level of water and a high flow of

water, that difficult surveys during the night. For *Crocodylus intermedius* we classify in size classes using the Seijas and Chavéz (2000) proposal, with *Caiman crocodilus* we use the Ayarzaguena (1983) proposal.

The tributaries in both size of the boundary, caño Bayonero and caño Jesus in Colombia, and caño La Colorada and caño Agua Verde in Venezuela was evaluated during the night navigating in a small boat with a motor of 40 hp, using a spotlight to identify the crocodile species. We cover 80.6 km including 5 section of Arauca River (Fig. 2).

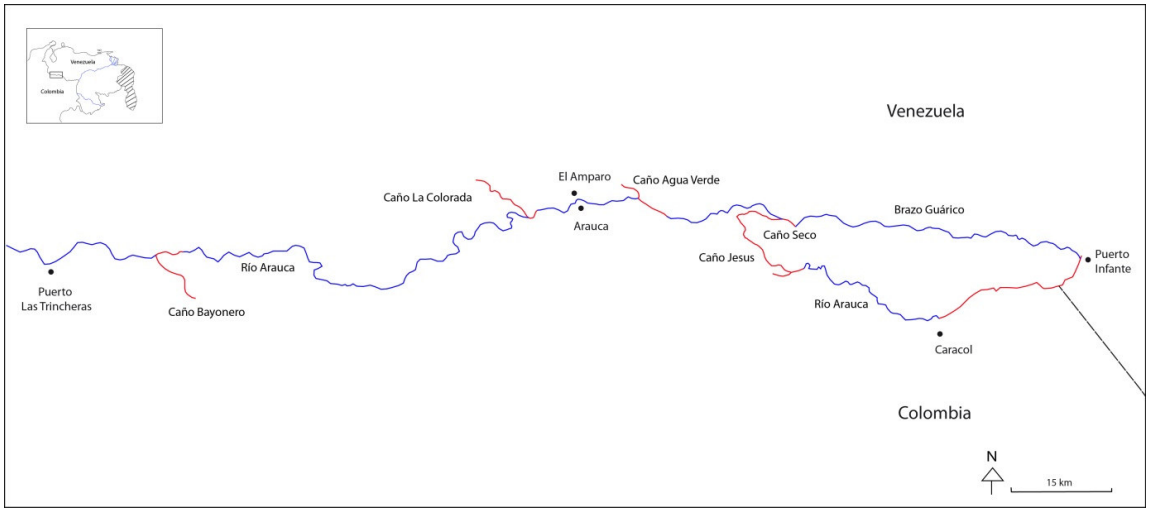

Figure 2. Surveys areas (in red).

All journeys was georeferenced through a GPSMap Garmin 78S using a Datum REGVEN (WGS-84).

Results

For crocodile survey we cover around 80.6 km, 51.4 km correspond to Arauca River and 29.2 km of tributaries (caños= creek). The vegetation around the water bodys is small forest called "galleries forest". No aquatic vegetation is in the Arauca River and in the creeks is like a plug that not permits the boat navigation.

The crocodile species observed was a *Caiman crocodilus crocodilus* (Table 1) and one *Crocodylus intermedius* possible class III in Caño La Colorada, this croc submerged quickly under the water and we found in the mean of the creek, typical place for *C. intermedius*. In Puerto Infante town we see a hatchling captured by a fisherman.

Locality	Type	km	Density (ind/km)	Class II	Class III	Class IV	Only eyes	Total	Aquatic vegetation
Survey 1	River	5.9	0.85	1			4	5	Not present
Caño Bayonero	Creek	2.7	5.56	3	1		11	15	Not present
Caño La Colorada	Creek	3.0	1.67	1	1	1	2	5	Not present
Survey 2	River	2.5	0.40	1				1	Not present
Caño Agua Verde	Creek	3.0	0.67				2	2	Not present
Survey 3	River	4.3	2.33	5	2		3	10	Not present
Caño Seco	Creek	17.8	2.98	10	10	33		53	Not present
Caño Jesús	Creek	2.7	1.11				3	3	Not present
Survey 4	River	10.0	1.50	2	1	1	11	15	Not present
Survey 5	River	28.7	0.42		3	9		12	Not present
	TOTAL	**80.6**		**23**	**18**	**44**	**36**	**121**	

Table 1. *Caiman crocodilus* observed during the survey.

Discussion

The Arauca River during the study present a high water level, strong current of water and few beaches was observed, without crocodiles saw in the beaches. In the creeks was similar the habitat characteristic, with vegetation in the borders.

During the day, when the big boat navigating the Arauca River, we could observe few *Caiman crocodilus* in the river, no more than 10 caiman, all close to the border. During the night survey, we report 43 caimans (35.53% of total), all located close the border and protect by the vegetation, and the size class show in Figure 3. In the tributaries or creek we identify 78 caimans (64.47%), all in the border and under the vegetation. The size class show in Figure 3. Both histograms are typical of population not under exploitation (Velasco and Ayarzagüena, 1995). The tributaries or creek are better habitat for *Caiman crocodilus*, because have a small current of water, the distance between borders is less than 10 mt and present high vegetation in the borders for protection the caimans.

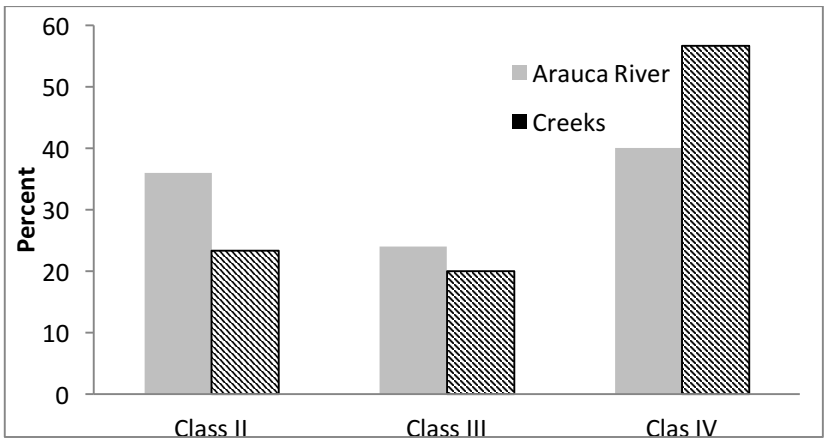

Fig. 3. Histograms of size class of *Caiman crocodilus*.

Only one juvenile of *Crocodylus intermedius* was observed in Caño La Colorada (1.5 m TL). In Puerto Infante town (Venezuela) a fisherman collected a hatchling of *C. intermedius*, this demonstrate that at less one couple are present in the area and other hatchlings. Conversations and interviews with the local people, they comment that saw few crocodiles in the area. In June 26, 2017 a big *C. intermedius* (3.98 m TL) dead appear between El Amparo and Arauca town in Arauca River (7°05'49.51"N, 70°5'32.67"N). All this suggested that is possible to believe that are present a small population of *Crocodylus intermedius* and reproductive.

Close to Puerto Infante town, is a Picapico creek with 17 km and present sandy beaches, but we not visited because the Venezuelan army don't permit to do it, evaluating the satellite images we suggest is a great place to found crocodiles.

Conclusion

The Arauca River apparently is a good place to maintenance a crocodiles population, especially *Crocodylus intermedius*, but is necessarily make a survey in dry season to ratify this comment, especially to determinate the differences on the habitat conditions, especial on presences sandy beaches for nesting.

The tributaries or creek on Arauca River show an excellent habitat for *Caiman crocodilus* and *Crocodylus intermedius* and could maintenance good populations.

We recommended continued the studies more dedicate and enforce on the tributaries or creek, especially on caño Picapico, in dry season.

The specie more abundant is *Caiman crocodilus*, with a density of 1.5 ind/km.

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Mobile Application (App) for Sustainable Management of Jacare do Pantanal (*Caiman yacare*)

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The development of a mobile application (App) that uses the Geographic Information System - GIS, allows the real-time georeferencing of the egg collection in the Ranching system in areas authorized for management, being a fundamental tool for project validation and transparency. In this way, the developed App works online and offline and allows the collection of data such as: a) geographic coordinates with a precision of up to 6 meters, b) photo of the nest, c) photo of the female, d) annotation of biometric data of the female, e) temperature, f) quantity of viable and non-viable eggs, g) identification of the transport box, h) nest distance to water, i) egg classification and information, j) shaded or sunny place, k) farm name, l) collection team, m) date and time, and finally, n) delimitation of the collection area. In the second year of its use, 100% of nests were collected with a total of 1336 nests and 32,270 viable eggs. In this period, the data collected showed that 9.49% of the females were close to their nests, 34.73% of the nests were exposed to the sun (floating vegetation) and 65.27% of the nests were under shading (capon or range of forest). The following averages were found by region: Castelo (30 eggs/nest), Paraguai Mirim 22 eggs/nest) and Tuiuiú (29 eggs/nest), with the average egg weight being 60 g and the mean nest temperature of 31°C. Through the evaluation of the data obtained, we can identify the productive potential of each region, nesting behavior, fecundity rate, stocking rates, egg weight and size, georeferencing of the preferred places for laying, material used in nests, mapping the areas to streamline the eggs collection. As a safety measure, the App allows online access to information by government inspection agencies, ensuring collection at source and quota, promoting transparency in the sustainable management of *Caiman yacare*.

Keywords: *Caiman yacare*, sustainable management, GIS, conservation

The Use of Drones in Conservation: A Methodological Tool for Surveying the Density of Caiman Nests

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The use of drones or unmanned aerial vehicles (UAVs) is a thriving aspect of conservation biology with the potential to revolutionize the way animals and habitats are monitored. Until recently, the location and distribution of crocodilians nests was focused on the use of helicopters, paramotors and aircraft for both the identification and validation of nesting sites. The methods chosen for crocodilians counts are subject to many interpretation difficulties when comparing densities observed in different habitats or with different visibility conditions and environmental variables. In this work, flights were performed with an UAV, with the objective of being able to estimate the density of caiman nests, quantify the potential of nests in the monitored area and evaluate if the harvest of nests in the province of Corrientes by YACARÉ PORÁ is within the acceptable limits for a sustainable program. In total, there were 52 flights (drawn from a total of 240 polygons); 36 flights in Nueve de Julio area and 16 in Malvinas area. On Nueve de Julio the average density was 0.054 nests.ha^{-1} minimum and 0.056 nests.ha^{-1} maximum. For Malvinas the average density was 0.157 nests.ha^{-1} minimum and a maximum of 0.203 nests.ha^{-1}. We extrapolated these densities only to the surface of the 240 polygons generated within the total study area and obtained a minimum of 804 nests and a maximum of 1356 nests in only 12,000 ha. The use of this technology allows us to carry out nest presence sampling, quantification and georeferencing, but the most interesting thing is that it can be treated as density and extrapolated to other sites with similar conditions.

Keywords: UAV, ecology, monitoring, wildlife

Capture, Mark and Recapture of *Crocodylus acutus* in Portete Bay

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Between 2007 to 2011 it carried out the research of capture, mark and recapture of *C. acuttus* in Portete bay. Objetive: to determine the number of animals present in Portete bay for to establish the conservation facts that assist the population recovery. **Metodology**: between 18:00 to 04:00 hours, using hand-lamps and hand free lamps were realizated rounds in small boats with motor of 12 hp, canoe and walking for the capture and recapture of individuals in establish transects in 2007. The capture of animals were according with the size: animals <1 m with the hand and animals >1 m with snare. It recorded the animals biometry total length (TL), snout-vent length (SVL) and the weigth (W) using a weighing scale of 300 g, 500 g, 25 kg and 200 kg. With the TL and W it determine Relative Fatness (Seijas *et al.* 2003) The gender was determinated by direct palpate in the sewer or using a clamp; it established the structure of class acording with Platt and Thorbjarnarson (2000) Finally, the animals were mark with microchip and cuts in the caudal crest. It used Recapture package to estimate if is a close or open population. **Results**: It obtained 178 individuals of *C. acutus.* The individuals of *C. acutus* moves to near habitats, located at the north of bays and the population presented a class structure well-balanced. It presented significatives diferences between class(KW: H'= 6.11; $p<0.05$), among years (KW: H'= 2.61; $p>0.05$) and the IGR did not present significatives differences among years (KW= 71.265; P= 0.0); the Rcapture package showed a closed population of 497.9 (441.6-554.2) and open population of 356 (315.6-396.4). **Conclusion**: The population of *C. acutus* is conserve with a class structure well-balanced viables for develop a conservation program. The animals presents a minor size than other population reporte, and according with the IGR, Portete bay is an healthy ecosistem with resources for the maintenance of the *C. acutus* population.

How to Estimate Population Size in Crocodylians? Population Ecology of American Crocodiles in Coiba Island as a Study Case

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Reliable estimates of crocodylian population size are desirable for both understanding the ecology and natural history of species as well as developing sound conservation and managements plans. However, choosing appropriate methods to estimate population numbers can be difficult due to the paucity of comprehensive analyses regarding their effectiveness, robustness, and applicability. We estimated the American crocodile population size in the southern tip of Coiba Island, Panama, using both spotlight surveys (Messel's and King's visible fraction estimations) and mark-recapture (POPAN formulation-superpopulation) methods. We assessed and compared the outcomes of these methods with the overall capture record for the study area from 2009 to 2013, evaluating their applicability, accuracy, strengths, and limitations. We also provide a novel approach to indirectly estimate (based on spotlight data) crocodylian population numbers based on the sampling distribution (via bootstrapping) with adjusted confidence intervals. Using historical and current capture data, we defined a minimum population size of ~112 non-hatchling animals in our study area, which was larger than both Messel's (19.00 ± 7.50 individuals) and King's (25.71 ± 7.25 individuals) population size estimates, revealing that these latter approaches clearly underestimate population numbers. We estimated a total population size of 243 (182-319) individuals based on our novel approach and a range of 147-257 individuals based on POPAN formulation grouping the data by sex and age groups. We analyze and discuss sources of bias in population size estimations for all methods used in the present study, providing recommendations to minimize and improve estimations. We also analyzed and compared population ecology attributes obtained in our study with what have been reported in other insular and coastal areas across the American crocodile range, increasing knowledge about the ecology of the species. We think our approach is promising and can bring some clarity regarding how to estimate this population attribute in a more cost-effective way. However, more studies must be done applying this method in different habitat/species conditions to fully assess its effectiveness, robustness, and applicability for all crocodylians.

Characterization of Ticks Found in *Caiman crocodilus fuscus* of the Hidroprado Dam, Tolima

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The objective of this study was to identify the hard ticks (Acari: Ixodidae) found in 10 individuals of brown caiman (*Caiman crocodilus fuscus*) at the Hidroprado dam. The ticks were removed manually, fixed in 70% alcohol with glycerin for subsequent taxonomic identification in the laboratory. A total of 33 ticks, 6 females, 4 males and 23 nymphs were collected. Two genus were identified through adults (*Amblyomma* and *Rhipicephalus*) and 4 species: *Amblyomma dissimile* (n= 7), *Amblyomma cajennense* (n= 1), *Amblyomma scutatum* (n= 1), and *Rhipicephalus sanguineus* (n= 1). The genus *Amblyomma* and *Rhipicephalus* prefer as hosts to domestic and wild mammals and birds, however, *Amblyomma dissimile* and *Amblyomma scutatum* is reported commonly in reptiles. Natural infestation of the *C. c. fuscus* by mites makes visible participation of the species as a host in the life cycle of the four species of tick in Hidroprado. Health level, the risk of exposure to these arthropods increases in individuals the vulnerability to suffer by mechanical action ulcerative damage of the mucous membrane and the dermis, which could allow infection and the entry of infectious agents such as bacteria, parasites, rickettsia, protozoa and viruses. Also, the subtractive action of ticks could cause severe anemia that generates susceptibility to other diseases, putting at risk the health of the individual and the population. From Public healt point of view, is relevant to highlight the parasitism in *C. c. fuscus* by *Rhipicephalus sanguineus* and *Amblyomma cajennense* due that are ectoparasites associated with domestic animals with high potential as disease vectors zoonotic, which indicates that Hidroprado ectoparasites with preference in domestic animals can enter into intimate contact with wildlife, creating new opportunities of parasitism and transmission of infectious agents potentials with the respective economic and social implications.

Keywords: *Amblyomma*, *Rhipicephalus, Caiman crocodilus*, Hidroprado, ticks, *Ixodidae,* Tolima

Ranching Protocol of Morelet's Crocodile (*Crocodylus moreletii*) in Mexico

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Since 2010 the *C. moreletii* monitoring program has confirmed that wild populations of the species in Mexico are stable, and holds potential for sustainable use with benefit of local communities and other key stakeholders of the species' value chain, whilst promoting habitat and species conservation. In this regard, the Mexican CITES Scientific Authority (CONABIO) coordinated the development of a "Ranching Protocol for Morelet's crocodyle in Mexico". The document was co-edited by Gabriel Barrios (expert on crocodile monitoring) and Juan C. Cremieux (expert on captive breeding) in collaboration with 13 experts. In August 2016, a consolidated draft was presented for consideration of the Specialist Group on Crocodilians in Mexico and international experts, including some from IUCN-CSG. Following the adoption at CITES-CoP17 of Mexico's proposal to lift the zero quota for wild Mexican specimens of Morrelet's Crocodile, this peer reviewed edition of the protocol establishes technical and scientific foundations for the development of management plans for those Management Units for the Conservation of Wildlife (UMA) that will carry out the Morelet's crocodile ranching activities in Mexico. Throughout its nine chapters, the protocol gathers experiences of ranching in other countries and expertise of national and international key specialists on: population, nest and habitat monitoring and management; estimation of sustainable rates of harvest by ranching; extraction and transportation of eggs; incubation (including details about infrastructure, equipment and materials needed); and hatchling-handling. All the information generated through the implementation of the protocol is systematized in an *ad-hoc* database designed by CONABIO, to follow-up and run various analyzes on performance and success of ranching activities within the country, among other variables.

Keywords: ranching, *Crocodylus moreletii*, protocol, database

Ecology and Conservation of Crocodilians in Northeasten Brazil: Projeto Jacaré, UFRPE

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Projeto Jacaré of the Federal Rural University of Pernambuco (UFRPE) emerged from the need for information on the populations of crocodilians in the Atlantic forest and associeted ecossystems in the metropolitan region of Recife, state of Pernambuco, proposing to know various ecological and conservationist aspects for the two species found in the region, *Caiman latirostris* and *Paleosuchus palpebrosus*. The project started with theoretical-practical training in 2013 and a subsequent recycling of the techniques in 2014, following independently until the present day. The work carried seeks to reach the comprehensiveness of the scientific community and population in general, by investing in lines of research, such as genetics, reproduction, space-timxe monitoring, environmental support capacity, trace elements and diet analysis, besides extension work and environmental education, through exhibitions and dynamics in schools. Concomitantly, the project operates in partnership with environmental institutions in order to provide technical and theoretical support, involving capture, management and release os the animals. The activities are carried out in a bimonthly systematic way, with the capture of individuals in water, through the use of funnel traps, manual and steel cable capture and indirect registration, by interception of the ocular globes by a concentrated light beam and subsequent collection of biological samples, in addition to attending occasional occurrences of animals rescued or found in risk áreas. Until now, 11 papers in scientific events and one book have been published and and two undergraduate degree completion papers were completed finalized, as well a several events, meetings and theoretical-practical courses. Projeto Jacaré also invested in the qualification of human resources, training 20 undergraduate students in the techniques of capture and containment and making them apt to the development of researchwith crocodilians. In total, 260 *C. latirostris* and four *P. palpebrosus* were captured, with 39 and two recaptures for these species, respectively. In partnership with fauna organs, 39 animals were rescued, with one recapture. In addition, 1493 other animals were visualized during night counts. In the northeast of Brazil, researchs with crocodilians are insipient and Projeto jacaré brings the proposal to elucidate and open discussions about the species in this region, based in a real diagnosy about its populations. With the development of scientific knowledge coupled with the investment in expanding and demystifying the vision of these animals from a popular perspective, the efforts made by the project aims to contribute with strategic actions for the conservation of the species of this locality.

Keywords: *Caiman latirostris*, enviromental education, herpetology formation, *Paleosuchus palpebrosus*

Crocodile Management in Sarawak, Malaysia: A Year after CoP17

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At the 17th meeting of the Conference of the Parties of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CoP17) held at Johannesburg (South Africa) from 24 September-5 October 2016, Malaysia successfully transferred its Saltwater crocodile (*Crocodylus porosus*) from Appendix I to Appendix II. The transfer allows wild harvest restricted to the State of Sarawak and a zero quota for wild specimens for the other States of Malaysia. The transfer was based on clear evidence, derived from long-term monitoring, that the number of wild crocodiles has significantly increased and that crocodiles are no longer threatened in Sarawak. Human-crocodile conflicts continue to exist and crocodiles are still seen as dangerous vermin. The transfer provides the opportunity for wild crocodiles to be utilized and to bring direct benefit to the affected population, this could lessen the negative perception on crocodiles as a useless and dangerous beast and indirectly ensure the conservation of crocodiles through sustainable harvesting and various other initiatives. Crocodiles are still protected in Sarawak and any harvesting of crocodiles or their eggs from the wild requires license from the Controller of Wild Life. The Management Plan for Estuarine Crocodile in Sarawak 2016-2020 has been developed and implemented to systematically manage and conserve crocodiles in Sarawak. Through this paper, the authors take the opportunity to share their experiences in managing and conserving crocodiles a year after the successful transfer to Appendix II.

Illegal Hunting of Caimans Related to Protected Areas, Access and Seasonality in Tocantins, Legal Amazon, Brazil

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Despite Brazilian and international laws imposed prohibition to illegal trade of caimans in the late 20th century, illegal hunting continues under subsistence or sport hunting motivations, focusing in caiman meat. However, it can favor illegal trade. Surveillance and seizure by the environmental agencies inhibits the illegal activity and contributes with information hunting data in their reports. In this study, we assessed the seizure reports about caiman specimens in the state of Tocantins, Brazil, aiming to characterize hunting and contribute for conservation and public policies. We analyzed 80 seizure reports of environmental agencies (IBAMA, NATURATINS and CIPRA) between 1992 and 2014. We clustered the data in macroregions according to local, hydrography, and protected areas. The recorded hunting data were hunting local, date, number and attribute of specimens. Hunting was seasonal, selective and small-scale. Illegal hunters are active during dry season (May to September), when there is high caiman density. We reported 134 caimans confiscated (mean= 5.83 ind./yr), identified as *Caiman crocodilus* (N= 32) and *Melanosuchus niger* (N= 5). Only 23 individuals had body sizes described, with an average of 1.50 m (range: 0.6-4 m). This mean size refers to animals of easy capture and slaughter, and produces satisfactory kilograms of meat (total of 223.8 kg seized). The hunting local were rivers of easy navigability and access. The region of Bananal Island, Cantão State Park, and surrounding municipalities concentrate the highest number of infractions and animals seized. Some seizures report packed meat in thermal boxes, suggesting later consumption or illegal trade. Thus, federal and state highway surveillance had high finding of caiman hunting and protected areas are main targets and sources of hunted wildlife. The mid-Araguaia River basin region and *C. crocodilus* had highest effort for hunting. Easy navigability and access are factors to consider in hunting. There are evidences that logistic and economic difficulties may affect surveillances and produce underestimates, notwithstanding highway surveillance acts efficiently to curb. Therefore, greater investment in surveillance and public policies in prohibiting illegal hunting is required. Interaction between environmental agencies and society is also necessary for awareness of wildlife conservation importance in protected areas. Yet, long-term population studies are essential to know caiman population status, focal area for surveillance, and possible impact of illegal hunting.

Keywords: environmental agencies, Araguaia-Tocantins Basin, crocodilians, hunters

Philippine Crocodile as Flagship Species for Community-Based Sustainable Tourism (CBST) Model in Siargao Island: An Integration of Critical Habitat and Species Protection

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The concept of community-based sustainable tourism was shared to the local stakeholders of Paghungawan Marsh, Siargao Island Protected Landscapes and Seascapes in southern Philippines. This aimed to encourage communities on the importance of integrating habitat and species conservation actions toward a resilient Philippine crocodile habitat. Resolving poverty alleviation, ecosystem resiliency, and knowledge development are the major focus of the project. In 2014-2017, the operational development of ecotourism has considered the Paghungawan Marsh to be the first ecotourism destination in the municipaltiy of Pilar. Sustained community-based participatory monitoring was exemplified in the demarcated strict protection zone of the marsh. As a result of the effective public education, the local government unit has adopted May as a special month for the conservation of Philippine crocodiles in the municipality of Pilar. Several government projects were installed by partner institutions in aid to the communities growing effort in conserving their ecosystem. These effort have led to the supplemental release of 29 juveniles *Crocodylus mindorensis* in July 2017. The inspiration among local residents are remarkable despite of fundamental challenges encountered in *C. mindorensis* conservation. These challenge still remain in ensuring the perpetuity of wild Philippine crocodiles being conserved through local initiatives.

Keywords*:* Philippine crocodile, sustainable, ecotourism

Madagascar's Crocodiles: A Combined Approach for Conservation

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In the last decade, numbers of wild crocodiles in Madagascar have undergone a dramatic decline. As a direct result, a CITES imposed international trade embargo was placed on all crocodile products from Madagascar from 2010-2014. However, other than the economic impact, the effect of this embargo on wild crocodile populations remains largely unknown. We present new research that will help inform a new management plan for crocodiles in Madagascar in terms of genetic diversity, population trends, and threats faced by wild Madagascan crocodiles, and the role that traditional cultures and customs may play in crocodile conservation. We document two closely related but distinct genetic lineages of Nile crocodile in Madagascar; one older lineage with a restricted distribution, the other with an island-wide distribution. We present findings from a case study investigating the dynamics of a crocodile population in an unprotected area near a major market and trading hub and how they respond to local pressures. We discuss our findings in terms of the potential ramifications for other crocodile populations in Madagascar that are exposed to similar pressures and the effectiveness of the international intervention. We also investigate how traditional belief systems and customs may better serve conservation efforts.

Keywords: conservation, *Crocodylus niloticus*, Madagascar

Project *Mecistops:* Scientific and Management Foundations for the Conservation of the Critically Endangered *Mecistops cataphractus*

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The West African Slender-snouted crocodile (*Mecistops cataphractus*) is one of the most threatened crocodylians globally. In 2013, we initiated the Project *Mecistops* - a captive-breeding, reintroduction, and capacity-building program designed to facilitate the recovery of severely depleted populations of *M. cataphractus*. Unfortunately, *M. cataphractus* is also the least known crocodylian in the world, a title that results in considerably hindered management. To start overcoming this, and lay the foundations for future reintroductions, habitat management, and community conservation efforts, we are conducting surveys to assess the current status of Slender-snouted crocodiles in the wild and evaluating the efficacy of Ivorian protected areas (PAs) to protect crocodilians. We have completed 1500 km of surveys in and around 8 PAs of varying protection level, and estimated a total 60 *M. cataphractus*. We observed 65% of specimens within national parks (Taï and Comoe), 28.3% in community conservation areas and 6.7% in Classified Forests. Slender-snouted crocodiles were only found outside of the core PA in one site (Taï), otherwise they were distributed uniquely within the confines of PAs. The main threat to *M. cataphractus* in Cote d'Ivoire is by-catch in both subsistence and small-scale commercial fisheries, though this species is hunted on a limited basis, which we also found to be more significantly concentrated inside than outside PAs. In 2016 we initiated a telemetry tracking program with wild slender-snouted crocodiles to establish baseline habitat use, home range, and survival parameters. To date we have tagged 19 individuals in Taï National Park ranging in size from 1.2-2.7 m total length. We track crocodiles every day for a month at a time, every other month. We have lost 3 crocodiles to poaching and another 2 transmitters have been ripped off in active or ghost fishing nets. We will present preliminary results for the other ecological parameters, though in general note that most individuals recognize the boundary of the national park and do not regularly, or ever, move beyond it. In addition, we will present a status update of the breeding production and captive management, as well as capacity-building efforts for protection of wild populations of West African Slender-snouted crocodiles in Côte d'Ivoire.

Management of Estuarine Crocodile (*Crocodylus porosus*) in Sarawak, Malaysia

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Abstract

Sarawak, one of the Malaysian States in the island of Borneo, is transected with numerous river systems and network. Due to its rugged topography in most areas rivers have become the main mode of transportation to local communities and providing foods and water to them. At the same time rivers and estuaries in Sarawak provide natural habitats for the increasing population of estuarine crocodiles. Unfortunately overlapping use of rivers by crocodiles and local people often ends up in Human-crocodiles conflict (HCC). Despite the conflict that seemed to be on the rise of lately estuarine crocodile is legally protected under the State's Wild Life Protection Ordinance, 1998, and was listed under CITES Appendix I which was recently been downgraded to Appendix II. Estuarine crocodile is an important biological resource that could generate revenue to the State of Sarawak while at the same time plays an important role in the ecosystem. Thus several management programs are now being implemented to ensure sustainable utilization of the species while at the same time aiming to reduce HCC. Some of the programs being put up for the species in Sarawak are;

1. Monitoring of crocodile harvest activities along certain rivers.
2. Resurveys and reassessment of crocodile population and distribution along certain rivers.
3. Conducting awareness program on crocodile among communities dwelling along crocodile-infested rivers.
4. Establishing Crocodile Removal Zones (CRZ) in a number of public places.
5. Culling and removing nuisance crocodiles.
6. Establishing a crocodile sanctuary which serves both as a rescue center for nuisance crocodiles as well as to provide tourist attraction and research on crocodile.
7. Enhancing research activities on crocodile for better management of the species.
8. Introducing crocodile-based tourism activities along a number of rivers in certain areas.
9. Installing crocodile warning sign boards at places of high crocodile densities to remind general public on potential danger posed by the predator when using rivers.

It is hoped that the implementation of the above programs would help general public, particularly local communities, to understand the crocodile better of it roles in the ecosystem and its potential to contribute to the economy of the State and its people, and not just seen as pests that must be eradicated.

Introduction

Sarawak, one of the Malaysian states in the island of Borneo, is transected with numerous river systems and network that provide transportation, food and water to local communities (Engkamat, unpublished report) (Fig. 1). At the same time rivers and

estuaries throughout the state also provide natural habitats for the viable and increasing population of estuarine crocodiles (*Crocodylus porosus*) (Cox and Gombek 1985; Engkamat, unpublished report). Malayan False Gharial (*Tomistoma schlegelii*) is also found to occur in Sarawak but the species is more confined to upper reaches of a number of rivers in freshwater swamp in some parts of southwestern and central Sarawak (Stuebing *et al*. 1993; Payne, pers. comm.; Engkamat, unpublished report). Overlapping use of rivers by local communities and estuarine crocodiles often ends up in human-crocodile conflict (HCC) which usually end up in several fatalities each year and, as a result in the follow-up process, dozens of the crocodilians were also killed (Borneo Post 2017; Anonymous 2017). Ritchie and Jong (2002) have described Sarawak as a land of legends and mysteries following a fatal attack on human by a mysterious white-backed crocodile in Batang Lupar area in 1982. Despite the conflict both estuarine crocodile and False Gharial are legally protected under the State's Wild Life Protection Ordinance, 1998, and was listed under CITES Appendix I. It was only recently (October 2016) that Estuarine crocodile in Malaysia was downgraded into CITES Appendix II which allows regulated commercial hunting and trading of the species in the Sarawak. Though estuarine crocodiles are seen as menace by some local people (Ritchie and Jong 2002) the species remains an important biological resource that could generates revenue to the state of Sarawak, and its people, while at the same time plays an important role in the ecosystem. Thus several management programs are now being implemented to ensure sustainable utilization of the species while at the same time reducing HCC.

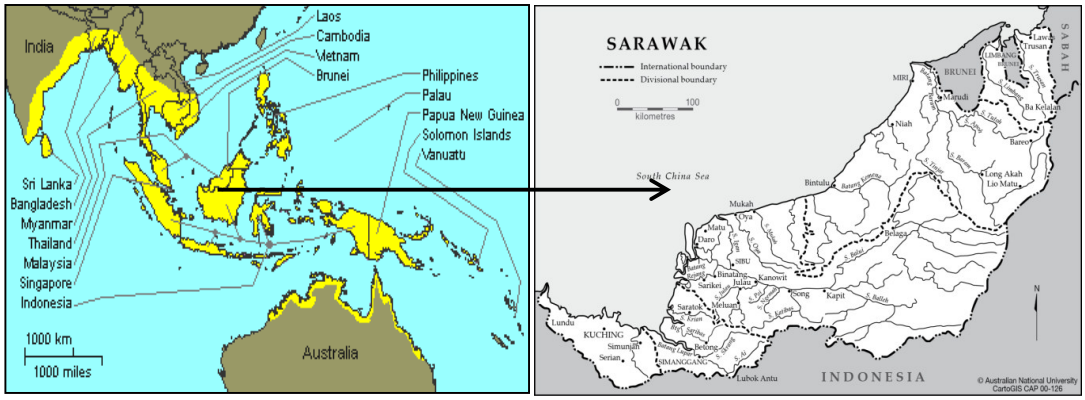

Figure 1. Sarawak, Malaysia.

Management Programs

Among some of crocodile management programs being implemented in Sarawak are;

1. **Monitoring of crocodile harvest (and crocodile culling) along certain rivers**

Apart from giving harvest quota and tags for crocodiles that they captured the hunters are requested to report to the Controller of Wild Life on their catch before the expiry date of their hunting licenses, which usually valid for six months. Periodic monitoring and patrolling by enforcement team are done, whenever necessary, along certain rivers allocated for hunting activities.

2. **Resurveys of crocodile population and distribution along certain rivers**

Hunting activities may significantly reduce population of Estuarine crocodile in their natural habitats to certain level. Thus to ensure that hunting is done on a sustainable basis rivers where commercial hunting was taken place need to be resurveyed to reassess status of the remaining population of the crocodiles. Hunting may need to be stopped along rivers where density of the crocodile falls under certain level.

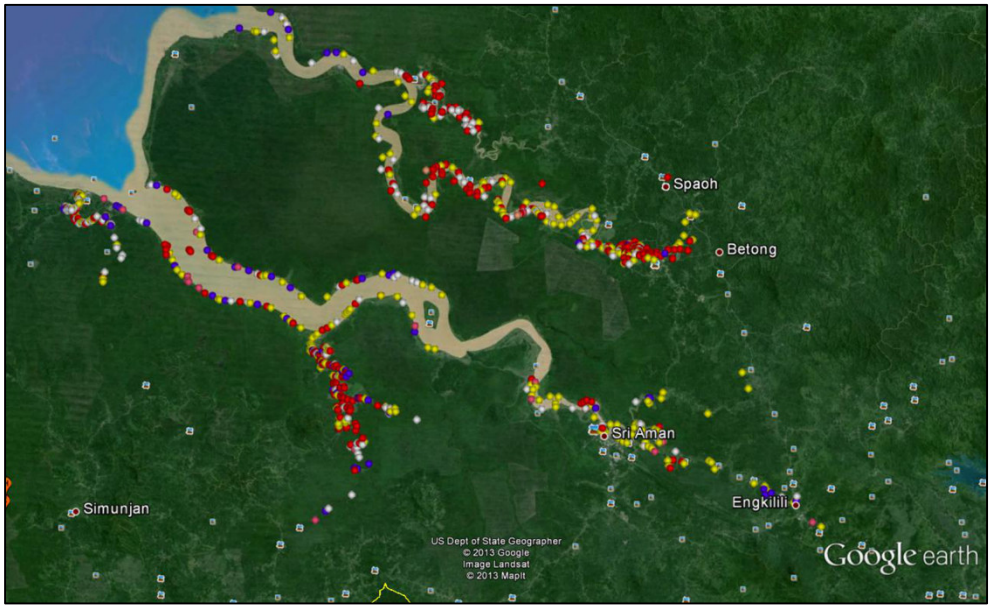

Figure 2. Distribution pattern of estuarine crocodile (based on Size Classes) along Batang Lupar and Batang Saribas rivers as of May and July 2013. The rivers would be resurveyed following commercial hunting within the area.

3. **Conducting awareness program on crocodile among communities dwelling along crocodile-infested rivers**

The program is being carried out by the Wild Life Division of the Forest Department of Sarawak, together with its counterpart from Sarawak Forestry Corporation, in some crocodile-infested areas and among school children of selected schools. The activities comprise of talks on crocodiles which is usually tied up with other important wildlife species for conservation and wildlife licensing procedures. Installation of crocodile warning signage (sign boards) at several locations in crocodile-infested areas is part of the program, and up to date at least 60 sites have been installed with the signage (Fig. 3).

Figure 3. Crocodile warning signs.

4. **Establishment of Crocodile Removal Zones (CRZ) in a number of public places**

Public and recreational areas such as waterfronts of major towns and recreational beaches have been declared as Crocodile Removal Zones whereby crocodiles that are found to encroach into these areas would be terminated or removed. The task would be undertaken either by enforcement team, or SWAT of the Forest Department or the Sarawak Forestry Corporation, or could be undertaken by licensed commercial hunters.

5. **Culling and removing of nuisance crocodiles**

The activities are usually done following crocodile attacks on human or along sectors of rivers heavily used by local communities while at the same time housing high density of the man-eaters. A total of 148 attacks, where 80 (54.05%) were fatal, have been recorded throughout Sarawak since 1982 until April 2018 (Fig. 4). The culling operation is usually done by the authorities or by someone duly appointed by the Government with closed supervision by the authorities. The operation usually aimed at individual crocodiles of above 8 feet long (Fig. 5).

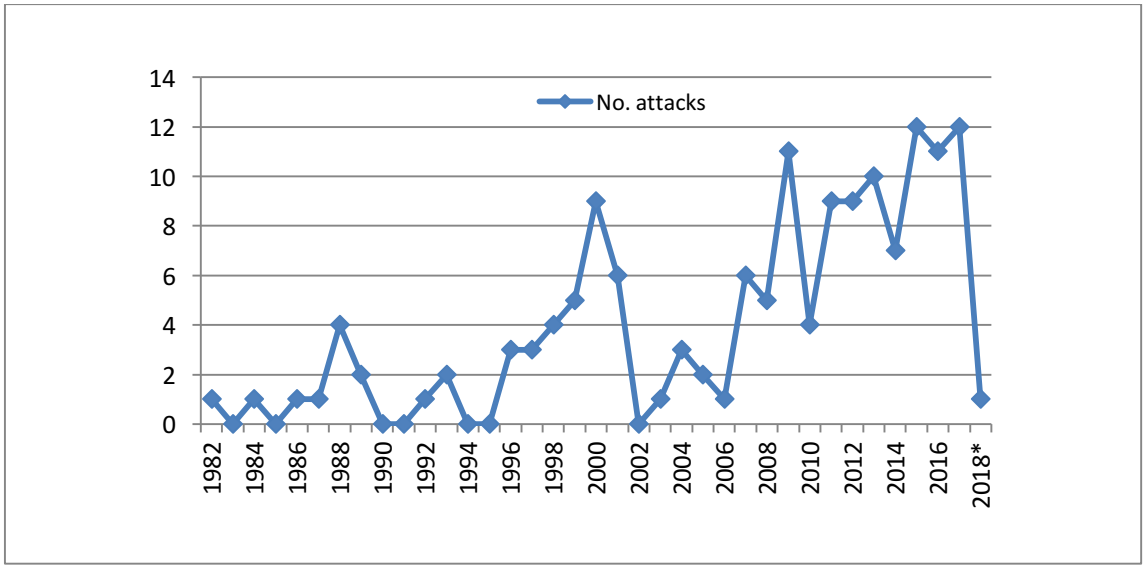

Figure 4. Number of recorded crocodile attacks throughout Sarawak from 1982 until April 2018*. (Note: Total number of attacks for 2002-2006 are not fully available at the moment).

Figure 5. Some of the crocodiles culled (killed) and removed after attacks on humans.

6. **Crocodile Sanctuary**

Forest Department of Sarawak is CITES Management Authority (MA) for the state, and it is an obligation for the MA to come up with a rescue center for CITES-listed species, and thus a crocodile sanctuary, which is now under construction process, is one of the rescue centers for Sarawak. It would not only cater for nuisance crocodiles taken from every part of Sarawak but also caters for croco-tourism and research purposes, as well as for education-awareness programs, particularly for school children.

7. **Enhancing research activities on crocodile**

Despite having high population of estuarine crocodiles, research on the species in Sarawak is still very limited. Proper management of the species depends so much on good research and thus Sarawak welcomes those who are interested in the species to submit their research proposals to the Controller of Wild Life, Forest Department, Sarawak for consideration of the research permits. This is not only applies to Estuarine crocodile but also goes to the Malayan False Gharials, a more elusive species of which still not much is known on its ecological and population status within the State.

8. **Introducing crocodile-based tourism activities along a number of rivers in certain areas**

Crocodiles are important resources in eco-tourism industry. A number of travel agents in Sarawak have introduced crocodile watching along certain rivers, rivers where hunting is prohibited such as areas in national parks, as parts of their eco-tourism activities. With a good and competent guide crocodile watching along river at night can be a very interesting event especially among crocodile enthusiasts. In the neighboring state of Sabah the event seems to be a lucrative business and has generated remarkably important revenue not only for the state but also for its local people involves in the activities such as in providing transportation, guides and home-stay (Stuebing *et al*. 1993). For Sarawak such activities are still at its infant stage but with the intervention, enhancement and assistance from the Government the activities could soon be developed as one of major eco-tourism attraction for the state.

Conclusion

With the adoption of the above mentioned management programs it is indeed our prime hope, and the hope of the state Sarawak, that sustainable utilization of estuarine crocodile would not only generate revenue to the state and its people but at the same time would ensure sustainability of the species while reducing its conflict with human, particularly with local communities dwelling along rivers. Regulated commercial hunting activities that are just introduced following its down-listing into CITES Appendix II should not cause too much worries among crocodile lovers and enthusiasts. Webb (1978) stated that animal's population in its wild habitats normally has various means of compensating for losses in numbers due to mortality or harvest by hunting while Bolton (1989) stated that when crocodile numbers in the wild have been reduced, for instance by hunting, the survival rate of young one would improve. In other word survival is partly depends on the numbers or on the density of crocodiles while losses are greater at the higher densities. As such crocodile would remain as one of important biological resources for Sarawak both in term of revenue generation either by mean of consumptive (eg. meat and its leather) or non-consumptive (tourism), or in term of its ecological roles in the ecosystem.

Acknowledgements

I would like to thank the State Secretary of Sarawak and the Acting Director of Forests, Sarawak for allowing me to attend this very important meeting on crocodiles. With this opportunity I would also like to extend my thanks to my staff and colleagues in the Wild Life Division of the Forest Department who have helped me a lot in the surveys and licensing activities all these while.

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Tracking the Critically Endangered Philippine Crocodile: an Ongoing Diet and Telemetry Study of Wild, Translocated, and Headstart Crocodiles

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Facing threats from habitat loss, destructive fishing methods, and unlawful killings, the critically endangered Philippine crocodile (*Crocodylus mindorensis*) is the most threatened crocodylian species in the world. Once distributed throughout the Philippines, *C. mindorensis* may now be restricted to just two wild populations - one on the island of Mindanao, and the other in northeast Luzon. In 2005, the Mabuwaya Foundation initiated a conservation and hatchling headstart program focused in San Mariano, Isabela Province, northeast Luzon. This multidimensional program has focused successfully on generating local community support for the conservation of Philippine crocodiles and increasing hatchling survival to augment the wild population. Until now, however, relatively few data exist on the spatial and foraging ecology of wild Philippine crocodiles and the long-term success of the Mabuwaya headstart program. We initiated a spatial and foraging ecology study focused on wild and headstarted adult and juvenile, translocated adult, and newly released headstarted yearling crocodiles. We are tracking individuals with VHF, GPS, and Iridium satellite transmitters to better understand habitat selection, dispersal patterns, and survivorship. We are also examining stomach contents and diet on all individuals. We have collected data on 13 adults and 7 juveniles caught in the wild, 10 (50%) of which were headstarted individuals released by Mabuwaya from 2007-2015. Preliminary stomach content analysis show a varied and seemingly opportunistic diet with consumption of snails (75%), birds (45%), fish (40%), rats (30%), insects (25%), snakes (20%), lizards (10%), turtles (10%), frogs (10%), and crabs (10%). Preliminary tracking results show adult crocodiles in particular range much further than previously thought, use previously unidentified habitats, and occupy sites previously thought to be absent of *C. mindorensis*. We will continue monitoring tagged crocodiles, and collecting additional ecological data, over the next year to better support critical management efforts and the long-term success of Mabuwaya's Philippine crocodile conservation program.

Survey of West African Crocodile (*Crocodylus suchus*) in Godro River in the Sitatunga Valley Natural Reserve of Benin

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Sèdjè-Houègoudo in a Sitatunga Valley Natural Reserve is one of the last sanctuaries of the Dwarf crocodile (*Osteoleamus tetraspis*) and the West African crocodile (*Crocodylus suchus*) in southern Benin. Sitatunga Valley is initiative of the Regional Center for Research and Education for Integrated Development (CREDI-ONG). Godro river stretches between longitudes 2° 42'46.24" and 2° 42'56.78"E and between latitudes 6° 78'68.29" and 6° 81'98.87"N. Discovered in 2013, the Godro pond has an important fishery resource as well as the crocodile of West Africa (*Crocodylus suchus*). The present study aims to know where and how the crocodiles of this pond make their nest for a better awareness of the population on the use of the pond. For four years, several raids, observations and capture have been made on the Godro pond for the collection of data on crocodiles. Since 2016, we have observed the invasion of this pond by ferns. Between 2013 and today the river is invaded by plant species characteristic of wetlands. Today, it is clear that almost the entire surface of this pond is covered by ferns, particularly *Diplazium sarmantii*. However, the above-mentioned species is found in its majority (99%) in swamp-dominated wetlands. Two small crocodiles (*Crocodylus suchus*) were captured, tagged (transponder) and released in the Godro River while three sub-adults with an average length of 1.5 m observed by the population from 2016 to 2017. Since 2013, nests (02 in particular) were noted by the local populations. Since studies have shown West Africa crocodile live in rivers, ponds, lake, we can say today that crocodiles of this environment are threatened by two factors: it is invading ferns that would make it difficult for the survival of this species of crocodile as well as the reconquest of the site through the increase of the human population around the site. Because, the site is sacred for the faithful of the Church of the Celestial Christianity. It is important to conduct studies on the size of the population of this species in the ecosystem and to check (by marking) if the individuals observed in the Sô River do not come from this pond?

The American Crocodile (*Crocodylus acutus*): An Indicator of Ecosystem Health in South Florida

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The American crocodile (*Crocodylus acutus*) is a flagship, federally threatened species and ecosystem indicator that directly links crocodiles to hydrologic restoration in the Florida Everglades. Here, a long-term mark-recapture study of American crocodiles was conducted in south Florida from 1978 to 2015. Over the study period, 10,040 crocodiles were captured, with more than 90% of captures being hatchlings. We estimated hatchling survival at 22%, which steadily increased with age to near 90% survival from age six onward. Body condition factor and growth of crocodiles were strongly stage-structured with younger crocodiles found in lowest body condition but growing fastest. Salinity affected body condition and growth, but area effects were stronger, illustrating that crocodiles captured in Northeastern Florida Bay where hydrologic conditions have been most altered were in lowest body condition and had reduced growth relative to western south Florida. Restoration efforts have been geared toward restoring a more historic estuarine ecosystem that is less prone to extreme hydrological conditions. American crocodiles illustrate the need for continued restoration efforts in NE Florida Bay to ensure health of the Everglades and Florida Bay.

Gharial Distributions and Abundances: Then and Now

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Historic accounts indicate that Gharial (*Gavialis gangeticus*) were common and abundant throughout the Indus, Ganges, Mahanadi, Brahmaputra-Meghna drainages. Large scale water control and extraction, widespread hunting, and intensive fishing during the 19th and 20th centuries reduced Gharial numbers dramatically throughout the species' former range. At present, the species has been extirpated from the Indus, Irrawaddy and most rivers and tributaries of the Ganges and Brahamaputra-Meghna systems. Today, Gharial are limited to only 14 widely spaced, restricted localities in north India and lowland Nepal. Only 5 subpopulations exhibit recent reproduction/recruitment. Nesting is commensurate with the estimated adult females only on Chambal River and at Katerniaghat Reservoir. Limited nesting, relative to adult females at these localities, occurs at Chitwan, Corbett, and on Gandak River. Nesting at Babai River (Bardia NP in Nepal) may have occurred previously, but not recently. The other 8 minor locations where Gharial have been sighted show no evidence of reproduction. The number of extant Gharial is estimated conservatively at ~650 (300-900 mature adults). The Chambal River subpopulation, inhabiting ~625 river km within the National Chambal Sanctuary, contains 77% of the global total (=500/650 mature adults; conservatively 425 females, 75 males). In 2017, the nesting on the Chambal River and its tributaries accounted for ~89% of the global total nesting (425/475). In 2018, the IUCN Red List re-assessment of Gharial, a decade after its initial listing as CR in 2007, recommends no change in status. In 2007, the primary basis for CR were low population numbers, <250 mature adults. In 2018, multiple counts estimate current numbers at 2-3X those previous. However, the recent increase is due entirely to a demonstrable increase in the Chambal subpopulation. Elsewhere, despite evidence of reproduction, numbers remain stable or have decreased. Of the 8 minor subpopulations, 3-6 will likely be extirpated within the next decade. The updated CR status of Gharial is based upon a 1) 94% exponential decline in adult numbers, within 3 generations (using 25 yrs/generation, = from1943), from>20,000 adults historicially (based on 1 Gharial/river km) to 650 adults today, and 2) 94% exponential decline in occupancy area from 80,000 km^2 historically to 4400 km^2 today. Continuing major threats include: dams/barrages, water extraction/irrigation, river inter-linking, fishing net mortality, sand/boulder mining, and introduced species. Conservation actions have included captive breeding and head-starting in past decades, but now require smart, site-specific programs with local river communities to reduce multiple threats *in-situ*.

Keywords: Gharial, distribution, abundance, conservation

Winter Feeding and Cold Weather Mortality in American Alligators Living Near their Northern Distributional Limit

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It has long been assumed that American alligators (*Alligator mississippiensis*) do not feed during winter months, particularly in the central and northernmost portion of their range where winter temperatures commonly reach 0°C or lower. This assumption is largely based on the idea that during such cold periods alligator metabolism is inhibited to the point assimilation of food is not possible. In addition, a combination of feeding trials and anecdotal observations indicate alligators will not take food during winter months. In January 2018, the coastal plain of North and South Carolina, USA, experienced an episode of unusually cold weather in which the mean daily air temperature over a 7-day period was below 0°C. Approximately two weeks later, dead alligators were opportunistically encountered at multiple localities in coastal North and South Carolina. By the end of February, over 50 dead alligators were reported. We examined a subset of these animals (n= 20) and found fresh prey in 14 (70%) stomachs. We also observed one alligator feeding on the carcass of another, and a game camera recorded an alligator feeding on an unidentified bird when air temperatures were below 0°C. Collectively, these observations indicate some alligators do feed during winter months near their northern distributional limit. It is unknown if this feeding behavior contributed to the observed alligator mortalities, as stomachs of several dead animals were empty or contained prey remains that could have been retained from warm weather feeding (e.g., bones, scales, scutes).

Comparative Phylogeographic Studies between *Caiman latirostris* and *Caiman yacare*

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Caiman latirostris and *Caiman yacare* are distributed in a large region in South America. *C. latirostris* has a wider distribution, but in some localities both species cohabit in sympatry. There are data about their structure, genetic variability and mating system, but there is still no comparative phylogeographic data between them. Because there is a possibility of hybrids between both species (synchrony in their reproductive biology, morphological similarities and coexistence in sympatry), this analysis becomes especially important to provide data on their taxonomic and conservation status. In this study we used mitochondrial sequence data to evaluate the correspondence between the phenotypic characteristics of *C. latirostris* and *C. yacare* with the specific genetic assignment studying different populations of their distribution. We analyzed 60 *C. latirostris* and 37 *C. yacare* samples through the cytochrome b gene. We also included in the study three samples of hatchlings whose taxonomic classification was doubtful. Sequences were aligned using MAFFT 7, and edited with MEGA 7.0. The number of haplotypes (n), haplotype diversity (h), number of segregating sites (S) and nucleotide diversity (π) were estimated using DnaSP 5. A parsimony haplotype network was generated using NETWORK 5.0.0.1, and the geographical distribution of haplotypes was graphed using PhyloGeoViz and Qgis 2.14.3. In a first stage we analyzed all the samples together, and the haplotype network showed a clear differentiation between both species, and the three unclassified samples showed to belong to the species *C. yacare*. Then, we analyzed the samples of each species separately, and the results showed variability parameters lower in *C. latirostris* (n= 12; h= 0.492; S= 57; π= 0.01288) than *C. yacare* (n= 10; h= 0.745; S= 29; π= 0.00661) considering that, proportionally, we studied many more samples of *C. latirostris.* Tajima's D was negative (-0.24816; -0.98831) and non-significant for both species, but Fu and Li's test showed a positive value in *C. latirostris* (0.77987) and negative (-1.11934) in *C. yacare,* although both values were non-significant. These data show relation with the life histories of these species: *C. latirostris* suffered important genetic drift events that reduced its genetic variability; their population sizes increased thanks to conservation programs, and currently shows a fairly homogeneous structure, intermediate genetic variability and few rare alleles. On the other hand, *C. yacare* was less subject to bottleneck events and has remained with higher values of genetic variation; conservation programs are more recent in this species, and currently shows signs of population expansion.

Keywords: *Caiman latirostris*, *Caiman yacare*, phylogeography comparative-conservation programs

eDNA as Tool to Evaluate and Monitor Critically Endangered Flagship Wetland Species in Upper Guinea Forest Protected Areas

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Many threatened species exist at low densities and are difficult to detect or monitor due to their shy and cryptic behaviors. This poses a challenge for conservation managers who must have data on the status of these species to adapt their protected area management actions. Environmental (e)DNA is a cutting-edge technique that involves the extraction of DNA left behind in the environment (such as water or soil) by target species. Utilisation of such technology enables the effective deployment of limited conservation resources needed to gather information simply by eliminating the need for hundreds, if not thousands, of man hours in the field hoping to catch a glimpse of the animals. We are evaluating eDNA as a means to detect and monitor three highly endangered species endemic to the wetlands of the Upper Guinea forest zone in West Africa: the Critically Endangered (CR) West African Slender-snouted crocodile (*Mecistops cataphractus*), the Endangered (EN) pygmy hippopotamus (*Choeropsis liberiensis*), the West African Dwarf crocodile (*Osteolaemus* sp. aff. *tetraspis*; unevaluated, likely EN). Specifically, we aim to: 1) develop species-specific quantitative PCR (qPCR) assays that will amplify short mtDNA sequences from the 3 target species so that they may be detected in eDNA samples, and 2) implement our developed eDNA primers and probes as a tool to identify critical habitat for the 3 species of interest, estimate population distribution, and give an indication of species abundance within, and even around, the protected areas of Cote d'Ivoire. We have successfully developed species- specific primer and probe pairs for all three- target species. Each assay was optimised using both tissue and environmental (water) samples taken from zoological collections in North America and Europe. Rigorous testing for cross- species amplification was also carried out using tissue and environmental samples from captive and wild animals. In 2017, we sampled a wild control site - Hana River, Taï National Park, Cote d'Ivoire - where the three-target species are known to be present. We here present the results of these efforts and discuss the hurdles to using eDNA for monitoring of highly threatened crocodilians.

Behavioral Ecology of Crocodilians: the Missing Links from Mating System to Dispersal

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In terrestrial vertebrates there usually is an evolutionary relationship among behavioral ecological processes such as mating system, dispersion, sex determination of embryos, parental care and dispersal patterns. In crocodilians the knowledge of such evolutionary ecological processes - and their possible relationship, whether it exists - has still relevant gaps. Although multi-paternity has been detected, polygyny appears to predominate. Dominant males usually have larger territories including a certain number of reproductive females which dispute the best nesting habitats. Sex determination of embryos is temperature-dependent (TSD), with generally early occurrence during the incubation period. Females usually invest a considerable amount of energy and time in parental care from nesting period through the first year of hatchlings' age. Dispersal pattern is rather unknown due to the operational difficulty in tracking the young during dispersal. Gene flow - and consequently the evolutionary process itself - is based on the relationship between mating system and dispersal pattern. The relationship between female social hierarchy, nesting habitat, TSD, and dispersal pattern should be prioritized in future studies of crocodilians' biology either for their conservation, sustainable use or control. The development of novel molecular analyses (e.g., DNA markers) might be useful on such a task.

Status and Conservation of Mugger in Protected Area and Unprotected Area in Shivpuri, Madhya Pradesh, India

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Crocodile species were greatly depleted worldwide and India is no exception. There are three crocodilians species found in India, fresh water crocodile Mugger (*Crocodylus palustris*), Gharial (*Gavialis gangeticus*), and the Saltwater crocodile (*Crocodylus porosus*). Mugger is a medium-sized crocodile that mostly inhabits freshwater lakes, ponds, sluggish rivers, swamps and marshes. Under Indian Crocodile Project, launched during 1975, about 24 Crocodiles Sanctuaries have been established where crocodile reintroduction programmes have been taken up. Due to conservation management populations of crocodiles especially mugger have been increased in different states of India. The crocodiles are also living in unprotected water bodies, sometimes near urban areas which lead to conflicts between humans and crocodiles. Shivpuri in north Madhya Pradesh is a small town with historical importance. The forests surrounding the town are once a hunting ground of erstwhile kings of Sciendia dynasty. A patch of forests has been declared as Madhav National Park. There are two lakes, the Chandpata and Madhav lakes, present in the National Park. Large numbers of muggers, residents in the lakes, have been receiving protection. There is a water body inside the town, named as Jadhavsagar Lake surrounded by human habitation, where large numbers of muggers are present. A sewer drainage canal is passing on the northern side of the Jadavsagar lake. The field studies conducted in the Madhav National Park, Jadhavsagar Lake and in the sewer canal revealed presence of large number of muggers. The mugger population is high in Jadhavsagar lake and in the sewer canal an unprotected water body. There are more than 40 muggers in the Jadhavsagar lake. When the lake is becoming dry during the summer all mugger enter into the sewer canal, which is 8 to 10 feet deep. Although the water in the canal is polluted muggers are living in the sewer canal for survival and also getting food in the sewer water. While Human Crocodile Conflict may be an increasing issue in many areas such conflict is not reported in the study area. There are reports of stray crocodiles reach urban area through the sewer canal but local residents are taking care of these stray crocodiles and releasing them back in the lake. Status of mugger in Shivpuri town also provides an opportunity to local residents to observe the muggers in the vicinity of the town and contribute to crocodile conservation. This study has generated a very useful data on conservation requirements of crocodiles in urban areas both in protected and unprotected areas.

Keywords: Mugger, protected area, distribution, polluted water body

Human-Crocodile Conflicts in Mexico; an Update

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Human-crocodile conflict (HCC) in Mexico was reported in 2014, including a brief history of the study of HCC and the first estimates of HCC. There were 153 cases reported up to early 2014, involving all three species found in the country (*C. acutus*, *C. moreletii* and *Caiman crocodilus chiapasius*) and data on undetermined species. We update the information for Mexico, reporting a total of 317 cases up to Sept 25, 2017. We report 212 cases involving *C. acutus* (66.8 %), *C. moreletii* 87 (27%), undetermined species 15 (4.7 %) cases and *Caiman crocodilus chiapasius* only three cases (0.95 %). We analyze the increasing numbers of cases reported at both national and state by state levels. We propose some factors involved with the HCC, related to American crocodile including the increasing numbers and the size of the crocodiles, and the intensity of human activities in the crocodile habitat like fisheries, tourism, habitat fragmentation, and destruction by the tourist sector.

Keywords*:* HCC, Mexico, factors involved

Evaluation of Nuisance Alligator Management in the Southeastern United States and Examination of Human-Alligator Conflict

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The American alligator (*Alligator mississippiensis*) is an iconic North American species, a keystone predator, and an important part of many state economies. As human development encroaches into more alligator habitat, conflict between *A. mississippiensis* and humans becomes inevitable and a necessary aspect of regulation for the state wildlife departments that fall within the alligator's range. Through communication with state natural resource departments dealing with alligators, extensive review of incidents documented by those departments and examination of recorded human-crocodylian interactions in the United States as a whole, this investigation seeks to better understand the current nature of human-crocodylian conflict and those programs that must deal with these conflicts. Investigation of these issues has indicated that improved nuisance alligator data collection upon immediate management of a nuisance individual, implementation of more intentional communication of program effectiveness, and a push toward cooperation between the programs of multiple states may lead to the development of more effective *A. mississippiensis* management programs.

Keywords: nuisance, human-alligator conflict, wildlife management, Alligatoridae

The European Croc Network: It's Purpose

Ashley Pearcy and Agata Staniewicz

Two years ago, at the CSG meeting in South Africa, some Europe-based researchers realized we were isolated from each other, only engaging every two years at the CSG meetings with some even, just by chance, finding the CSG. Most regions within the CSG engage with each other throughout the year. We hoped, in arranging this meeting, we could find both experts we know, find people doing work independent from the CSG and make available a knowledge network for people interested in pursuing work with crocodilians in research, husbandry and industry. Because one of the main aims is to the help young people pursue their career, we make the meeting cheap, with student-friendly pricing and free camping, to be as inclusive as possible. This way the only real cost to the students is traveling to the meeting. We have had two meetings, the first at Crocodiles of the World in the UK and the second at Krokodille Zoo in Denmark with growing success and reach. This year's meeting will be at La Planète des Crocodiles in France. The general set up is a few talks from already active experts and young researchers- including how they started their work with crocodiles from Europe and any needs, collaborations and/or opportunities they might have. We also host a speed dating session, where our participants engage with every other participant to encourage networking across communities and disciplines. As part of these meetings, we also make available opportunities for internships, volunteer programs, and potential graduate positions from around the world. This not only gives opportunities for our participants but also to smaller organizations globally to bring in funds and/or help. We are currently seeking more presence from industry people at the meetings. Europe is one of the regions where industry is still shrouded in negativity and a result of this meeting is to revive the understanding of the support of industry. We are also looking into expanding the format to include more sessions where people can present their work or follow-up work in a poster session. Finally, we are always looking for sponsors to help with this event to keep the costs minimal for the participants.

AZA Orinoco Crocodile Regional Studbook

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The purpose of the Association of Zoos and Aquariums (AZA) studbook is to document the pedigree and entire demographic history of each animal within a managed population among AZA member institutions (AZA-accredited institutions, Conservation Partners, and Certified Related Facilities). In 2011, the Crocodile Advisory Group from the AZA nominated the first author to be the studbook keeper for the Orinoco crocodile due the species IUCN/Red list Critically Endangered category, and the efforts to preserve it done by The Dallas World Aquarium (DWA) since 1999. The process begun taking the Population Management 1 course: Data Acquisition and Processing to get trained. Several letters were sent to different institutions asking historical and current information about their specimens. With the species and taxon reports received, a chronological worksheet started to emerge. One hundred forty-six Orinoco crocodiles were recorded; from these, 53 live specimens are located in 14 US collections: 10 adult males, 31 adult females and 12 undetermined juveniles. All the founders came from Venezuela. First importation to the US was in 1968 by the Cincinnati Zoo (male) and Miami Metro Zoo (female). Oldest live specimen in the studbook is a 45-year-old female at the DWA which is also the most productive. Oldest Orinoco crocodile hatched in the US is a 38-year-old female belonging to Zoo Miami and on loan to Everglades Alligator Farm. Breeding has occurred at the DWA and Gladys Porter Zoo (GPZ). There are four blood lines in the US: "DWA" and "GPZ" in Texas, Zoo Miami "ZM" and Saint Augustine Alligator Farm "SAAF" in Florida. There are 35 Orinoco crocodiles that belong to the DWA (66%), 18 belong to GPZ (26.4%), three to SAAF (5.7%) and one to ZM (1.9%). The importation of young males from Venezuela has been considered to open the blood lines. The conservation plan of the species seeks to produce genetically viable hatchlings to maintain a long-term non-wild reproductive population (+99 years), although it also considers the preservation of natural habitat in Colombia and Venezuela as well as *in situ* research. To house species belonging to a studbook should be considered a commitment so the exhibition of these can generate resources that help the conservation of habitats and the development and continuity of research that helps the existence of the species in its natural habitat in the long term.

Keywords: Orinoco crocodile, studbook, captive population

Human-Crocodile Conflicts in Mexico with American and Morelet´s Crocodiles: When and Why?

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Human-crocodile conflicts (HCC) impact conservation of crocodylians because people fear and sometimes kill dangerous crocodiles. We report when and why HCC happens, with *Crocodylus acutus* (189 events) and, *C. moreletii* (84 events) in Mexico. We found that HCC is distributed unevenly through the year. Data for two species shows that 71.9 % of events occur from April to September (53% *C. acutus* and 18.9% *C. moreletii*) and 22.1% in cooler months and dry season. Both species have more HCC reported during the rainy season, coinciding in part with reproductive events. We analyzed the data by latitude, which affects the timing of reproduction in both species. *C. acutus* shows peaks during nesting; in June when hatchings are present, and in September during hatchling care. This pattern is more evident at higher latitudes >18N^{o}, not at latitude lower than 16N^{o}. We found the lowest values during *C. acutus* mating season. This might be explained by stress and sex hormones, and body temperature. Our data suggest that *Crocodylus acutus* attacks are associated with territory defense (nesting and incubation), and parental care (hatching and hatchling care). HCC in *Crocodylus moreletii*, is not so clearly defined by reproductive events. This species attacks during mating to incubation, but less during hatching and the hatchling care period. HCC during reproductive events between species are not correlated statistically. Surprisingly, the incidence of fatal attacks is not correlated with the number of attacks. Human deaths in *C. acutus* are higher in September during the period of hatchling care. Deaths caused by *C. moreletii* occur during mating, nesting, are low when hatchlings are present and again higher during cooler and dryer months. We conclude that HCC varies due both to species behavior and to the different timing of life history events due to latitude and season and that overall analyses of HCC should take these factors into account.

Keywords: Human-Crocodile Conflict (HCC), Mexico, *Crocodylus acutus*, *Crocodylus moreletii*

The Use of *Caiman latirostris* as a Flag Species for the Conservation of Biodiversity in Environmental Education Actions in Brazil.

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Caiman latirostris is a species that has great cultural value in Brazil. The species is present in myths and folklore and in several aspects of Brazilian popular culture. It is considered a fundamental species for environmental homeostasis and a symbol of the struggle for the conservation of biodiversity in Brazil. Among the caimans that occur in Brazil, the broad snouted caiman stands out due to the complexity that permeates its conservation in the country. Most areas of natural occurrence of the species have been drastically altered or suffer from negative influence of anthropic activities. Hunting, drainage of water bodies, urban expansion, agriculture, livestock, diseases and endocrine disruptors are constant threats to the conservation of the species in Brazil. The Caiman Project - Jacarés da Mata Atlântica works on five fronts for the conservation of the species, which are: research applied to conservation, public policies, training of young researchers, rescue and rehabilitation of caimans and environmental education. The present work aims to present the environmental education actions within the scope of the Caiman Project, using *Caiman latirostris* as a flag species for the conservation of biodiversity in Brazil. In 2017, the Caiman Project published the book "O jacaré-do-papo-amarelo - Guide to Environmental Education", an e-book available for free download that aims to enable people to use *Caiman latirostris* as a flag species in environmental education actions. All environmental education activities in the Caiman Project are based on this book. Transmitting information about caimans and the Atlantic Rainforest to society is as difficult task. Even more complex is to construct a change in the perception and the attitudes of people in face of the problems related to the conservation of nature. Therefore, actions must be conducted with clear objectives that can be achieved in activities that are often punctual, but which need to be outstanding enough to the point of provoking a reflection and adoption of sustainable attitudes. In this sense, the Caiman Project carries out environmental education activities for different publics, always using the most appropriate methodology for each age group and socio-environmental condition. The activities are based on: Environmental education program in public and private schools; Use of games and children's play; scientific communication program; use of images for environmental awareness; thematic park for environmental education of children, youth and adults aiming at the conservation of caimans and Atlantic forest as a whole. Through these approaches the Caiman Project is collaborating with the conservation of caimans and the construction of a sustainable society in Brazil.

Keywords: environmental education, conservation biology, environmental awareness

Hematology and Biochemical Values of Wild and Captive Broad-snouted Caiman (*Caiman latirostris*) in Brazil

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Caiman latirostris is considered a fundamental species for environmental homeostasis and a symbol of the struggle for biodiversity conservation in Brazil. In spite of the efforts to preserve the species in Brazil, little is known about the etiology, epidemiology, pathogenesis, diagnosis and treatment of the diseases that occur in the natural environment and the impact they cause to caimans populations. It difficults the establishment of programs to maintain the population health of *Caiman latirostris*. In this context, hematology and biochemistry are basic but very important tools for evaluation and monitoring of the population health and the establishment of hematological and biochemical reference values are fundamental. The objective of this study was to establish the hematological and biochemical reference values of wild and captive *Caiman latirostri*s in different size classes in southeastern Brazil. In total, 120 specimens of *Caiman latirostris* were sampled. Of these 103 *in situ* and 17 *ex situ*, all clinically healthy. Blood samples were collected from the occipital venous sinus. Immediately after collection blood smear was performed and blood was stored in tubes containing lithium heparin at 4 to 8ºC. Blood counts were performed less than 6 hours after collection. Plasma was obtained after centrifugation and stored at -20ºC until the biochemical parameters were analyzed in the conservation research laboratory of Marcos Daniel Institute. The reference values were determined after evaluation of normality by the Kolmogorov-Smirnov test. For parameters with normal distribution the reference interval was defined as the mean ± 1.96 standard deviation. For asymmetric parameters the interval was defined as between the 2.5th and 97.5th percentile. Hematocrit, hemoglobin, hemoglobin, MCV, MCH, MCHC, leukocytes, heterophils, monocytes, eosinophils, thrombocytes, glucose, total proteins, albumin, globulin, albumin / globulin ratio, uric acid, urea, creatinine, triglycerides, total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gammaglutamyltransferase (GGT), potassium, sodium, chlorine, calcium, phosphorus, creatine kinase (CK) and lactate. Hematology and biochemistry are essential tools for the medicine of crocodilians, as it provides valuable information for assessing the health profile of a population and helps in the diagnosis of diseases. Although hematological and biochemical parameters may vary depending on the physiological, clinical and environmental condition, the present work contributes to the use of laboratory tests in *Caiman latirostris* as a tool for *in situ* and *ex situ* health evaluation.

Keywords: clinical pathology, population health, conservation medicine

A Review of Communal Egg-laying Between Crocodilians and other Reptiles

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Communal egg-laying could be defined as the egg oviposition by at least two gravid females sharing the same nest area or nest cavity throughout the nesting season. Conspecific communal nesting has been reported frequently, while interspecific communal nesting has remained somewhat opaque; therefore, our studies were motivated to enhance the little information about communal egg-laying between crocodilians and other reptiles. We searched in the following databases: ISI Web of Science, SciELO Citation Index, BioOne, Science Direct, Scopus and Redalyc. In each of the databases, all records containing the words: crocodilians, nesting ecology, reproductive ecology, communal oviposition or communal egg-laying, in the title, abstract, and/or keywords fields were reviewed. We also reviewed exhaustively herpetological journals on ecological and natural history studies on crocodilians. We considered anecdotal observations from both non peer-reviewed papers and personal communications by members of the International Union for the Conservation of Nature Crocodile Specialist Group. We found records of 15 turtles, 6 lizards and 4 snakes using crocodilian nests as egg-laying sites. The majority of the reports referred to the American Alligator *Alligator mississippiensis* nests (14 commensal nesting), followed by the Broad-snouted caiman *Caiman latirostris* (3 species) and Black caiman *Melanosuchus niger* (one species). In the Family Crocodylidae, the reports of secondary nesters are few, but three of the four Neotropical *Crocodylus* interact with other reptiles, mainly turtles of the genus *Trachemys.* The rare use of crocodilian nests by squamates, and some kinosternid turtles suggest a random communal oviposition, and it could be explained by the unavailability of nesting habitat during water level fluctuations in the aquatic environment. The year-to-year variation of turtles nesting in crocodilian nests suggests that crocodilian nest construction coincides with the nesting season of other nesting reptiles. Finally, we discussed the secondary nest attendance as an adaptive hypothesis for communal egg-laying between long-lived reptiles.

Keywords: crocodilian, nest attendance, nesting ecology, reptiles, testudines

Anatomical Description of the Thoracic Limb Autopod of *Caiman latirostris* (Daudin, 1802) (Crocodylia: Caimaninae)

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Caiman latirostris shows a wide geographic distribution, being found in six countries in South America. An anatomical description of the bones of the left carpus and manus of a 1.5-meter animal is conducted here. The specimen was donated to the Instituto Marcos Daniel (IMD) by the private breeder Criadouro 2C, located in Cachoeiro de Itapemirim-ES. The phalangeal formula of *C. latirostris* is 2:3:4:4:2, from digit I to V. Radiographic images of the live animal were also used in the description. The first (distal) phalange of digit I is an ungual. The second phalange of digit I has wide epiphyses with a narrow diaphysis. Starting with digit III the phalanges show less disparity between the width of epiphyses and diaphysis. The metacarpus II is longer than metacarpus III. Despite having less bones, the digit II is slightly longer than the digit III. The species shows a set of four carpal bones, like the genus *Melanosuchus*. The main bone of the wrist comprehends the fusion between the radiale and the intermedium. This later bone is similar in morphology to the ulnare. Both have wide proximal and distal epiphyses in comparison with the diaphysis, giving them an hourglass shape. The intermedium + radiale bone has a slightly concave proximal end and a flat distal extremity. Yet, this resembles the structure observed in the ulnare. However, there is a difference between these bones. The intermedium + radiale has a medial projection that articulates with both the ulnare and the pisiform (i.e. the third bone of the carpus). The distal articular surface of the intermedium + radiale faces the metacarpi and the proximal one meets the radius proximally and the pisiform medially. The proximal epiphysis of the ulnare touches the medial projection of the intermedium + radiale, whereas its distal end articulates with the distal carpal 4. This fourth bone of wrist represents the fusion of the carpal bones that articulate with the metacarpi III, IV and V. As future perspectives, a higher number of specimens will be analyzed to understand the variation in morphology in *C. latirostris*, specially concerning the ontogenetic changes within the species.

Keywords: morphology, osteology, front limb

Body Condition Analysis Over Time in *Caiman latirostris*

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Climatic variables can affect the body condition (BC) of individuals because they directly influence the natural environment by modifying the availability of food. BC is an indicator of nutritional reserves; it can be calculated using indices that relate size and weight, which can be directly related to environmental parameters such as precipitation and temperature. We could then assume that, within a population, reproductive females would possess the best BC, since they should provide part of their energy to producing their progeny (at a high energy cost). In addition, the BC of reproductive females, like that of the rest of the population, would be affected by climatic variables. Using the Body Condition Index Scaled Mass Index (SMI) we compare the BC between reproductive (R) and nonreproductive (NR) individuals, and whether there were differences in BC between years, and if those differences could be explained by climatic variables. We calculated the SMI for 335 individuals of *C. latirostris* captured from 2001 to 2016, in the province of Santa Fe (Argentina). We classify the animals in NR (n= 262): Class I, II, III (males and non-breeding females -proven by ultrasonography-) and class IV; and R (n= 73): class III females with eggs (verified by ultrasonography) and females found next to their nests. We analyze the data using analysis of variance and principal component analysis. We observed that the average population BC was 4.35 (max: 6.90; min: 1.92; CV= 16.08). The R's BC was greater than the rest of the population ($p<0.0001$), 42.5% of the R individuals present values of SMI greater than 5, but only 10.5% of the NR reach values greater than 5; for both R and NR individuals, the SMI varied between years ($p<0.05$) but was not related to the environmental variables tested. The bigger BC of reproductive females (R) could be due to the excess of energy stored to be used for reproduction. Variation on BC among years shows that the species has the ability to store more resources from the environment in some years. Environmental variables analyzed in this work were not related to BC, possibly other environmental variables (water level, habitat complexity, number of days with low or high temperature), or intrinsic variables (habitat use, competition), could be affecting BC.

Keywords: body condition, *Caiman latirostris*

Gharial Communication: Acoustic Signaling via "Pops"

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Living crocodilians communicate using visual, acoustic, chemical, and/or tactile signals. As adults, Gharials (*Gavialis gangeticus*) vocalize only rarely, and reportedly do not produce infrasound. On-going ecological and behavioral studies of the largest remaining wild population of Gharials, living in the Chambal River, north India, indicate that Gharials breed at well defined arenas established by dominant males, and then reproductive females assemble and nest in large colonies nearby. Detailed behavioral observations, supported by 24hr acoustic and still/video imagery, at breeding and nesting sites, at multiple locations in successive years, have documented complex social interactions, including acoustic signaling between and amongst adults, as well as young. Male Gharial produce an explosive, concussive "pop" sound underwater, in 1-3 short, loud audible bursts. A "pop" is always sudden and high volume, resembling a stoppered bottle being opened rapidly, like a wine bottle being uncorked. We used hydrophones and aerial mics, to record the "pop" signals of 15+ male Gharial, totaling in excess of 500 samples. Spontaneous recordings were obtained as Gharials behaved normally under natural conditions. At one site frequented by a breeding male, with few females (<10 in 2017) vs. many (>30 in 2018), "recruiting" pops directed at females predominated in 2017, whereas "challenging" pops were frequent in 2018, when other males were present. In 2018, at this site, overall pop frequency more than doubled, relative to 2017. During hatching and afterwards (~4-6 wks), at each crèche site, a guardian male "pops" often to 1) alert and recruit hatchlings, and 2) announce his presence and location to females in the vicinity of the creche. Each male Gharial produces a stereoptypic and characteristic series of "pops," performed underwater, and consisting of 1, 2, or 3 pops. Temporal patterning, rather than frequency differences, appears to be the primary feature of this unique signal, not known in other crocodilians, that presumably facilitates individual recognition. The "pop" or syllable duration ranged from 0.013 to 0.023 seconds, and the time interval between syllables ranged from 0.103 to 0.555 seconds. Distinctive low and high frequencies were characteristic of each pop, ranging from >100-2400 Hz to >10,000-22,000 Hz. Immediately preceding a pop, infrasound is produced. Signal analyses: Raven Pro & Avisoft software. This is an interim progress report for 2017-18.

Keywords: acoustic, Gharial, signaling, infrasound

Genotyping Gharials: Efficacy of "Next Generation Sequencing" vs. Conventional Approaches

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The Gharial (*Gavialis gangeticus*) is a Critically Endangered, river dwelling crocodylian, with a piscivorous diet, a specialized elongated and narrow snout, and a distinct phylogeny. The documented drastic decline (>90%) in the species' geographic range, as well as in its numberical abundance, are strongly suggestive of severe genetic bottleneck(s) in the recent history of the few extant populations today (~5; total adults= 600-900). On-going ecological and behavioral studies of the largest remaining wild population of Gharials, living in the Chambal River, north India, indicate that the Gharials nest in large colonies. When nests at these sites hatch pre-monsoon, hatchlings remain together for 4-6 weeks in large creches (100-1000+ young) guarded by adults, typically multiple females and a single adult male. Detailed behavioral observations, supported by 24hr acoustic and still/video imagery, at these large creches, at multiple sites in successive years, have documented complex social interactions. These are frequent amongst creche members, and involve young-young, young-adult, and adult-adult interactions. Here we outline a strategy for genotyping the Chambal Gharial population in order to 1) examine the underlying genetic bases for creche behaviors, 2) provide baseline "relatedness" values within and amongst Gharial populations, 3) test the efficacy of using "Next-Generation Sequencing" (NGS) vs. conventional genotyping for such analyses, and 4) set the stage for scaling up "genetic" monitoring of all extant Gharial populations. In 2017, we collected ~3000 eggshells and tissue samples from a subset of breeding adults, at nine colonial nesting sites along ~100 km of the lower Chambal River. As a "proof of concept" in the first instance, we are isolating DNA from samples from one large creche site (~34 nests), which likely contained eggs sired by 2-4+ males from nearby breeding sites. Microsatellite markers (18) will be screened, and polymerase chain reactions optimized, and the samples will be genotyped with 15 markers via NGS. To assign clutch parentage, adult profiles will be matched to egg profiles, clutchwise. Additionally, capillary electrophoresis based genotype data of a small sample of egg shells from each nest will be calibrated along with few complete nests. The number of adult males and females will be estimated using a double sampling approach. The use of NGS over conventional method of genotyping with double sampling approach will be evaluated for cost effectiveness for the accuracy and precision of population inferences. This is an interim report of progress to date.

Keywords: Gharial, next generation sequencing, genoptyping, relatedness

Hybridization Between Threatened Species of Central American Crocodiles: a New Hope or a Dead End?

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Hybridization can generate novel combinations of genotypes to facilitate rapid adaptation to new environments, but it can also lead to the loss of one or more lineages by extensive introgression. Escalating rates of hybridization between the American crocodile (*Crocodylus acutus*) and Morelet's crocodile (*Crocodylus moreletii*) in Belize has raised concerns among conservation stakeholders regarding the preservation of each species' genetic integrity. In addition, both species of crocodiles are listed as highly threatened within Belize, and decreased protection due to ambiguous conservation laws for hybrids increase the vulnerability for both the animals and their environment. High levels of introgression between hybrids and both parental species have been recorded in sympatric areas throughout Central America, revealing evidence of multigenerational hybrids across the Gulf of Mexico and parts of the Caribbean. While this localized hybridization event has been characterized as an ancient process, anthropogenic impact may be speeding up and spreading the hybrid boundaries both beyond historical hybrid zones and farther than the boundaries of any one species. Using existing genetic data, I will study the evolutionary history of these species with a special focus on inferring gene versus species trees. If hybridization between *C. acutus* and *C. moreletii* is indeed ancient, then the natural influx of genetic variation could possibly enhance genetic health and species-wide adaptability. However, if this spread is anthropogenically induced, then artificial shifts of pure species habitats into hybrid zones may rapidly displace parental species and drive one or both species to extinction via genetic swamping. For future work, I will examine the phenotypic variations between hybrids and each respective parental species. Preliminary data has exhibited a possible resurgence of F1 or F2 hybrids in Belize that may due to relatively current habitat destruction of key (nesting) habitats. While the driving mechanism for hybridization in Belize is still unclear, looking at the physiological mechanisms amongst each lineage may explain the reasons behind the crocodiles' interspecific breeding, which thus can be used for preventative measures of further developing hybrid swarms.

Synthesis of Hydroxyapatite from *Caiman yacare* Egg Shell: a Perspective Biotechnological in Health

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All people are exposed to bone damage every day either by domestic, work or traffic accidents. The old-aged are more affected by lesions because they suffer loss of bone density, such as osteoporosis, in some cases small accidents, as falls, might to cause fractures. In works, accidents in construction are more frequently and severe, which has about 20,000 accidents per year in Brazil. In addition, the brazilian traffic are 400 thousand people affected per year as sequels mainly in bone levels. For recovery and strengthening of the skeletal system, post-traumatic or prophylactic, there are several calcium-based products, an important agent in bone care. However hydroxyapatite (HAP) from animals eggshells are a calcium compound that has biocompatibility and bioactivity well described in literature. The objective of this work is to synthesize HAP from *Caiman yacare* eggshell. To start the eggshells were donated by Caimasul company located in Corumbá-MS - Brazil. The shells were sanitized by 2% sodium hipochlorite (NaClO) during 2 h and then calcined. The synthese was carried out by humid route with phosphoric acid (H_3PO_4). The reaction of addition was controlled with pH between 9 and 10 using pHmeter (TECNAL Tec-2). Were used 250 g of eggshell dried in a drying oven (Nova Ética Model 400 / 2ND-300) at 70°C for synthesis. The eggshells were taken to muffle (Fornitec F1-M) at 1000ºC for 5h for CaO formation. The next step was solubilize the CaO in distilled water (1:3) to react and product calcium hydroxide ($Ca(OH)_2$). After this step, phosphoric acid was added to $Ca(OH)_2$, 1:13 (w:v). As result was observed that the calcination of 1g of eggshell generated 0.57 g of calcium oxide (CaO), from each 1 g of CaO can synthesize 3 g of HAP, which 1.2 g is calcium. We conclude that the synthesis of HAP was performed with a final yield of 235%, due to reaction of addition, which can be used as raw material and for development from mineral supplements, biomaterial, prophylactic for bone diseases, implants for tissue regeneration, healing and filling for aesthetic corrections.

Keywords: synthesis, hydroxyapatite, egg, Pantanal alligator, biotechnological application

Cyanobacterial Cell Population and Colonial Form as Factors Affecting Bacterial Biological Control Response

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The incidence of toxic cyanobacterial blooms has increased in recent years due to anthropogenic impacts. Climate change is believed to be a further driver of such blooms in the environment. Biological control is now commonly applied and has also been explored in the control of toxic cyanobacteria. Most studies have indicated a lytic effect of predatory bacterial isolates on the targeted cyanobacterial cells, resulting in subsequent cyanotoxin release into the aquatic environment. Among the aquatic life impacted, are reptilian populations in cyanotoxin filled waters. Crocodilian farms are of global significance from an economical and conservation perspective. Eutrophication of breeder ponds is however a constant challenge faced by crocodilian farmers. A recent publication by Singo *et al*. (2017) has directly shown the vertical transmission of microcystins from older crocodiles to their eggs. The study found microcystins were directly linked to mortality, with accumulation of this toxin in the yolk of unhatched eggs. This study examined the effects of biological control using bacterial isolates with a predatory effect on *Microcystis* sp. The findings indicate that the population and the colonial form of the cells may affect their response to bacterial control agents. Using *Bacillus mycoides* as a reference predatory isolate, *Microcystis* sp. isolated from a crocodile farm was exposed to different bacteria isolated from bloom waters, over a four day period. Changes in intra and extracellular microcystin concentrations were monitored using the Envirologix ELISA microcystin detection kit. Microcystin concentration was reduced in water samples with higher population, colonial cells as opposed to lower populations with unicellular Microcystis sp. This indicates a possible threshold in stress impacts that needs to be observed in the potential treatment of cyanobacterial blooms to prevent complete cell lysis. Alkaline phosphatase measurements generally indicated cell stress, as activity was reduced in the presence of the control agents, although this did not correspond with the changes in toxicity. Unicellular, lower abundance ($<4x10^6$) cells were more sensitive to the biological control agents as opposed to higher abundance, colonial cells ($>4x10^6$) cells. Eco toxicity testing using *Daphnia magna* indicated that 40-60% reductions of microcystin at concentrations of ±4 μg/ml resulted in greater survival of daphnids, as opposed to minimal changes at lower concentrations (±1.5-2 μg/ml), which showed no significant difference in comparison to controls. The greatest toxicity reduction was observed in colonial Microcystis treatments. Our data indicate the potential of bacteria-based biological control on crocodilian farms, and the importance of dosage intensity for effective remediation.

Breeding the Cuvier's Dwarf Caiman *Paleosuchus palpebrosus* at the Dallas World Aquarium

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Cuvier's Dwarf caiman *Paleosuchus palpebrosus* is the smallest of all living crocodilians. Males can reach 1.4 meters of total length but females barely 1.2 m. Due to the hardness and completely bony scaled skin, this is one of the less important crocodilian in the skin industry and because of this, one of the less studied. The species is distributed in ten countries of South America. In the past 20 years, there has been an important amount of *P. palpebrosus* exported from French Guiana to different countries. The species breeds relatively simple in captivity but there are some questions about the artificial incubation of the eggs. In 2017, an 8-year-old pair of *P. palpebrosus* (♂950 mm and ♀920 mm of total length) housed at The Dallas World Aquarium (DWA) mated without being noticed, and on 5 July the female laid 10 eggs at the bottom of her 212-gallons acrylic tank. The eggs were underwater but still covered with mucus and were found a couple hours after been deposited. Eggs were measured and averaged 46.9 g, 57.3 mm of maximum, and 37.5 mm of minimum diameters. All were placed in a plastic box with moisten perlite and covered with sphagnum moss. The incubator temperature was set at 30°C and Relative Humidity >92%. On day 1, a macula was observed, and from day 2 this grew up to a band. Band growth mas measured the first two weeks and was noticed to increase rapidly at the beginning. Incubation lasted 90-91 days and all the eggs developed normal hatchlings. Hatchlings were measured averaging 29 g and 206.7 mm in total length. This is the first time the DWA bred this species and also was the first time this pair reproduced. The egg mass represented 11.6 % of the female body mass (4.04 kg). Comparing to other egg masses and female body masses the authors have recorded: *Crocodylus intermedius* egg mass represented 2.38% female body mass, and *Crocodylus moreletii* 7.05%. The weight of the female *P. palpebrosus* was 0.02% of the female *C. intermedius* weight, but the *P. palpebrosus* eggs were just the 68% the size of the eggs of *C. intermedius*.

Keywords: Dwarf caiman, breeding, captivity

Assessing Crocodile Species Niche and Developing Adaptive Conservation Strategies Under Climate Scenarios in West Africa

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Climate change is likely to exacerbate the current threats to freshwater ecosystems biodiversity, yet studies on the potential impacts of climate change on crocodile species that inform management planning are needed. The study started in January 2018 in the Complex Pendjari Biosphere Reserve - W Biosphere Reserve in Benin (West Africa). The research aims to address how far climate change will impact range of suitable habitats for threatened crocodile species (*Mecistops cataphractus, Crocodylus suchus*, and *Osteolaemus tetraspis*) in Benin. Specifically, it aims at (i) examining crocodile species distribution and abundance in the study area, (ii) determining morphological characteristics of crocodile species, (iii) to evaluate the influence of environmental factors on the crocodile species, (iv) characterizing the current niche of the three crocodile species and (v) predicting the impact of climate change on the distribution (2050) of the three crocodile species. Crocodile species distribution and abundance will be acquired through fieldworks by coupling pedestrian methods of crocodile census and the use of drones. Morphological characteristics of crocodile species will be measured. Physico-chemical characteristics of waters, level of human pressure, area protection status, climate covariates, etc. will be recorded. Bioclimatic variables will derive from monthly temperature, humidity and moisture index obtained from Wordclim database. The stable isotopes will be used to identify the current niche and the mix effect model will be used to forecast distribution (2050) of the three crocodile species, under present and future climate models. Finally, the overlap between their predicted habitat suitability map and the current national protected areas network will be estimated. Findings will inform on the species conservation / protection effectiveness and gaps at horizon 2050. Ultimately, the results will provide information on potential suitable habitats where conservation and protection actions towards the concerned species could be concentrated in the future. Besides, the study will provide with strategies to enhance the inherent adaptability of crocodile species and ecosystem processes, and reduce trends in environmental and social pressures that their increase vulnerability to climate variability. The project is expected to cover a period of 4 years.

Keywords: crocodiles, niche, vulnerability, predicted habitat suitability

Evidence of Cryptic Lineages within a small South American Crocodilian: the Schneider's dwarf caiman *Paleosuchus trigonatus* (Alligatoridae: Caimaninae)

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The Schneider's Dwarf caiman *Paleosuchus trigonatus* is one of the smallest living crocodilians. Due to its broad distribution, cryptic behavior, and small home range, the species should be well suited to study of phylogeographic patterns on a continental scale. Therefore, in the present study we have tested the hypothesis that species with a wide geographic distribution in Amazonia should contain different lineages and present population structure within this drainage. Phylogenetic reconstructions of the mitochondrial gene cytochrome b revealed the existence of two well-supported lineages within *P. trigonatus* clade-haplogroups Amazonia and Guiana. Divergence time estimates between lineages using a fossil and secondary calibration methods recovered mean times of 5.6 and 4.1 Ma, situated in the Late Miocene, Early Pliocene, respectively. The hypothesis that the Guiana lineage was founded from the Amazon lineage is supported by demographic metrics and relatively low genetic diversity of the Coastal and upper Branco populations when compared to the Amazon basin populations. *Paleosuchus trigonatus* presents a population structure among different drainage basins in the Amazon along an east-west gradient, with a sharp transition in haplotype group frequencies to the east and west of the Negro and Madeira Rivers. This study provides strong evidence for the existence of two deeply divergence lineages within *P. trigonatus*, fostering important material for discussions about the future conservation status of *P. trigonatus* and these lineages.

Keywords: Dwarf caiman, population genetics, biogeography, diversification

Discriminating Populations of *Paleosuchus trigonatus* (Caimaninae: Alligatoridae) through Microsatellite Markers Retrieved by Next Generation Sequencing

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The development of microsatellites was very laborious, time consuming and with no success guaranteed. Currently, the use of next-generation sequencing to develop specific microsatellite loci for non-model species have revolutionized the fields of population genetics and evolutionary biology. We isolated and characterized 10 new microsatellites loci for *Paleosuchus trigonatus* using ION TORRENT Personal Genome Machine. We tested the transferability of these loci to three related species of Caimaninae and used these bi-parental markers to test population structure and investigated the genetic diversity of two populations of *P. trigonatus* that are under the impact of hydroelectric powerplants on the Madeira and Xingu rivers. We used an adapted ddRADseq protocol to obtain a reduced representation of the genomes of four dwarf caimans sampled across the distribution of the species and after we filtered putative polymorphic microsatellites loci. We screened 32 *P. trigonatus* from two populations, Madeira (N= 16) and Xingu (N= 16). We investigated the transferability for three related species: *Paleosuchus palpebrosus* (N= 5), *Caiman crocodilus* (N= 6) and *Melanosuchus niger* (N= 6), cross-amplifying successfully with good levels of polymorphism. The genetic diversity of *P. trigonatus* was low for both Madeira (*He*: 0.535 ± 0.148) and Xingu (*He*: 0.381 ± 0.222) populations, similar what was reported for other crocodilian species. The set of these 10 loci were sufficiently polymorphic to be used in future mating systems or kinship studies in *P. trigonatus.* Using DAPC analysis with a set of nine microsatellites loci we were able to separate the four species of Caimaninae studied and with all 10 loci we detected a shallow genetic structure between Madeira and Xingu populations of *P. trigonatus*. The AMOVA and STRUCTURE analysis using *locprior* model corroborate the putative shallow genetic structure between these populations. For the first time, specific microsatellites loci were developed for a crocodilian species using next-generation sequencing. Our set of microsatellites also represent the first toolkit of bi-parental molecular marker that will be available for *P. trigonatus*, since the species have no specific microsatellites nor have they been cross-amplified using loci developed to other species. These novel molecular markers will be also useful in conservation genetics and phylogeographic studies of *P. trigonatus*, since they improve our ability to monitor the putative effects of dams on the loss of genetic diversity.

Keywords: cross-amplification, genetic structure, Madeira River, Xingu River

Predation on Eggs of Schneider's Dwarf Caiman, *Paleosuchus trigonatus* (Schneider 1807), by Armadillos and other Predators

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Nests of Schneider´s dwarf caiman, *Paleosuchus trigonatus*, were located in the forests around three streams that drain into the Xingu River, Brazilian Amazonia, in October 2014. Camera traps were installed at the edge of four nests to document predators and female parental care. At two nests, females unsuccessfully defended their nests against one or more giant armadillos, *Priodontes maximus*, and nine banded armadillos, *Dasypus novemcinctus*. Both armadillos' species responded to the attack by fleeing and returning on the opposite side of the nest by going around the tree under which the nest was located. Giant armadillos have never before been recorded consuming caiman eggs and their diet has been described as consisting mostly of ants and termites. Another species of armadillo, *Cabassous unicinctus*, was also registered digging into a nest and probably consuming eggs though it is generally considered to be primarily insectivorous. A tayra (*Eira barbara*), lizard (*Tupinambis teguixin*) and coati (*Nasua nasua*), were also registered taking eggs from nests during the day, but we obtained no registers of nest defense by caimans during the day. The three nests were attacked after 60 days of incubation when the eggs were well developed.

Keywords: nests, parental care, predators, *Paleosuchus*

Where are the Black Caimans (*Melanosuchus niger*, Spix 1825, Alligatoridae) in the Xingu River Basin? Shrinking Distribution Map of *Melanosuchus niger*

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The Black caiman, *Melanosuchus niger*, is the largest crocodilian in Brazil and reaches a length of up to 6 meters, which makes it easily visible. The published distribution of this species includes the entire Xingu basin except for the headwaters of the rivers and tributaries of the Xingu River in the state of Mato Grosso. Several nocturnal surveys along the river's upper, middle and lower regions confirmed the occurrence of this Black caiman only in the lower Xingu, below the large Volta Grande waterfall. The reduced area was inserted into the IUCN distribution map.

Keywords: conservation, Bback caiman, occurrence, habitat

In Search of the Thermal Niche of *Paleosuchus palpebrosus*

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The coexistence of multiple crocodilian species has led to hypotheses about how they divide their niche. While *Paleosuchus palpebrosus* is smaller as an adult than its sympatric species, they overlap in much of their ecology. Contrary to its sympatric species, however, *P. palpebrosus* is a complete thermoconformer, and the possibility of a thermal niche has been proposed but not thoroughly investigated as a means by which *P. palpebrosus* may be able to expand their niche compared to sympatric or syntopic species. Standard metabolic rate (SMR) is a reliable measure of the minimum energy necessary to keep an individual alive, and therefore provides a possible link between organismal physiology and environmental limitations on performance. Since SMR varies with body temperature in ectotherms, including crocodilians, we compared SMR in two temperatures (18 and 31^{o}C) in *P. palpebrosus*, *Caiman crocodilus* and *C. latirostris*. We found that *P. palpebrosus* has a lower SMR at both temperatures, whereas studies on blood oxygen affinity of the haemoglobin revealed no differences between the three species, indicating a need for further research into cardiovascular parameters such as heart rate. The generalist approach to habitat selection combined with a slower SMR likely allows *P. palpebrosus* to thrive in border habitats, perhaps as an adaptation to avoid competition with larger crocodilian species. This result directs future research for identifying niche separation into deeper physiological studies of how oxygen is processed in *P. palpebrosus* to better understand how it co-exists with other species. It may also lend insight into speciation and distribution.

Sustainable Management of the Jacaré do Pantanal (*Caiman yacare*): GIS as a Tool for the Inclusion and Income Generation of Riverside Communities in the Southern Brazilian Pantanal

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This study was conducted near the confluence of the Paraguay Mirim and the Taquari Rivers. Being an inhospitable and inaccessible region, daily lives are extremely difficult, especially in the generation of income. The annual collect of eggs from Pantanal caiman nests is a major source of income for these riverine families. With the use of GIS, we developed a mobile application (APP) to aid in the precise location of the nests, as well as in the generation and storage of geo-referenced data in a way much more efficient and accurate than possible through previous methods, what allowed us to delimit the territories where it is permitted to collect eggs in the proper season. Interviews in the communities showed that, out of a total of 33 collectors, 20 are beginners with ages up to 25 years. It is easy to notice the interest of young people in joining the activity, since 14 of those beginners had just made their very first collect. Aiming to include the entire community, there were lectures to guide them in the use of the APP and instructions aimed to the formation of teams more capable of effectively carrying out the collection of eggs and the recording of data with the APP, given that every team consists of at least one young literate. The records of the first year of collection with the use of the APP showed that, out of the 747 nests collected, 82% were successfully registered. Now in its second year of application, the APP registered 100% of the 1336 collected nests. Through the evaluation of the data obtained both from records maintained since 2013 and with the APP, it was possible to identify the productive potential of each region and map the areas overlapping the locations of nests found in each year with the incidence of females in the reproductive period. Thus, one can direct the collectors in order to streamline the course of egg collection and significantly contribute not only to income generation but also to the environmental awareness of the riverside community, as well as improving the sustainable conservation practice of the species and preserving the local culture for future generations.

Keywords: *Caiman yacare*, sustainable management, GIS, riverside

Hunting of the Broad-snouted Caiman (*Caiman latirostris*, Daudin 1802) in the Municipality of Campina do Monte Alegre, Southwest of the State of São Paulo, Brazil

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Much appreciated for their meat, caimans are hunting targets in many Brazilian regions. It is believed that urbanization and the progressive loss of rural traditions have led to a decrease in hunting in some of these regions. This work aims to elaborate a current overview of the poaching of caimans in the municipality of Campina do Monte Alegre, southwest of the state of São Paulo. The municipality is located within the High Paranapanema River basin and has agriculture, livestock and forestry as the main economic activities. After identifying poachers through informal conversations with the local population, we applied a semi-structured questionnaire containing 12 questions about poaching habits and the Broad-Snouted Caiman behavior. Additional information was obtained through open interviews. Ten poachers were located and agreed to participate anonymously in the survey. They were all men ranging from 24 to 79 years (mean age 45 years). According to the results poaching occurs all year long in ponds and dams. They usually catch only one caiman at a time, using firearm (n= 4), hook (n= 5) and loop trap (n= 1). The frequency of poaching events also varies among interviewees occurring monthly (n= 4), every three months (n= 3) or every six months (n = 3). In many cases (n= 6) the caiman is not the main target, being hunted only when the opportunity arises. Appreciation for the animal meat is the main reason alleged for hunting (n= 10). Most respondents (n= 8) alleged they would not be willing to pay for an annual hunting permission, nor would they change their habits if the hunting became legal. We conclude that illegal caiman hunting is a practice still present in the region, but apparently occurring at low intensity and in opportunistic situations. Respondents also claimed they did not pass on their hunting habits to younger generations, corroborating the hypothesis that this is a declining custom. The identification of other poachers with the increasing of interviewees number can give a greater reliability to the identified trends in this preliminary study.

Keywords: conservation, monitoring, population ecology, poaching

Differences in Distress: Distress Call Structure and Production of Crocodile in Belize

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Crocodilian distress calls are behaviorally significant repeated chirps generally emitted by juvenile crocodilians to elicit a defense response from nearby conspecifics. In spite of their significance to crocodilian ecology and juvenile survivorship, distress calls, and acoustic communication, is relatively understudied. In this study, we analyzed the acoustic structure of distress calls from wild American Crocodiles (*Crocodylus acutus*) and Morelet's Crocodiles *(C. moreletii*) in Belize with the objectives of: 1) identifying and comparing the temporal and spectral parameters of juvenile distress calls of the two species, 2) comparing call variation among size classes of American Crocodiles (i.e., hatchling, juvenile, sub-adult, and adult), and 3) investigating call production on a gradient of human disturbance among study sites with high to low human impact. Our findings illustrate that American and Morelet's Crocodile distress calls have strong frequency modulation and are comprised of multiple harmonics in a downsweeping pattern. In spite of similarities in call structure, spectral parameters (maximal frequency, first quartile frequency, end frequency, slope of first quartile, slope of last quartiles) differed significantly among juveniles of the two species ($P<0.05$), but temporal (total duration) parameters did not ($P>0.05$). Overall American Crocodile distress calls were lower in frequency, but had greater modulation in the first slope. Comparison of distress calls among American Crocodile size classes determined both spectral and temporal parameters (total duration, first quartile duration, maximal frequency, first quartile frequency, end frequency, slope of first quartile, slope of last quartiles) differed significantly among size classes ($P<0.05$). Our results determined that hatchling distress calls are higher in frequency and strongly modulated, whereas calls produced by sub-adults and adults showed little modulation, are lower in frequency, and have greater overall duration. In addition to measuring call parameters, we recorded all instances of call production by American Crocodiles captured during this study at sites ranging from high to low anthropogenic disturbance. American Crocodiles, of all size classes, produced distress calls more frequently at sites with higher anthropogenic activity ($P<0.05$). Moreover, comparison of call parameters of juvenile American Crocodiles also varied among sites in relation to level of human disturbance. Calls recorded at sites of high anthropogenic impact have increased duration and less modulation, which may adversely affect response to emitted distress calls. Our results indicate that anthropogenic activity may be a driver for increased distress call production and alteration of call parameters at high human-impacted sites.

Key Words*:* American crocodile, Belize, bioacoustics, crocodilian, sound

Modeling American Alligator Population Dynamics

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The American alligator was hunted extensively until a management program was started in 1967. The purpose of this experiment was to model the effects of human interaction and hurricanes on alligator population dynamics. A model was made using python code to test this. The model includes exponential growth, carrying capacity, aging, predator-prey interaction and human interaction in the form of farm release, egg collection and hunting. Without human interaction, the populations took three years to recover from a hurricane. Different variations of human interaction did not affect the recovery time. The populations were stable without human interaction. The hurricanes that occurred in Louisiana from 1950-2000 were modeled to see if multiple hurricanes in a row affected recovery. The recovery was longer when hurricanes came in quick succession. Modeling a historical hunting level (50% and 20% on alligator adults and young) caused the alligator adult population to be very low. No levels of farm release and egg collection could be used to manage this level of hunting, as increased farm release caused the alligator population to be over the carrying capacity, which made it drop because there were not enough resources. Management plans must balance alligator and human needs to be successful.

Keywords: American alligator, population dynamics

The CSG's Student Research Assistance Scheme (SRAS) and Fritz Huchzermeyer Veterinary Science Student Research Assistance Scheme (FHVS-SRAS) - the First 9 Years.

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The Crocodile Specialist Group's Student Research Assistance Scheme (SRAS) was established in 2009, with the specific goal of encouraging and assisting undergraduate and postgraduate students to undertake formal research on crocodilians, particularly field research. In 2014, in honour of the late Dr. Fritz Huchzermeyer, the Fritz Huchzermeyer Veterinary Science Student Research Assistance Scheme (FHVS-SRAS) was established, with a focus on veterinary science research.

As of May 2018, the CSG had received 158 applications for funding (152 SRAS, 6 FHVS-SRAS), of which 142 (89.9%) met the criteria, were reviewed, and funding was awarded (Table 1). One application was under review at the time of writing of this report. Of the successful applicants, one (Costa Rica, 2009) withdrew soon after starting their project, and returned the funding. Three applicants who were rejected in the first instance later applied with a different project and were successful. Of the 142 applicants, 57.7% were males and 42.3% were females.

Table 1. Fate of 158 applications for SRAS and FHVS-SRAS funding, as of May 2018.

Year	Approved Complete/Ongoing	Approved Withdrawn	Under Review	Unsuccessful	Totals
2009	20	1	-	-	21
2010	13	-	-	3	16
2011	9	-	-	-	9
2012	11	-	-	-	11
2013	11	-	-	5	16
2014	15	-	-	1	16
2015	16	-	-	1	17
2016	4	-	-	2	6
2017	25	-	-	2	27
2018	17	-	1	1	19
Totals	141	1	1	15	158

Qualifications being sought by 141 funded applications were: PhD (38.1%), MSc (38.1%), BSc (21.6%) and Post-doctorate (2.2%). Four students applied for funding twice, as they had continued on with higher qualifications [PhD to Post-Doc (N= 1),

MSc to PhD (N= 3)]. The application currently under review is also from a former SRAS recipient.

Students from 35 countries have undertaken, or are currently undertaking, studies in 38 countries. In terms of research location (CSG region), most studies have been in Latin America & the Caribbean (Table 1). Six countries [Argentina (18), Mexico (14), Brazil (13), USA (12), South Africa (9), Colombia (8)] make up 52% of SRAS/FHVS-SRAS projects to date.

Most students have carried out studies in their own country/region (Table 2). Of particular interest, few (1%) studies, as expected, have been carried out in Europe, but 10% of SRAS applicants have been from Europe.

Table 2. Percentage of approved SRAS and FHVS-SRAS applications by "Study Location" and "Student Origin", 2009 to May 2018.

Region	Study Location	Student Origin
Latin America & the Caribbean	55%	49%
East & Southern Africa	11%	11%
West & Central Africa	8%	4%
North America	8%	15%
East & Southeast Asia	8%	4%
South Asia & Iran	5%	5%
Australia & Oceania	4%	3%
Europe	1%	10%
No. of approved applications	142	142

Most approved projects focused on one species (N= 121; 86.4%), 14 (10.0%) involved two species, 2 (1.4%) involved three species, 2 (1.4%) involved 5 species, and one (0.7%) covered 23-24 species (based on museum specimens).

The most commonly studied crocodilian species were: *Crocodylus acutus* (24 studies), *Caiman latirostris* (24), *C. crocodilus* (18), *C. niloticus* (15) and *Alligator mississippiensis* (13), followed by *C. porosus* (8), *C. moreletii* (8), *Mecistops cataphractus* (8), *C. suchus* (8), *Osteolaemus tetraspis* (7), *Melanosuchus niger* (6), *C. intermedius* (6), *Gavialis gangeticus* (6), *A. sinensis* (5), *C. rhombifer* (5), *Paleosuchus palpebrosus* (5), *C. yacare* (5), *C. siamensis* (4), *P. trigonatus* (4), *C. mindorensis* (3), *C. palustris* (3), *Tomistoma schlegelii* (3), *C. johnstoni* (2) and *C. novaeguineae* (1).

Most funding of SRAS and FHVS-SRAS projects has come from CSG reserves, with specific contributions from: Bergen Aquarium ($US25,000 in January 2009); Bjornparken Zoo ($US2500 in October 2017); and, three individual CSG members.

In August 2016, a questionnaire was sent to 98 SRAS students, and responses were received from 89 (91%) of them. Review of the questionnaires was undertaken by the CSG's Future Leaders Working Group (FLWG), which provided a series of

recommendations that are currently under review by the CSG Executive Committee.

There is little doubt that the SRAS and FHVS-SRAS initiatives have been important to students undertaking studies on crocodilians.

Copulatory Directional Asymmetry and Implications for Crocodylian Reproduction

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Abstract: Directional asymmetry or laterality in a species' behavior can provide evidence for brain lateralization. Structural and functional asymmetries have been documented in birds, reptiles, and amphibians. In addition to providing valuable information about the evolutionary origin of brain laterality, these behaviors can inform investigations on reproductive preference, anatomical asymmetries and biological adaptations contributing to reproductive success. This study investigated directional asymmetry in the courtship and copulatory behavior of Cuban crocodiles, *Crocodylus rhombifer*. We examined the approach and mounting side-preference (left or right) of three *C. rhombifer* males: two from the Smithsonian National Zoological Park (NZP) in Washington, D.C. and one from the Madras Crocodile Bank Trust (MCBT) in Tamil Nadu, India. Our results revealed that *C. rhombifer* males do exhibit individual-level directional approach and mounting asymmetries. However, a consistent side-choice behavior is not likely at the population level as both right and left male approach tendencies were observed, and subsequent mounting side did not always agree with the preceding approach. Further research investigating the contexts of female receptivity cues and mounting permissiveness is needed. These findings have significant implications to inform the study of the reproductive behavior in Cuban crocodiles--specifically, as it relates to the study of behavioral lateralization, or handedness, in archosaurs, the evolution of brain lateralization in vertebrates, and functional copulatory anatomy of crocodylians.

Introduction

The evolutionary origin of brain lateralization, once considered a trait unique to *Homo sapiens*, is a crucial issue in modern neuroscience (Bisazza *et al*. 1998). Evidence of brain lateralization, which can lead to lateral asymmetries in species behavior, are well documented in mammals and birds (Bradshaw and Rogers 1993; Rogers 2017; Rogers and Workman 1993; Ward and Hopkins 1993; Waters and Denenberg 1994,). Structural and functional asymmetries are more recently being documented in reptiles and amphibians (Biasazza *et al*. 1996a, 1998), however in-depth evidence of related behavioral asymmetry (also known as lateralization: left-right differences in the response to the same stimulus) at the individual and population levels are lacking. These comparative studies in non-mammalian or non-avian vertebrate species are important in that they contribute to our understanding of the evolutionary origin(s) of brain lateralization.

Published investigations of behavioral lateralization in crocodylians are absent despite the behavioral complexities observed in their courtship and subsequent breeding behavior (Garrick and Lang 1977; Staton and Dixon 1977). During copulation there are

opportunities for behaviorally-driven laterality-specifically male side-preferences first while following/approaching and then subsequently in mounting and copulating with the female. Phalli function to transfer male gametes directly to the female reproductive tract, thus increasing the chance of reproductive success via internal fertilization (Kelly 2002). The male crocodylian phallus is stored inside the cloaca until copulation when it is everted via a system of muscle contractions and tendons (Johnston 2014; Kelly 2013). The everted phallus is inserted into the female cloaca and the terminus of the semen-conducting sulcus is placed in close proximity to the opening of the female's reproductive tract (Moore and Kelly 2015).

However, in order for copulation to occur, a male must approach from behind and mount the female either to the left or the right of the female's tail, allowing the alignment of cloacas and intromission of the everted penis (Augustine 2017; Garrick and Lang 1977; Staton and Dixon 1977). Male crocodylians, similar to snakes and lizards, are faced with this "unilateral choice" during copulation of assuming a left or right mounting side. The "sidedness" of copulation may be a random event at each occurrence, be side-biased due to male behavioral/neurological/anatomical asymmetries, be influenced by female interactions (Brennan 2016), or this sidedness may depend on random environmental factors (Bisazza *et al*. 1998), such as positioning of the female within the environment.

Studies examining the lateralization of sexual behavior have documented a side-preference in birds (Workman and Andrew 1986), fish (Neville 1978) and amphibians (Green 1997). This suggests that sexual behavior may be a good indicator of behavioral asymmetry in other species. This study examined the approach and mounting side-occurrences of male Cuban crocodiles, *Crocodylus rhombifer*, and the resulting compliance or rejection of the male's advances by the females. We acknowledge the behavior of the female and environmental factors within the enclosures may have contributed to the mounting side-preference in this species; however, this preliminary study evaluated male side-preference during the courtship approach and copulation positioning specifically.

Methods

The Smithsonian's National Zoo (NZP) has housed five *C. rhombifer* in two adjacent enclosures along the east wall of the Reptile Discovery Center (RDC). One exhibit (B2) houses a trio of *C. rhombifer* (M2, F2, and F3) and the other (B3) houses a pair (M1 and F1). Both *C. rhombifer* exhibits are approximately 72 m^2 and feature large pools ~0.9 m deep that cover roughly half of each exhibit space. The pair housed in exhibit B3 consists of a captive-hatched female (F1), approximately 35 years in age, and a captive-hatched male (M1) approximately 30 years in age. The trio in B2 is comprised of two females: a younger female captive-hatched in 1980 (F2) and an older, wild-caught female estimated to have been 58 years old in 2016 (F3). The male in this trio was captive-hatched in 1970 (M2). Both males have a history of genital problems; M1's specimen record has a medical note revealing an "old minor trauma to penis and superficial abrasions" when he arrived at NZP in 1991. M2 has had a partially prolapsed penis for approximately 10 years. All three females breed seasonally with their respective male partners and lay viable eggs (Augustine and Watkins, 2015). In May 2016, the two females housed in B3, F2 and F3, fought with the older female, F3, sustaining an injury to the upper left jaw. In June of that same year, F3 was removed from the enclosure leaving just the pair, M2 and F2, for the 2017 breeding season. Therefore, data collected in 2014 was from three breeding pairs (M1&F1, M2&F2,

M2&F3), and the data from 2017 was collected from two pairs of crocodylians (M1&F1, M2&F2).

Data was collected during two breeding seasons at NZP, 2014 and 2017. Video footage taken during a previous study (Augustine et al. 2017), for which Cuban crocodiles were observed randomly from 18 March through 20 May 2014 between 0800 and 2015 hours, was reviewed and scored for copulatory behavior. In 2017, copulatory behavior was recorded from 25 February through 31 May between 0700 and 1300 h. In both instances, observations were collected *ab libitum* from the guest viewing area in front of the two adjacent enclosures (Fig. 1). Within this 48-day time period, approximately 500 viewing hours of footage were recorded. During observations, the observer did not wear a zoo uniform in order to eliminate potential animal response bias to the zoo uniform. Repeated human presence was determined to not pose interference with this study due to the fact that the crocodiles are habituated to the continual presence of humans in front of their exhibit (Vliet 1989). The identification and sex of each individual was known prior to this study, and their physical characteristics made identification possible from the observation location in the guest viewing area. Behavioral observations were recorded with a JVC Full HD camera (JVC, Kenwood Corporation, Long Beach, CA, USA) and a Samsung WD1100F Smart Digital camera (Samsung, Sheffield, UK). Recorded footage was later reviewed and analyzed.

Behavioral observations also occurred at the Madras Crocodile Bank Trust (MCBT) in India where a group consisting of one male, captive hatched in 2005, and four female, captive hatched in 2005 and 2007, *C. rhombifer* are housed off-exhibit in a large outdoor enclosure measuring approximately 75 m^2. The enclosure has a semi-circular pond measuring 11.5 m at its longest and 7.4 m at its widest, and the ratio between land and water is 1:2. The group was observed from March 2014 onward, typically during daylight hours (0700-1900). The animals range from 1.9-2.4 m and were differentiated by observers based on tail clippings. Recordings from 2014 and 2015 of the animals were made using a Fujifilm SL300 HD camera (Fujifilm, Tokyo, Japan) and later reviewed.

Approach and mounting data was statistically analyzed for deviation from a 50/50 random probability using Pearson's chi-squared (X^2) test (Graph Pad).

Results

Out of 155 observations, 27 did not result in successful copulation (17.4%). Males approached females from the right (55.5% of all observation, not significant from random) and were more likely to mount females from the same side they approached (70.3%; X^2= 19.5; d.f.= 1; P value (two-tailed) <0.0001) rather than switching sides (29.7%). Individuals did significantly favor one side for approaching (3 of 3) and to a lesser extent for mounting (1 of 3) (Table 1). Mounting did not alternate in consecutive breeding and side preferences is not correlated with copulatory success. The male at MCBT only attempted to breed with the two larger of the four females in the enclosure during the study period. Furthermore, the male attempted to mount one female from the left seven out of 24 observations with six being unsuccessful. This male attempted to mount this same female from the right 17 times, 41.2% of which were successful.

Table 1. Approach and mounting preferences of three male *Crocodylus rhombifer* at the National Zoological Park (NZP) in 2014, 2017, and 2018 and Madras Crocodile Bank Trust (MCBT) from 2014 to 2015. X^2: * = P<0.05; ** = P<0.001.

	Location (number of cohabitating females)	N	Approach preference (%)		Mounting preference (%)		Total unsuccessful (%)
			Right	Left	Right	Left	
Male 1 (M1)	NZP (1)	72	70.8%**	29.2%**	58.3%	41.7%	4.2%
Male 2 (M2)	NZP (2)	56	26.8%**	73.2%**	50.0%	50.0%	8.9%
Male 3 (M3)	MCBT (4)	27	77.8%**	22.2%**	70.4%*	29.6%*	70.4%

Discussion

Structural asymmetries exist in the brains of more basal vertebrates (Braitenberg and Kemali, 1970; Harris *et al*. 1996) and brain lateralization can affect animal behavior (Bradshaw and Rogers 1993; Rogers 2017). Laterality in animal behaviors can take place at either the individual or population level (Deneberg 1981; Lehman, 1981), and a population is considered lateralized if more than 50% of the individuals perform in the same direction (Bisazza *et al*. 1998). Examples of lateralization in a wide range of vertebrates include escape and courtship behavior in fish (Bisazza *et al*. 2000; Cantalupo *et al.* 1995, 1996; Neville 1978), forelimb use and pivoting direction in toads (Bisazza *et al*. 1996b; Sovrano 2007), turning bias in tadpoles (Briggs *et al*. 2016; Dadda 2005; Oseen *et al*. 2001), courtship behavior in newts (Green 1997; Marzona and Giacoma 2002), eye use in lizards during aggressive displays (Deckel 1995, Deckel and Fuqua 1998; Hews and Worthington 2001; Hews *et al*. 2004) and predatory behavior (Robins *et al*. 2005), and coiling direction and side dominance in snakes (Amaral 1927; Heinrich and Klaassen 1985; Roth 2002). In these examples, laterality has been document in both the population and individual levels. These types of observations can contribute to the study of asymmetry in brain structure and function and its evolutionary origins, as it remains unclear if these traits evolved independently or whether they reflect basic homology (Bisazza *et al*. 1998).

Investigations into the behavior of crocodylians have revealed complex behavioral repertoires (Garrick and Lang 1977; Garrick *et al.* 1978; Lang 1987; Vliet 1989; Thorbjarnarson and Hernández 1993), providing several opportunities for individual animals to make a choice, left or right. Most notable is the side to which a male

approach and mounts a female during courtship and copulation. This preliminary study shows evidence that individual *C. rhombifer* likely display a strong side preference when approaching and a possible side preference when mounting a female crocodile, but this lateralization is only present at the individual level since approach directionality was not consistent among the three males. Alternatively, females may present to mates in a particular manner that increases the frequency of a male's behaviors on a specific side. Since an approach is a more female-independent male behavior and mounting is a direct and cooperative male-female physical interaction, this may explain why we observed robust approach lateralization for all males, but only one consistent mating lateralization. Although, the right cerebral hemisphere dominates sexual behavior and is usually exhibited on the left side of the organism (Malashichev and Wassersug 2004), this study showed that two of the three *C. rhombifer* males exhibited a right-side approach preference and, though to a lesser extent, maintained that preference when mounting.

While there was no correlation between approach and mounting side preference and successful copulation, the male housed at MCBT was unsuccessful 70% of the time, 42.2% from the right and 87.5% from the left. These animals were around 10-12 years of age during this study (SVL between 1.9 and 2.4 m). The age, experience level and high number of female *C. rhombifer* cohabitating at MCBT could be a contributing factor to their lack of successful copulation, as this was not the case with the older animals housed at SNZP. Male and female crocodylians raised in captivity attain sexual maturity several years earlier than animals in the wild (Joanen and McNease 1980; Lance 1989) and head-started Orinoco crocodiles, *Crocodylus intermedius*, were documented reproducing in the wild at no greater than eight years of age (Rivas and Owens, 2002), captive American alligators, *Alligator misissipiensis,* reach sexual maturity at six years of age (Joanen and McNease 1980), a pair of Saltwater crocodiles, *Crocodylus porosus,* first reproduced when the female was six and the male was ten (Isberg *et al*. 2005), and female Yacare caiman, *Caiman crocodilus yacare,* generally reach sexual maturity between 10 and 15 years of age (Campos *et al*. 2014). The MCBT animals were younger animals with less experience, never produced eggs, and this may have affected their rate of successful copulation. Furthermore, this high number of females within this enclosure may also be influencing the success rate of his advances.

Morphological asymmetries have been documented in the American alligator, *A. mississippiensis*, where in males, the right testis is generally larger than the left, and in females, the right ovary (and oviduct) is usually larger and contains more follicles of preovulatory diameter than the left (Lance 1989). These asymmetries have not been reported in other crocodylian species, but this may be attributed to a lack of detailed reproductive tissue masses investigations rather than an alligator peculiarity (V. Lance *pers. comm*.). However, variations in individual levels of handedness do not correlate to pre-existing morphological difference between sides in the individual (Seligman 2000). For example, red-sided gartersnakes were found to have larger reproductive structures on the right, but used their hemipenes equally (Shine *et al*. 2000). It has been suggested however that previous mating history may influence side-preference or hemipene use (Shine *et al*. 2000). In this study, when consecutive mating was observed between a pair, there were no clear rotation or alternation of side choice observed.

Even if testis size is asymmetric, the delivery of gametes does not show asymmetry. The ductus deferens from both epididymis deliver semen to a common vestibule that marks the proximal beginning of the penile sulcus- an open groove along the ventral surface of the phallus that conducts the semen to the distal glans (Johnston, 2014;

Moore and Kelly, 2015). While across crocodylian species, penile glans show species-specific morphological variation (Johnston *et al*. 2014; Moore *et al*. 2016; Zeigler and Olbort 2007), they all are bilaterally symmetrical and the sulcus terminates at the distal medial aspect of the glans. Thus, contributions from asymmetrical-sized testes would pool to produce a single ejaculate.

It is unclear how the male crocodylian glans interfaces with the female cloaca during copulation and how the glans shape(s) may facilitate insemination. Female oviducts terminate in the middle chamber of the cloaca, the urodeum, with narrow openings to the cloaca lumen (Grigg and Kirshner 2015). In the absence of detailed copulatory mechanics studies, we can put for two general hypotheses: first, the sulcus releases semen into the urodeum lumen and the oviducts take up the gametes from this general insemination site or, second, the distal glans interacts with the individual oviducal openings in a way to facilitate a more direct insemination. A key question resulting from our evidence of lateralized copulatory mounting in Cuban crocodiles is if the side of male mounting has any bearing on the position of the distal sulcus in regards to oviducal openings and, in turn, the success rate of effective gamete transfer? While one of the three males observed in this study showed a right-side mounting bias, a significant number of left-handed mounts were also observed for each animal. Therefore, to better understand a role for potential male reproductive behavior lateralization, a more detailed understanding of copulatory interactions and insemination mechanism is needed to determine a potential impact of mounting side choice or alteration.

We acknowledge several limitations to this preliminary study, but would like to present them as opportunities for future research. The initial and most notable limitation is sample size which is stifled by challenges to studying this species in the wild and the relatively few breeding pairs or groups available for observation. Recruitment and data collection for a more robust data set continues in an effort to strengthen this research. Second, the influence of enclosure and female preference were not considered in this preliminary study, but could certainly be playing a major role in the male's side-preference and resulting success of the copulatory attempt. Female positioning within the enclosure, chemical cues, social conditions, age/experience and behavior responses to male advances should all be considered in future studies. Lastly, the injury to both M1 and M2's genitals could have also been a factor.

Despite the preliminary nature of this study, we hope to promote the use of captive specimens to advance the study of evolutionary questions in future research. Although captive crocodylians experience different stimuli than their wild counterparts (Brazaitis and Abene 2008), their reproductive behaviors remain consistent with those of wild individuals (Brazaitis and Watanabe 2011) making them ideal subjects for this type of research. These types of observational studies can contribute vast amounts of data to reproductive studies on species that are challenging, if not impossible, to document in the wild. Zoological institutions, crocodylian farms and ranches, as well as other facilities housing crocodylians are in a unique position to conduct such studies thereby contributing to datasets on brain lateralization in vertebrates and the functional copulatory anatomy of crocodylians. Furthermore, these types of investigations can contribute to research on reproductive preference, anatomical asymmetries and biological adaptations contributing to reproductive success in certain taxa.

Conclusion

Once thought to be unique to the human species, brain lateralization is documented in an increasing variety of taxa, contributing to our knowledge about its evolutionary origin. Despite the lack of empirical studies on crocodylian behavioral lateralization, there is a distinct pattern of lateraled behaviors appearing in archosaurs. With more data, we hope to provide evidence of brain lateralization in crocodylians based on behavioral asymmetries. It is important to consider the possibility that these lateralizations derived independently from common selection pressures and are not a highly conserved trait, but instead originated independently in certain taxa.

This study aims to illuminate the ability of zoos to contribute data towards complex animal evolutionary advancements in research through simple observational studies that inform and promote further basic biology research. Potential future directions of this type of observational research include: assessing reproductive anatomy in the context of mounting side preference, female chemical cues that could be directing the male's advances, investigating insemination success within the context of copulatory behavior, and more. These types of observations are more easily documented in zoological facilities or farms and can promote the use of captive specimens for research. The results could contribute to the study of reproductive behaviors in crocodylians, and, in this case, the evolution of brain lateralization in vertebrates.

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Helicobacter Detection in the Oral and Cloacal Swabs from Orinoco Crocodile (*Crocodylus intermedius*) in Venezuelan Wild and Captive Populations

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Helicobacter species can colonize digestive tract of animals and humans and have been associated with diverse gastrointestinal diseases; however this genus has not been studied in crocodiles. Our objective was to detect *Helicobacter* genus and *H. pylori* species in the oral and cloacal swabs from Orinoco crocodiles of two wild (Cojedes River System and Capanaparo river) and two captive breeding centers (CBC; Masaguaral Ranch and UNELLEZ) populations. The bacterial DNA in swabs was confirmed by *16S rRNA* gene amplification using PCR. Samples positive for bacterial DNA were tested for *Helicobacter* spp. using *16S rRNA* genus-specific primers. Samples positive for *Helicobacter* genus DNA were then tested for *H. pylori* DNA, amplify *glmM* gene. Bacterial DNA was found in 100% of the crocodiles oral samples (10 wild and 10 captives), and in the 95% of cloacal samples of crocodiles (10 wild and 9 captives). In wildlife populations, *Helicobacter* spp. wasn't detected, whereas in CBC, *Helicobacter* was detected in 10% (1/10) of the oral samples, and 66.7% (6/9) of the cloacal samples. *H. pylori* was found in two Orinoco crocodiles. Two Helicobacters non-*H.pylori* amplicons from cloacal samples were sequenced [~260 base pairs (bp)] and had low similarity (≤97%) with *Helicobacter* sequences reported. This is the first report of the high prevalence of *Helicobacter*, including *H. pylori* in *C. intermedius* from CBC.

Keywords: *Helicobacter*, Orinoco crocodile, captive breeding centers, Venezuela

Dispersion of a Group of Schneider's Smooth-fronted Caiman (*Paleosuchus trigonatus*) Hatchlings on a Stream within a Forest Fragment in Manaus, Brazilian Central Amazonia

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Our ability to evaluate behaviour of wild vertebrates has experienced significant improvements over the last decades, allowing researchers to monitor and map detailed movement patterns, even for highly cryptic species. Schneider's smooth-fronted caiman (*Paleosuchus trigonatus*) represents one of the least known crocodilian species within the Amazon Basin, and the only one that inhabits closed canopy small forest streams in *terra firme* forests. We herein report the spatial dispersion of a clutch of recently hatched *P. trigonatus* located within a 700 ha urban forest fragment in Manaus, Brazil. On September 29th 2012 we encountered a recently-made *P. trigonatus* nest at the margin of a first order freshwater creek during a caiman nesting survey. On 26 December 2012 we revisited the site and found that the nest had successfully hatched. We did not find infertile or egg shell fragments inside or around the nest. On 10 January 2013 we returned to the nest area and searched for hatchlings during a spotlight survey on foot 200 m upstream and downstream of the nest site. We encountered a total of eight hatchlings (four upstream and four downstream), with remarks that one found at the most upstream location being dead on margin with no apparent cause of death. We assumed that all hatchlings found came from that same nest, because no other nests were found in that area. The hatchlings were dispersed within the creek, with some level of aggregation between downstream individuals. Five of the hatchlings were individually dispersed through the waterbody spread at similar distances from the two nearest hatchlings (mean: 49 ± 8 m; range: 40-60 m), whereas the three most downstream hatchlings were closer together in the same section of the waterbody (within 5 m from each other and 50 m away from nearest upstream hatchling). Maximum distance between individuals was 285 meters and mean distance to nest was 56 ± 30 m (range: 15-91 m). No adult caiman was observed in the survey area during this period. Long term effort following marked individuals in the wild is crucial to understand the population dynamics of *P. trigonatus*. Urban forest fragments are more and more common within the Brazilian Amazonia and so the continuity of our caiman research in Manaus, the most central area of the Amazon Basin, represents a perfect opportunity to highlight the pressures of urban expansion on environment and behaviour of Amazonian crocodilians.

Keywords: Amazonia, Alligatoridae, movement, nest

Body Size Stages of Morelet's Crocodiles (*Crocodylus moreletii*)

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Classification in the Morelet's crocodile (*Crocodylus moreletii*) within size classes based on ecological and morphological similarities may not be associated with the species ontogeny related changes. Age or size of first reproductive behavior is not precisely known in *C. moreletii*, but changes in allometric patterns and relative cranial size between juveniles and adults could be used as an indicator of sexual maturity. In this sense, Regression Trees were performed to analyze the relationship between size and age of 1266 crocodiles, using simple linear models and general linear models with sex and condition (captivity and wild) as factors. Total length (TL), snout-vent length (SVL) and cranial length (CL) were used as predictors and the logarithm of weight as response. Our results indicate the following: i) four stages of size were determined using all predictors (TL, SVL and CL) exhibiting well-defined thresholds; ii) no significant differences in length (TL, SVL and CL) and weight (logarithm) relations were observed between sex and condition; iii) significant difference was observed in the allometric relation TL-CL between sexes ($P<0.001$) and among size stages ($P= 0.01$). These results indicate that size stage thresholds seem to correspond to important ontogenetic changes in *C. moreletii*, and also that sexual maturity is more related to size than age within this species, that exhibits sexual dimorphism particularly in size stage IV (adults).

Keywords: dimorphism, GLM, ontogenetic size stage, size class

Responses of Juvenile American Alligators to Contact Calls

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Crocodylians are known for their ability to vocalize throughout their lives in a variety of social and ecological contexts, particularly during their vulnerable juvenile life stage. However, few studies have analyzed juvenile calls in laboratory settings, fewer still have analyzed them across large sample sizes or with respect to sex and body size, and no studies to date have analyzed crocodylian vocalizations with respect to human conditioning in captivity or animal personality profiles. This study tests juvenile American alligators' (*Alligator mississippiensis*) ability to respond via movement and callback vocalizations to pre-recorded conspecific contact calls across a large sample size (n= 36), and tests for correlation between response rates and juvenile body size and sex. Seventeen and 16 individuals out of a total of 36 responded via movement toward the source of pre-recorded vocalizations across the first and second experimental trials, respectively, where as none responded through movement toward control sounds; 75% of juveniles who vocalized in return did so only to contact calls rather than controls (though the latter difference was not significant given that only four vocalized in Trial 1, and only a single animal vocalized in Trial 2); there was also no significant difference in movement toward recorded vocalizations across size or sex. However, one particularly vocal individual whose upbringing in captivity was known was identified as a possible unique personality profile given its propensity for callback vocalizations in comparison to its fellow study subjects.

Keywords: alligator, vocalizations, contact calls, juveniles

Population Status of *Crocodylus acutus* and *Crocodylus moreletii* in Área de Protección de Flora y Fauna Yum Balam, Quintana Roo, Mexico

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Crocodiles accomplish specific regulatory functions for the ecological balance. The objective of this study was to determine the population status of *Crocodylus acutus* and *Crocodylus moreletii* based on their distribution, density (encounter rates) and demographic structure (class sizes and sex ratio). The surveys were conducted in the Area de Protección de Flora and Fauna Yum Balam (Quintana Roo, Mexico), using an aluminium flat bottom boat equipped with outboard motor (15-25 HP). Our results show that the distribution was mainly aggregated and dispersed. We estimated a total abundance of 114.8 individuals over a combined distance of 8.9 km in three waterbodies, and observed an encounter rate of 12.89 individuals/km (including both species). The demographic structure of *C. acutus* is composed mainly by juveniles (28%), followed by yearlings (24%), sub-adults (24%) and adults (5%); while *C. moreletii*'s was represented by adults (22%), juveniles (17%) and sub-adults (6%). The sex ratio for *C. acutus* had a bias towards females (0.8♂:1.2♀), while for the *C. moreletii* was observed the parity (1♂:1♀). The results can be used to develop management plans and conservation programs for both species in the study area.

Keywords: abundance, conservation, distribution, population structure

Identification of Microbial Flora in the American Crocodile Oral Cavity (*Crocodylus acutus),* Cañas, Guanacaste, Costa Rica

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The incidences of human contact with populations of the American crocodile (*Crocodylus acutus*), have increased considerably in the country, generating a problem at the social level. Likewise, these animals have the ability to adapt in various habitats that go from fresh, brackish or salt water, acquiring thereby a wide microbial flora. Therefore, the objetive of this study is to determine the General characteristics of the microbial flora present in the mouth of the crocodile, in order to provide useful information in the area of public health. For that, we took a swab of the oral region of three was issues of the American crocodile in captivity and the samples were cultivated in different selective media to its further isolation and bacterial characterization by means of different respective biochemical tests. Generating 108 strains isolated and with respect to the percentage of Gram positive and negative at the end was fairly different with a 60% and 40% respectively. Also bacterial strains were classified into two group's cocci and bacilli, the latter was divided into genres: *Bacillus*, *Enterobacter*, *Pseudomonas* and *Aeromonas*. Concerning records of research on bacterial flora present in the oral cavity of the crocodiles American are very few, but this study showed relevant data with a high information content, although the identification it was only at the level of groups. Therefore, by comparing with other similar studies of bacterial flora in reptiles and similar species, demonstrates that there is considerable agreement regarding groups and genres found in such research, in this way the knowledge of the microbial flora of this specie may allow to generate a health protocol to achieve prevention against possible attacks to the human in the future.

Keywords: *Crocodylus acutus,* isolation, bacterial genus, pathogens

Review of Genetic Studies in Alligatoridae

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Over the past two decades studies incorporating genetic techniques have drastically risen in prominence in regards to crocodilians and have aided our understanding of a range of topics from behavior to development. The topics covered by these studies are as diverse as their methods. The multitude of information acquired requires a review and critique to aid in synthesis of information and identification of large-scale patterns. Due to the great volume of genetic studies on crocodilians, we focused our review on the family Alligatoridae. All alligatorids lack salt glands and with the exception of the Chinese alligator, all of them reside in the New World. These unifying characteristics make them ideal for asking questions about evolution and intraspecific population differentiation. First, we organized the studies into four different categories: characterization of molecular markers, studies of population genetic structure, genetic identification of mating systems, and studies on the genetic basis of development. Overall, population genetic studies have used a combination of molecular markers (e.g. microsatellites, mitochondrial DNA, RAPDs and allozymes), mating systems studies have identified patterns of paternity in multiple species, and genomic studies have begun exploring hormonal expression during the breeding season and embryonic development. We have identified several reoccurring issues among these studies. For example, many population genetic studies have suffered from small sample sizes. For American alligator studies in particular, there have been no samples collected in the inland portion or northern extent of their range. Additionally, the highest number of polymorphic loci used in any of the studies surveyed was eight. In conclusion, we suggest that greater sample sizes and more loci need to be sampled across all species within the family Alligatoridae. We also recommend future population genetics studies use genomic techniques such as RAD-seq to better resolve patterns of genetic differentiation.

Keywords: microsatellites, DNA, populations, caimans

Preliminary Analysis of Effects Caused by Exposure to Herbicide Glyphosate (Roundup®) in Neonatal Testis of *Caiman yacare*

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The two species of *Caiman* in Argentina are distributed in areas of agricultural activity. Moreover, such species are considered biological monitors of environmental pollution. Roundup® and its active ingredient, the glyphosate, are among the most widely used herbicides around the world. Studies on different species have focused on the effects produced by glyphosate exposure during embryonic development. It has been shown, not only, the herbicide's genotoxic capacity, mainly enzymatic and metabolic disorders, but also alterations related to reproductive strategies. However, there is little research which provides information about the toxicity at the reproductive system level. The aim of the research was to analyze possible alterations in testis of *Caiman yacare* exposed to glyphosate. Eggs were exposed to 250 and 1000 μg/egg of the glyphosate-based formulation (Roundup®) during embryonic development and also we used eggs as negative control. The eggs were incubated at a masculinizing temperature of 33ºC until hatching. The specimens were sacrificed according to the Ethics Committee protocol approved by the Universidad Nacional del Nordeste, then they were fixed in Bouin's solution and washed in 70% ethanol. The histological preparations of the testes were performed according to the conventional histological routine and stained with hematoxylin-eosin. The control testes showed differentiated seminiferous tubules, well defined basement membrane, and several spermatogonia and Sertoli cells. Leydig cells abundant blood vessels and extracellular matrix were observed in the interstitial tissue. The specimens which were exposed to 250 μg/egg of glyphosate showed tortuous seminiferous tubules, with disorganized spermatogonia and Sertoli cells towards the centre of the tubules. Neonates which were exposed to 1000 μg/egg of glyphosate showed poorly defined seminiferous tubules and disorganized germinal epithelium. The spermatogonia were found in the tubule centre without lying on the basement membrane, which was poorly defined. Even though these are preliminary results, we inferred that the differences found among the treated specimens and the control could be alterations caused by glyphosate. In addition, it is worth mentioning that future studies will be centered in the comparative analysis of different glyphosate concentrations in embryos and neonates of the analyzed species.

Keywords: *Caiman yacare*, testis, glyphosate, toxicity

Effects of Stress Conditions on the Immune System of *Caiman latirostris*

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The immune system (IS) is essential in the animals to monitor the ability to defend from infections, health status, and different changes in the external environment. Some stressors, especially hormonal factors, can affect this system.To test the effects of stress conditions on the IS of Broad-snouted caiman (*C. latirostris*), we elevated corticosterone level by either restraining animals or additionally injecting pituitary adrenocorticotrophic hormone (ACTH) and compared theirphytohemagglutinin (PHA) swelling response. Besides, we determinated the effects of these stressors on the others immunological parameters as differential white blood cell count (DWBC) and natural antibodies levels (NAb) titers. We compared all the immunological parameters 12, 24, 36 and 48 h after the immune challenge among three groups of caimans: (i) animals under restraint (n= 4), (ii) ACTH-injected animals (n= 4), and (iii) control-(n= 4). The results showed differences in PHA swelling response along the time but not among treatments, indicating a response independent of corticosterone levels. Nevertheless, it is interesting to note, that to respect to others authors, which found the higher response at 24 h after PHA inject, we found a late welling response at 36 h. On the other hand, we found differences in DWBC count, where basophils population showed an increase in animals under restraint stress (i) respect to the control; suggest that this treatment can cause inflammatory bodily reactions, due to the increase of this type of leukocyte. Besides, the results revealed an increase only in control animals of their NAb titers over time (at 48 h), moment where the adaptive response begins to unchain, indicating the possible inhibition in the immune response in animals treated. Finally, the corticosterone levels showed differences among treatments but only along the time, where animals-control-(iii) as well as animals under restraint stress (i) continued increasing their corticosterone levels over time, while the ACTH-injected animals (ii) reduced those levels at 48 h. This continuous increment of corticosterone levels in (i) and (iii) could be the result of the stress caused by mainteinance of animals in captivity. Nevertheless, the reduction observed in (ii) could be indicating an antagonist effect of the ACTH injection on this hormone over time.The result of this preliminary study allow broadening the knowledge about endocrine and immune effects caused by stress conditions, but this need to be continued. Overall, these results provide evidence of a late PHA swelling response, as well as being suggest that stress conditions in *C. latirostris* could leading to structural and functional changes to immune system could result in a reduction of resistance of the host.

Keywords: *Caiman latirostris*, immune system, stress conditions

The Crocodylia of Perú: Present and Future

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In 2013, Vásquez began the compilation of information generated about the Crocodylia in Peru, this work included scientific articles and publications, as well as the "gray" literature or secondary information considered relevant and available for alligators of the Peruvian Amazon. The American crocodile (*Crocodylus acutus*) was included, this work represents an important advance in the field that allowed to recover the lost time and to establish bases of knowledge of the Crocodylia in Peru. Subsequently and almost immediately, a series of population studies were initiated in Loreto, with the support of members of the CGS, important advances were made on the Amazonian caimans and a historical fact was achieved: The approval of the first two management plans for the purpose of use for *Caiman crocodilus* in Peru, the two National Reserves involved were those of Pacaya Samiria and Pucacuro, two important natural protected areas for the Peruvian Amazon. Subsequently, the Data Center for Conservation (CDC-UNALM) began with the work of population assessments in different points of Loreto in addition to those previously evaluated and included *C. acutus*, this study ended in the most important publication of the last years for the Crocodylia of Peru, as it clearly illustrated the situation of the populations, the threats they face and a modeling of potential habitat for the species. All the investigations were conducted between 2013-2016, all those involved in the different stages agree that there is still much to be done, illegal activity is the greatest threat and only by promoting management programs in collaboration with the communities will it be possible to advance in the right direction, there is an important resource potential, proof of this is that in recent years there has been an incessant trade of the different species in the markets of Loreto and there is no other initiative that can help reduce the impact of this activity that the management programs by regulating the existing activity. Unlike in previous years, now there are greater technical and professional capacities, as a result of the many trainings given to park rangers, professionals, students and fishermen or hunters in the management areas. All that remains is to find strategic allies to continue with the initiatives and achieve more than what has already been achieved so far.

Experimental Consequences of Low Temperatures on *Caiman latirostris* Inmune and Endocrine Systems

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Low temperatures have an inhibitory effect on animal metabolism, causing organisms to respond with physiological mechanisms to reestablish their homeostatic condition. Corticosterone is a glucocorticoid synthesized and secreted under stress conditions in order to stabilize metabolic functions and maintain the immune response of individuals. This hormone depends on the same metabolism for its synthesis, so if the latter descends to very low levels the organism is unable to synthesize it and, therefore, generate a regulatory response. For ectothermal animals, such as crocodiles, the release of corticosterone is the main compensatory response against the decrease in metabolism in low temperature conditions, but for a certain threshold of temperature the organism is unable to continue its synthesis and the metabolism ceases. In *Caiman latirostris* tolerance range and corticosterone blood levels at low temperatures are unknown. The objective of this study was to determine the lowest temperatures in which the concentration of corticosterone is maintained at adequate levels to maintain a stable metabolism and immunological parameters. Thus, 6 hatchlings and 6 juvenile were exposed consecutively to decreasing 3 temperatures during 24 hr each: 25°C (baseline and control), then to 17°C and lasts 8°C. Blood samples were taken from all animals to measure corticosterone plasma concentrations. In addiction total and differential white blood cell count, natural antibody titers and complement system activity were measured. Results showed a total white blood cell count and natural antibodies titers were significantly lower in juveniles exposed at 8°C compared to control and 17°C. Activity of complement system was lower at control and 8°C. Similarly, proportion of heterophils increased significantly between temperatures control and 17°C, and decreased between 17°C and 8°C. There was no significant change in immunological parameters of hatchling between control and 17°C. The results of corticosterone are processing to the date. For both groups of treatment, the period of 24 hours at 17°C did not produce a change in the immunological parameters, this may be due to the action of corticosterone. On the other hand, the period at 8°C generated a decrease in the immunological parameters in juveniles even death in some hatclings, which may be related to the inability to continue synthesizing corticosterone due subsequently stopping metabolic activities.

Keywords: hormone, immunology, stress, crocodiles

Optimizing Protocols for High-quality RNA Extraction from Blood and Liver Tissues of *Caiman latirostris* (Broad-snouted Caiman)

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Transcriptomic information provides fundamental insights into biological processes and can be used to determine which genes are up- or down-regulated (as transcribed messenger RNA: mRNA) in cell, tissue, organ, or organism under specific physiological conditions or in response to an environmental perturbation, such as exposure to a toxic chemical. Extraction of high quality RNA is a challenging step mainly in non-traditional organisms, and protocols for preservation and extraction need to be adjusted in many cases. Our objective was to optimize preservation protocols for isolation of high-quality and quantity RNA from blood and liver tissues on broad-snouted caiman through the comparison of absorbance ratios and RNA integrity number (RIN) values to assess RNA quality. Four preservation treatments were tested: 1) flash freezing (N_2 liquid) and storage at -80°C; 2) RNAlater® conservation with a progressive cooling (room temperature, refrigerator, storage at -20°C and storage at -80°C); 3) preservation in TRIzol® reagent and storage at -80°C and 4) direct extraction with TRIzol® from fresh cells. Our results showed higher RNA quality and quantity in liver than blood tissue in the four different preservation protocols. Moreover, RNAlater® conservation was inadequate for blood because of RNA degradation while liver tissue preserved in RNAlater® showed good quantity and quality of RNA but its concentration was higher with other preservation methods. TRIzol® treatment was the most efficient procedure for an adequate RNA quality, quantity and integrity in *C. latirostris* blood and liver tissues, both through immediately extraction or -80 °C conservation. This protocol was stablished for both tissues and is now being used for transcriptomic studies in both tissues.

Keywords: gene expression pattern, RNA conservation, RNA isolation

Evaluation of Immunological Parameters in *Caiman latirostris* Embryos Exposed to Different Pesticide Mixtures

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The aim of this study was to evaluate the potential effects of embryonic exposure to different pesticide mixtures on immune system of *C. latirostris*. The study was performed in the "Proyecto Yacaré" (Gob. Santa Fe/MUPCN) facilities. Sixty eggs from 5 different nests were equitatively distributed into 4 experimental groups and exposure to pesticide mixtures by spraying the incubation material in the early stages of embryonic development: 1) vehicle control (3.5 ml of ethanol); 2) Glyphosate (Roundup Full II® -GLY- 2%) + Chlorpyrifos (Lorsban* 48E® -CPF- 0.8%); 3) Chlorpyrifos (Lorsban* 48E® -CPF- 0.8%) + Cypermethrin (Atanor® -CYP- 0.15%); 4) Glyphosate (Roundup Full II® -GLY- 2%) + Cypermethrin (Atanor® -CYP- 0.15%). The eggs were incubated in an artificial incubator, under controlled conditions of temperature of 31 ± 1 °C and 95% humidity. At the hatching time, blood samples were obtained from the spinal vein of all neonates; and aliquots of the collected blood were used for measures of total (TWBC) and differential (DWBC) white blood cell count. The remaining sample was centrifuged at 2500 × g for 15 min and stored at -80°C until be used for the determination of Natural Antibodies levels (NAb) titers. The provisional results for the analyzed parameters (TWBC, DWBC and NAb titers) no showed differences between exposed and control animals. This could be consequence of a lower sensibility of these variables in *C. latirostris*. However, it could also be expected that successive exposures can generate a significant difference. These results suggest that a higher number of parameters must be incorporate for a better understanding of the immune response of *C.latirostris* when they are exposed to the aforementioned agrochemicals.

Visitors Perception of Laguna del Carpintero to the Moreletii Crocodile (*Crocodylus moreletii*) in Tampico, Tamaulipas México

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In Mexico, the distribution of *Crocodylus moreletii* ranges from Tamaulipas to the Yucatan Peninsula and its habitat is very close to human populations in the southern area of Tamaulipas. Therefore, the objective of the present study was to document and to determine the perception of visitors and residents near the Carpintero Lagoon on the moreletts crocodile to assist in the planning of future environmental education programs for their management and conservation. The field work was carried out in Carpintero Lagoon, located in the municipality of Tampico at the south of the state of Tamaulipas. A survey was carried out based on the study factors and 200 people were randomly chosen among the Lagoon visitors, the data was analyzed statistically by the following factors: Gender (female and male), Age (>30, <30>50 and <50), Season (summer, fall and winter) and Category (locals, tourists and merchants). The analysis of the information was carried out with the Office Excel 2016 and IBM SPSS statistics 22 programs. It was observed that more than half of the surveyed women perceive the crocodile as a dangerous animal, and are unaware of data such as the species that inhabits in the Lagoon or the main function of *C. moreletii* in their habitat, in the case of young people under 30 years, 30% does not know the protection status of the crocodile and most of the three age groups believes that it would be better to relocate the crocodiles. The results obtained suggest that the visiting population of Lagoon, lacks sufficient knowledge about this species and their great ecological and commercial value.

Keywords: *Crocodylus moreletii*, Tamaulipas, perception

Does Corticosterone Exposure during Embryonic Development Affects Sex Ratios in *Caiman latirostris*, a Reptile with Temperature-Dependent Sex Determination (TSD)?

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It is known, that the sex of broad-snouted caiman embryos is regulated by temperature during a critical period of the embryonic development; and the interplay between temperature and steroid hormones can direct the phenotypical embryos´ sex. Recently, stress and glucocorticoids (GCs) -stress-related hormones- have considered as potential modulators of the gonadal differentiation process. For example, cortisol manipulation result in sex inversion in fish larvae; and high corticosterone levels bias offspring sex ratio towards males in some lizards. In this context, we aim to assess whether corticosterone (the main GC produced in reptiles) can mediate between temperature and sex differentiation of *Caiman latirostris*. As a first step, we incubated embryos at 32 ± 0.2°C (a transitional range in temperature that would produce 70% females). Different doses of corticosterone (1.4 ppm and 0.014 ppm) were topically applied to the eggshell at stage 20, prior gonadal differentiation. None of treatments had affected embryos survival. The expected sex outcome was skewed to males by high corticosterone dose. In addition, hatchlings from corticosterone treated eggs, at low dose, were the earliest to hatch and were also the longest and heaviest. Our results suggest a role of corticosterone in the masculinization process of broad-snouted caiman. They also provide a possible link between stress experienced by a reproducing female and a trade-off involving enhanced growth or male biased sex ratio, and hence the fitness of her offspring.

Keywords: transitional temperatures, corticosterone, males

Human-Crocodile Interaction (*Crocodylus moreletii*) in Madero City, Tamaulipas, México

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The Morelets crocodile plays an important role in the ecosystems where it lives. The human at the time of invading the habitat of the crocodile causes a human - crocodile interaction that leads to a risk of the population near the bodies of water. The general objective is the identification of the zones where some type of human-crocodile interaction occurs in Madero city, Tamaulipas. A compilation of records of crocodile sightings in the last seven years (2010-2016) by the civil protection and fire protection authorities of Madero city, Tamaulipas was generated. Once with the data, a series of risks mapping were carried out where the human-crocodile interaction was determined; in the same way the risk distance from the human home to the body of water was determined, where the factors of year and season were evaluated. It was observed that there was a total of 253 sightings of which the majority were in a range of 100 to 500 m from a body of water. The risk mapping indicates that the probability of a sighting outside a body of water is greater in years of greater rainfall. In the period from July to August (summer) the human-crocodile interaction increased since in the southern zone of Tamaulipas these are the months of greatest precipitation. It was observed that the year of 2011 was the year with most of the sightings (60 sightings) and the one with the least sightings it was the year 2012.

Keywords: *Crocodylus moreletii*, Tamaulipas, riskmap

Acute Exposure of *Caiman latirostris* to the Glyphosate Commercial Formulation Roundup® Full II (66.2%): Evidence of Genotoxicity, Oxidative Stress and Endocrine Disruption

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Glyphosate-based herbicides, mainly the formulation Roundup® (RU), are the most widely used herbicides worldwide in extensive agricultural activities, causing toxic effects in non-target organisms, as we already demonstrated for the native crocodile species *Caiman latirostris*. We previously demonstrated that subchronic in vivo exposure to RU induces genotoxic, oxidative damage and enzymatic imbalances at the lower concentration recommended for application the in soy crops (2%) and less. The aim of this study was to evaluate the acute toxicity of the same formulation in order to determine if these effects are observed after a short-term exposure to a higher concentration and the possible underlying mechanisms. We evaluated genotoxicity through the presence of Micronuclei (MN) and other nuclear abnormalities (NAs): *buds*, notched nucleus (NN), binuclei erythrocytes (BiN), eccentric nuclei (EN), and total nuclear abnormalities (TNA); oxidative damage to DNA through the modified comet assay by using endonucleases ENDO III and FPG and plasma corticosterone determination. Eight juvenile males of *C. latirostris* were distributed in two experimental groups: a negative control (NC) and a treated group exposed to the herbicide (RU). Animals were maintained under controlled conditions of temperature (30 ± 2ºC) into plastics containers (0.57 m^2 base surface) and the exposure was performed by voluntary immersion in water, at a concentration of 8 mg/l of RU during one week. After exposure, blood samples were taken to all animals for the application of the biomarkers previously mentioned. The results indicated a significant increase of the FMN and BiN and the oxidation of purine and pyrimidine DNA bases, with respect to the NC ($p<0.05$ in all cases). Animals exposed to glyphosate showed a significant increase in plasma corticosterone concentration. These data and revealed that a subchronic exposure (2.5 months) to low concentrations of RU herbicide formulation produce similar effects than a short-term exposure to a concentration at least 3-fold higher (acute exposure). This study, together with previous reports, demonstrated that animals receiving some events of direct exposure in wild populations living in the very proximity of crops, as well as those receiving constant exposure lo low pesticide residues, can both have alterations in their genetic material/physiology and therefore, could have consequences for the population development in the future.

Keywords: biomarkers, crocodilians, toxicity

In Ovo Exposure to Endosulfan Modified Thyroid Histofunctional Biomarkers in Prepubertal Juvenile *Caiman latirostris* Females

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Thyroid hormones play an important role on development, growth and reproduction in vertebrate species. It is suggested that endocrine disruptors wildlife exposure, particularly during prenatal life, could affect thyroid function having consequences later in life. *Caiman latirostris* is highly sensitive to endocrine disruption but little is known about *Caiman latirostris* thyroid histofunctionality and hormonodependence and its sensibility to endocrine-disrupting chemicals. Endosulfan is an environmentally ubiquitous pesticide classified as xenoestrogen. Previously we have demonstrated that Endosulfan *in ovo* treatment modified T3/body weight ratio and induced a decreasing trend on T3 plasma levels in *Caiman latirostris*. In rats, neonatal exposure to Endosulfan increases ERα level in uterus and mammary gland. Moreover, Endosulfan has been detected in *Caiman latirostris* eggs demonstrating natural prenatal exposure. The aim of this study was to characterize thyroid histofunctional biomarkers in juvenile males and females *Caiman latirostris* and to assess the effect of prenatal exposure to Endosulfan on thyroid histoarchitecture and histofunctionality. Eggs from low anthropogenic intervention areas were exposed to Endosulfan or vehicle (ethanol) prior to temperature sex determination. Vehicle treated eggs were equally distributed and incubated at male or female producing temperature. Eggs exposed to 20 ppm of Endosulfan were incubated at female-producing temperature. Hatchlings were raised under controlled conditions until juvenile stage. Upon sacrifice, thyroid glands were dissected, formaldehyde-fixed and processed until paraffin embedded. Histofunctional parameters (percentage of the gland occupied by stroma, epithelium, or follicular colloid; follicle density and follicular size) were assessed using histological sections stained with PAS. Follicular size was categorized into three groups, small, medium and large follicles. Estrogen receptor-alpha (ERα) expression was revealed by immunohistochemistry and expressed as percentage of positive cell. None histofunctional parameter but percentage of large follicles showed sexual dimorphism. Thyroid gland follicular epithelia express ERα suggesting that it could be a target of endocrine-disrupting chemicals possibly acting through steroid hormone pathway. Thyroid glands from Endosulfan treated female animals showed a decreased follicular colloid area and increased ERα epithelial expression. Based on previous results on T3, these findings suggest that Endosulfan interferes with thyroid hormones plasma concentration, and colloid depletion could be the response mechanism for maintaining circulating thyroid hormones level. As far as we know, this is the first time that *Caiman latirostris* thyroid gland histofunctionality is described. Additionally, this study alerts about the effects of Endosulfan environmental pollution on *Caiman latirostris* thyroid homeostasis.

Keywords: reptile, thyroid gland, endocrine disruption, Endosulfan

Phenotypic Variability and Heritability of the Cephalic Region of *Caiman latirostris* (Broad-snouted Caiman)

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The study of the cephalic shape of crocodilian is relevant in ecology, systematics, evolution, and conservation fields. Most studies about morphology and crocodilian growth conducted so far are only related to the size of the animals but they do not consider the shape. Geometric morphometric tools have succeeded not only to implement the quantitatively assessment of morphological changes but also a qualitative assessment through recovery of the shape under study independent of size. The environmental conditions experienced during early ontogeny affect embryos. Thus, environmental conditions during embryogenesis can induce phenotypic variation in animals. Temperature is a particularly important factor in determining developmental rates and final size in ectotherms. In this study, the dorsal cephalic region of 210 *Caiman latirostris* hatchlings was analyzed from seven populations in Santa Fe, Argentina, to detect intra and inter-population phenotypic variability, and to determine the heritability (h^2) of biological shape and size, using newly available geometric morphometric tools. The principal component analysis showed two configurations of cephalic shape that could be related to sexual dimorphism. In the canonical variate analysis, Procrustes distances between groups indicated that there are differences in shape among populations. Furthermore, the method of partial least squares indicated a covariation between cephalic shape and environmental variables. Regarding to centroid size (CS) of the skull we found significant differences among populations, moreover the partial least squares was also significant, which indicates that there is covariation between CS and environmental variables. Besides, estimates of the heritability of shape (h^2= 0.8756) and size (h^2= 0.9141) were high. The high heritability values of the shape leads to conclude that cephalic shape of *C. latirostris* hatchlings is mainly influenced by the genetic information underlying ("origin nest effect") which could imply strong response to selection. The size also is mainly influenced by the underlying genetic information, but the environmental characteristics also play an important role, apparently, the most influential factor on the size would be the incubation temperature. Studies like this are useful in animal breeding programs: breeders should consider the fact that they can achieve larger sizes in animals in which the artificial incubation conditions eggs are handled after harvest, in temperature particularly. Another important issue is to consider the heritability values of centroid size of the animals that are subject to artificial selection pressures to obtain a larger size, since their response will depend on the proportion of genotypic variance the population have.

Keywords: Broad-snouted caiman, morphology, quantitative genetics, phenotypic variability

Development of a Molecular Method for Sex Identification in Broad-snouted Caiman (*Caiman latirostris*) Hatchlings

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Several years ago it was shown that all crocodilian species studied so far; including *Caiman latirostris*, have temperature-dependent sex determination systems (TSD). In the species with TSD it is believed that sex is determined by a differential methylation, between males and females, of temperature sensitive genes. Methylated DNA alters gene expression by preventing the binding of transcription factors or by favoring the union of repressors. It has been shown that the DNA of males and females treated with a restriction enzyme sensitive to methylation amplifies genes under evaluation only when methylation protects the DNA from digestion by the enzyme. These differences in sex-specific methylation could be used to identify the sex of individuals in species with TSD. In crocodilians, the genitals are hidden inside the cloaca in a state of rest. For a correct identification of sex, the male genital organ of an immobilized specimen must be protruded from the cloaca, and then compared with the female clitoris which is also erectile. Because the female and male genital structures are very similar morphologically at early stage of their life, sexing is very difficult. Also, sex identification can be carried out through the observation of sexually dimorphic characters, such as size or other morphometric measurements. However, direct comparisons are usually not possible, and these differences are only applicable in adult specimens. Then, a correct sexual identification is difficult in the embryonic and juvenile forms, and this information is of great importance for field work, ethological studies, to evaluate strategies of reproduction and population dynamics. The objective of this work is the development of a fast, safe and reliable sexing method for *C. latirostris* based on the analysis of the *fezf2* gene that is differentially expressed in male and female reptiles from its hyper methylation in females. Blood samples were taken from males and females of juveniles specimens of *C. latirostris,* in which the sex was determinate from the observation of genital structures. DNA was isolated from blood and treated with the restriction enzyme *Hpa*II. Then, the gene *fezf2* was amplified by PCR in all DNA samples using two primers pairs: one pair designed to turtle *Chrysemys picta*, and other pair designed by our work group using Primer3 and BLAST from putative sequences of the *fezf*2 gene from *Alligator mississippiensis* available in GenBank. The PCR products are being analyzed by electrophoresis on 1.5% agarose gels and 4% polyacrylamide gels stained with plate nitrate solution.

Keywords: *Caiman latirostris*, sex identification, molecular techniques

Genotoxicity of Agrochemicals Evaluated in *Caiman latirostris* Using ISSR Markers

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Due its life habits, Broad-snouted caiman (*Caiman latirostris*) is constantly exposed to the action of agrochemicals. This work aimed to evaluate possible genetic alterations caused by pesticides in *C. latirostris*, using inter simple sequence repeats (ISSR) markers. To achieve this goal, 27 Broad-snouted caimans from an environment presumably without contamination, and 25 individuals from an agricultural-livestock area, both from the province of Santa Fe, Argentina, were analyzed. In addition, an experimental work was carried out with 33 individuals of approximately one-year-old, divided into two enclosures: one of them was sprayed with a mixture of widely used pesticide formulations (glyphosate, cypermethrin and chloropyrifos) at concentrations recommended for their application in soybean crops, and the other with tap potable water (control). In the case of experimental tests, blood samples were taken of all animals before and after exposure to obtain the data of each individual pre and post treatment. DNA samples were amplified with a set of Operon® ISSR markers, from which markers 8 and 36 were chosen as variability indicators due to the large number of amplified bands (118 loci in total). The amplified PCR products were analyzed in 4% polyacrylamide gels stained with silver nitrate solution. Gels photographs were taken for elaboration of matrices, and subsequent analyses were performed with TFPGA 1.3 software, to estimate parameters of variability (Heterozygosis expected -*He*- and Percentage of polymorphic loci -*P*-); and Genetic Identity (*I*). Results obtained from caimans living in natural environments without contamination (control) showed differences with exposed environment samples in *P* values (*P* control= 88.98; *P* exposed environment= 94.91), but *He* values were similar (*He* control= 0.391, *He* exposed environment= 0.383). The genetic identity between both sample pools was 0.937. On the other hand, the results from experimental samples showed no differences in control samples before and after treatment, but some differences were observed in animals exposed to the agrochemicals between the values before and after treatment (*He* pre-treatment= 0.272 vs. *He* post-treatment= 0.349; *P* pre-treatment= 61.86, vs. *P* post-treatment= 80.51). These results suggest a tendency to the increase the values of genetic variability parameters in the animals experimentally and environmentally exposed probable due to genotoxic effects of pesticides. We consider appropriate to expand the number of markers analyzed as well as the number of sampled individuals to confirm the present results.

Keywords: *Caiman latirostris*, genotoxicity, molecular markers, ISSR

Crocodile Night Survey: Detectability and Relationships with Environmental Variables

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Night survey is the most used method for surveying crocodile populations. It is used to confirm the presence of crocodilians in monitored sites, to estimate abundance and also to determine population size structure. However, the results of these counts are usually variables and related to environmental characteristics of the place and at the time of monitoring. These conditions can modify the monitoring results in a significant way. In our research, we monitor monthly for 3 seasons (2015-2018), a *Caiman latirostris* population located in "El Fisco" reserve (Santa Fe Province, Argentina), which has a lake of 300 ha and a nearby canal of 4.5 km of length. In the counts, water and air temperature, water depth, wind direction and intensity, and moon phase were measured. In addition, to determine the sensibility of the method, animals were released from the ranching program (Proyecto Yacaré) in successive seasons (25% of the estimated population), counting the population before and after the releases. After 24 nocturnal monitoring events in the lagoon, we obtained very variable results, ranging from 78 to 314 animals sighted per night. No increase in the population was observed and even fewer animals were counted in some counts after the releases. It was observed that the number of counted animals increases, as the temperature and depth of the water fall. The monitoring schedules carried out coincided with those of maximum detection observed in previous telemetry works (20.8 $\pm$ 11.6%) of animals possibly visible between 2200 h and 0300 h in the lagoon. Our monitoring showed an average of 180.25 $\pm$ 72.78 animals per night, which could indicate only a small percentage of the population present in this place. However, it is necessary to consider that caimans also use the canal and that a monitoring in this environment could increase the percentage of animals visualized. Therefore, knowing the interference of temperature, water level and monitoring schedule regarding the detectability of caiman population studies and monitoring programs should carefully consider the conclusions regarding the abundance of the animals based on the results of night counts. This variability of the counting data can lead to gross errors when making management and conservation decisions, as well as regarding the environmental impact analysis.

Keywords: *Caiman latirostris*, monitoring, detection, abundance

Molecular Characterization of *Trypanosoma* sp. in *Caiman crocodilus fuscus* (Spectacled Caiman) and *Crocodylus acutus* (American Crocodile), Panama

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Four species of trypanosomatids have been molecularly identified in South American caimans; however, to our knowledge, reports of these parasites for other species of Neotropical crocodilians are limited. Although there is no evidence that trypanosomatids are pathogenic in crocodilians, in humans and other animals they are. Trypanosomiasis is a problem of great economic importance in animal farming. There are reports of cross-infections, but the role of the crocodilians as natural reservoirs is unknown. Trypanosomatids that parasitize Panamanian *Caiman crocodilus fuscus* (Spectacled caiman) and *Crocodylus acutus* (American crocodile) are unknown. This is an ongoing project and the aim of this poster is to present the project to the international community for the purpose of obtaining feedback from CSG's experts. We consider if the Panamanian crocodilians are parasitized by trypanosomatids, the latter can be identified by the Polymerase Chain Reaction (PCR) and sequencing of new generation of SSU (rRNA-ribonucleic acid-ribosomal-small-subunit) and gGAPDH (glyceraldehyde-3-phosphate-dehydrogenase) genes, which is a much more efficient technique than traditional amplification and cloning. We seek to standardize the techniques of preparation and sequencing of amplicons genomic libraries of trypanosomatid's SSU and gGAPDH genes, in order to know and evaluate the diversity of trypanosomatids in the populations of crocodylians; to know the relationship between the trypanosomatids of Panamanian crocodylian species with those of other species and to determine the pattern of distribution of these parasites in Panama. The project methods include: 1) collection of blood samples from *Caiman crocodilus fuscus* and *Crocodylus acutus* in the main watersheds of Panama; 2) collection of blood samples from domestic animals that share the habitat with the crocodilians; 3) collection of possible vectors such as ticks, gadflies and bed bugs; 4) preparation and parallel sequencing of SSU and gGAPDH amplicons in a MiSeq System. Laboratory work is conducted with the Smithsonian Tropical Research Institute lab protocols. The information will be collected in a geo-referenced database, which will give the opportunity to generate distribution models of the trypanosomatids that are found. The analysis of the data will allow establishing if there is cross infestation between the species of crocodilians included in the study or between crocodilians and other wild species and domestic animals. To date, we have collected 250 blood samples, 50 samples of possible vectors. We are in the standardization of the amplification of the SSU and gGAPDH genes.

Comparison in the Composition of Fatty Acids of Two Cuts of *Caiman latirostris* Meat Bred in Captivity: Perspectives for a Greater Valorization of the Resource

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Fat is the most variable component in the chemical composition of the meat of many species; since its disposition is strongly determined by the balance between intake and metabolic requirements. Strong differences in lipid composition between muscles have been reported in different domestic species and in wild species raised in captivity such as: capybara, rabbit, Nile crocodile, otter, etc. In alligators, fat represents 6% of the weight of the carcass and is constituted mainly of unsaturated fatty acids of long chains with low cholesterol content. Little is kwon about the distribution of these fatty acids between the different parts carcass, as well as nutritional differences between the different muscles. The objective of this work was to evaluate fatty acid profile of meat of *M. ílio-ischio caudalis* and *M. occipito-cervicalis medialis* (tail and neck respectively) from *Caiman latirostris* (Broad-snouted caiman) bred in captivity, with the aim of establishing differences between these parts that can provide greater economic benefits in the production system of *C. latirostris* meat. Both cuts were taken from animals produced by Proyecto Yacaré/Yacaré Santafesinos. The fatty acid composition in the two cuts was determined by the methyl esters method and calculated the proportions: PUFA/SFA, n-6/n-3, and Δ-9-desaturase activity. We found evidence that the profile of individual fatty acids in tail and neck cuts are different for fatty acids: C14:0, C16:0, C18:0, C18:1, C18:2, C20:5 and C22:6. The polyunsaturated fatty acids (C18:2, C20:5 and C22:6) were higher in the neck than in the tail, which presented higher values of short chain saturated fatty acids (C14:0, C16:0). These differences, especially in C18:0, C18:1, C18:2 and C20:5 affect the sum of fatty acids: SFA, MUFA, PUFA, n-6 and n-3; as well as the proportions: PUFA/SFA and n-6/n-3, which presented higher values in the neck tissue. The tail showed high Δ-9-desaturase activity compared to the neck, which suggests that the high presence of unsaturated fatty acids in the neck are essentially due to their direct incorporation through diet and not to de novo synthesis. This results suggest that fatty acids distribution varies between cuts; furthermore, this difference may be related to muscle function. High presence of polyunsaturated fatty acids in the neck can provide added value to this cut of the carcass, since it can be sold as a meat with cardioprotective characteristics and nutraceutical.

Keywords: Broad-snouted caiman, lipid, fat, consumer

Sebekia Are You There? Elucidation about his Presence in *Caiman yacare*

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The pentastomes are a taxon of obligate endoparasites found into the respiratory system of terrestrial vertebrates. Within reptiles, they have been detected in snakes, lizards, turtles and crocodiles. In the respiratory tract of the definitive host, parasites must be capable of attaining sexual maturity. The objective of this work was to identify and characterize specimens of these parasites found in a wild individual of *Caiman yacare* from the *Esteros del Iberá*, an extensive wetland in the province of Corrientes, in northeastern Argentina. Six specimens of the Family Sebekidae were extracted from the lung lobes. Morphological study found that all individuals were adult sexually mature females with two pairs of retractable hooks on each side of the mouth and lots of eggs inside. The pentastomes fixed in ethanol (70%) and mounted directly with lactophenol. Parasites were observed with Scanning Electron Microscope JEOL JSM- 35C equipped with a digital image acquisition system Sem Afore brand. For the measurement of the different specimens, TPS DIG 2 version 1.11 is used. Thanks to the scanning microscopy, we observed that the specimens do not have spines on their hooks, they have 2 teeth inside their oral cuadre. Usually, the identification of species are based of the hooks and copulatory spicules of the males, but our animals were females. Today we are working on the identification of the specimens, comparing the different reports for Sebekia in South America. Unfortunately, we only had six parasites to confirm the species by moleculars technics. The aim of the present work is to enrich our knowledge of the biology of pentastomids by analysing the specimens. This is the first report on the presence of the genus *Sebekia* in *Caiman yacare* living in Argentina, and the second for Latin America, as *S. oxychepala* has been previously recorded in caimans from Corumbá, in the state of Mato Grosso, Brazil.

Keywords: *Caiman yacare*, *Sebekia* sp., lung, pentastomes, parasitism

Preliminary Report of the Presence of Millipedes (Diplopoda: Spirobolida: Rhinocricidae) in Nests of *Caiman latirotris*, Santa Fe, Argentina

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Diplopods are known as millipedes and are part of the Subphylum Myriapoda. This is the first report of the presence of millipedes in *Caiman latirostris* nests in the province of Santa Fe, Argentina. Millipedes are among the most diverse groups of terrestrial organisms, with more than 12,000 species described and an estimated 80,000 to be described. They are important members of the soil fauna in warm temperate regions, are detritivores and eat plant remains and fragments of organic matter. Their habits turn them into important factors in the decomposition of organic material, thus promoting microbial activity. Despite being the third largest class of arthropods after Insecta and Arachnida (Golovatch *et al*. 1995), their taxonomic knowledge is very incomplete, as many of their groups are under revision. A total of 10 specimens were found during the ranching season (29°40'59.26"S, 60°54'3.38"W), only two of them were males. The specimens were maintained and preserved in 70% alcohol for later identification. They were observed with binocular magnifying glass BS- 80 BOECO. Previous works show the presence of a species of the Family Rhinocricidae, *Argenticricus nodulipes*, in our province (Estancia La Geraldina, Verhoeff, 1941) as well as in the province of Córdoba (Lax *et al*. 2011, pers. comm.). The diplopods cannot be identified easily, sometimes or even at high taxonomic levels. Because most species are diagnosed on the basis of the morphology of the male genitalia even adult females are difficult to be assigned to specific species. The objective of this work is to identify the species found at the time of egg harvest. For this, we are working with the Myriapoda collection belonging to the Provincial Museum of Natural Sciences "Florentino Ameghino" of Santa Fe and different specialists in this field.

Thermal and Spatial Niches of Sympatric Species of Caimans

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Living crocodilians share many morphological and ecological traits, potentially competing when in sympatry. In Amazonia, up to four species of caimans may occur in sympatry, but little is known about their interactions, especially those involving *Paleosuchus palpebrosus*, one of the smallest and least studied caimans. Here we compare the thermal and spatial niches of a crocodilian assembly in the Culuene river, the main tributary of the Xingu river, in Gaúcha do Norte, Mato Grosso, Brazil. Between 22–25 July 2015, we visually sampled caiman populations along a 10 km section of the Culuene river and four oxbow lakes. From each individual we recorded the species, body temperature, habitat, water and air temperatures, and geographical coordinates of the location of first sight. Body temperatures were recorded with an Amprobe IR-750 infrared thermometer gun with a laser pointer; water and air temperatures were recorded with thermocouples connected to the IR-750. Habitats at river margins were classified as riparian forest, shrubs, sandy bar, or river bank. We recorded 203 caimans: 24 *P. palpebrosus*, 133 *Caiman crocodilus*, and 46 unidentified individuals. Almost all individuals of *P. palpebrosus* were found in the river (22 individuals, 91.66%), whereas individuals of *C. crocodilus* occurred both in the river and oxbow lakes. During dry season, oxbow lakes present a lower water level, leaving the fish denser, what could explain the domain of the larger species. *Caiman crocodilus* was seen more frequently in river sections bordered by riparian forest (76, 57.14%), while *P. palpebrosus* in sections bordered by shrubs (16, 66.66%). The observed niche overlap (pianka= 0.68) falls between 95% of null distribution of simulated values of niche overlap, indicating that there is no spatial niche segregation between the two species. Mean body temperature of *C. crocodilus* (26.15 ± 1.57 C) was significantly higher (t= 4.04, df= 72, P<0.05) than *P. palpebrosus* (24.43 ± 1.53 C). There was no difference between the two species in water (t= 1.26, df= 23.41, P= 0.21) and air temperature (W= 653.50, P= 0.05). We propose that *C. crocodilus* has greater thermal inertia, which causes it to take longer to lose heat, maintaining higher average temperature than *P. palpebrosus*, regardless of air and water temperature. Differences in body size largely account for thermal differences between these two species, but are not correlated with habitat use, thus allowing their coexistence.

Keywords: *Paleosuchus palpebrosus, Caiman crocodilus,* species co-occurrence, Amazonia

Fatty Acid Profile of Muscle, Fatty Body, Adipose Tissue and Serum of *Caiman latirostris* in Captivity

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Lipids are important constituents of cell membranes and play a major role in metabolic processes. They are composed of fatty acids (FA) of different chain lengths that may be saturated or unsaturated. The FA profile in the adipose tissue is modified through diet. The aim of this study was to compare the FA profile of four tissues (muscle, serum, fatty body and adipose tissue) of *Caiman latirostris* bred for two years by Proyecto Yacaré. The FA profile was determinate by gas chromatograph. Data were analyzed by similarity analysis (ANOSIM), non metrical multidimensional scaling (NMDS) and principal component analysis (PCA). The results show that FA profile of adipose tissue and fatty body were similar (regarding to polyunsaturated and saturated FA), and they were different from the serum and muscle. The muscle is characterized by the presence of linolenic, eicosapentaenoic and docosahexaenoic acids (fatty acids that are of great importance for health and are recommended for human consumption), while the adipose tissue and the fatty body have FA such as linolenic, eicosatrienoic and docosatrienoic and saturated fatty acid like myristic. The serum was characterized by polyunsaturated fatty acids such as arachidonic, docosadienoic, linoleic, and saturated fatty acids like stearic. In the muscle we find different polyunsaturated fatty acids in comparison with the fatty body and adipose tissue. This would be related to the different functions of the tissues. The knowledge of the fatty acids composition of these tissues could define possible uses of them and allow modifying the diet to improve FA profiles.

Keywords: Broad-snouted caiman, polyunsaturated fatty acids, fat

Reduction of the Oxidative Stress of the Fatty Body of *Caiman latirostris* by Enrichment of the Diet with Flax

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It is recognized the high economic value that crocodile leather has in the fashion industry. Currently, meat is beginning to be valued as a food of special interest for human nutrition, as a source of high quality animal protein, low fat content and balanced fatty acid profile. However, there are other derivatives of these animals, which are discarded as the fat body and other minor lipid deposits, which could be used as sources of natural oils with potential applications, both in food and in cosmetics. The objective of this work was to compare the oxidative state of *Caiman latirostris* fat deposits bred in the Proyecto Yacaré, with three diets: Control (C): ground chicken head and dry balanced feed 70%/30%; Flaxseed (GF): control+10% ground flaxseed and Oil Flaxseed (OF): control+10% flax oil. The enriched diets were offered once a week, for 15 days. After these periods, the samples of the fatty body and the minor adipose deposits were collected to determine the peroxidative damage through the substances reactive to thiobarbituric acid. In addition, the activities of key enzymes involved in the prevention of oxidative damage such as Catalase and Glutathione Peroxidase were measured. Significant differences were found in GF and OF groups respect to control group. Lipoperoxidation of fats decreased four times in GF and OF groups respect to C. The activities of the enzymes Catalase and Glutathione Peroxidase increased double in both groups enriched with flaxseed compared to the control. On the other hand, no differences were found in the variables studied between GF and OF groups. From the results obtained we can conclude that the enrichment diet of the caiman (with ground flaxseed or flaxseed oil) would be an important tool for the preservation of fats and their properties.

Keywords: Broad-snouted caiman, polyunsaturated fatty acids, fat, flaxseed

English Edition of The Crocodylia of Cuba

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Research on crocodile ecology and conservation in the Cuban Archipelago has been deeply improved during the last decades due to the activity of a group of specialists: Manuel Alonso Tabet, Roberto Ramos Targarona, Roberto Rodríguez Soberón and John B. Thorbjarnarson. The Wildlife Conservation Society together with the University of Alicante (Spain) agreed to compile and edit the information issued by the group, to generate the monograph "LOS CROCODYLIA DE CUBA". This first, Spanish edition, was introduced at the 23rd Working Meeting of the Crocodile Specialist Group in Lake Charles, Louisiana. In the last few years, the continuation of research in ecology, molecular genetics, population biology of crocodiles in Cuba, has led to new results and updates, which demand a second edition. The excellent translation into English by Regina Anavy gives us the possibility of making the second, updated edition in English available to a wider range of readers. With the editing work practically finished, the authors are looking for a publishing house and the necessary support for its publication.

Keywords: Crocodylia, Cuba, monograph

Sensory Quality of Yacaré Meat: Descriptive Attributes and Consumer Perception

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In Argentina, consumption of wild animals has increased in the last two decades with a demand for export. In the Litoral region (Formosa, Corrientes and Santa Fe), programs of broad snouted caiman (*Caiman latirostris*) have been implemented for its conservation and sustainable use with a production of meat and leather, which represents an incentive for regional economic development. The physico-chemical characterization of the meat, as a first measure for its commercialization, has revealed that is a meat low in fat and cholesterol, where the total of fatty acids present corresponds to polyunsaturated fatty acids of the family ω-3 and ω-6. These and other characteristics make yacare meat a product with high commercial value, as it can contribute to the list of cardioprotective foods. In spite of the above, there are few studies that are known about the sensory characteristics of this meat. The objective of this work was to analyze the acceptance and preference of tail cuts of broad snouted caiman by consumers, as well as the sensory description (appearance, flavor, taste and texture attributes) by trained panelists. Tail meat cuts from individuals of *Caiman latirostris* bred in captivity were cooked in a pan with little oil and salt. Chicken and fish meats were used as comparative samples. The affective tests: acceptance (9-points hedonic scale) and preference ranking were evaluated by 65 consumers. Descriptive sensory analysis was determined by a panel of 8 trained specialists. Attribute intensity ratings were evaluated using different references in an unstructured linear scale of 150 mm. Data were analyzed by ANOVA, and correlation analysis between sensory variables. The correlation analysis between the sensory variables from affective tests and the descriptive analysis showed that texture, taste and juiciness were the main attributes that have the greatest impact on the acceptability and preference of meat samples. Chicken was the most preferred meat (49%), followed by fish (34%) and finally by yacare (17%). Yacare meat was characterized by its bitter taste, greater fibrousness and hardness, as well as low fatness and flavor. Although yacare meat is nutritionally an excellent source of proteins and essential fatty acids, using this type of preparation its acceptability is lower when compared to chicken and fish meats. This aspect could be to improve with alternative preparations trying to reduce the negative attributes (fibrosity and hardness), and that highlight positive attributes such as taste and juiciness.

Keywords: caiman meat, descriptive analysis, preference, acceptance

Impact of Variable Incubation Regimes on Genital Differentiation in Hatchling Alligators

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How different are male and female hatching alligators? The external genitalia of adult alligators are quite different by sex, as to be expected. However, at hatching, this morphological difference of the clitero-penis (CTP) is subtle, with males only being slightly more developed than females. While the gonadal sex of American alligators is determined by egg incubation temperature in the nest, development of external genitalia (clitoris or penis) is a more developmentally delayed, androgen-dependent process showing greater morphological variability within a given sex. Previous research has determined that a constant nest incubation temperature of 33^{o}C determines testis development and male genital formation. In contrast, 30^{o}C results in ovary development and clitoral formation. However, it is unclear how intermediate and/or fluctuating incubation temperatures could influence formation of external genitalia. We investigated this question in week-old hatchlings incubated under the following conditions: 1) Constant male producing temperature (33^{o}C); 2) constant female producing temperature (30^{o}C); 3) intermediate promoting temperature (31.2^{o}C); and, 4) intermediate promoting fluctuating temperatures one (31.2 +/- 0.6^{o}C) and 4) intermediate promoting fluctuating temperature two (31.2 +/- 2.8^{o}C). To better characterize morphological differences across incubation temperatures, we quantified lateral and ventral CTP dimensions of glands height (maximum vertical width at mid-section of the head), glands width (widest point of glands), and curve distance (from beginning of sulcus to end of tip). The results we present here expand our understanding of "normal" alligator CTP sexual dimorphisms. Our project illustrates how the environment intimately shapes crocodilian biology and increases our ability to detect altered development in wild populations.

Keywords: genitalia, sexual dimorphism, alligator, incubation

Analysis of *Sebekia mississippiensis* Intensity Across the St Johns River Indicating Parasitic Site Preferences within the Host *Alligator mississippiensis*

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While most parasites do not compromise crocodilian health, pentastomids such as *Sebekia* spp. are truly parasitic causing mortality or morbidity among relatively healthy crocodilians. In addition, *Sebekia* spp. are the most common endoparasite with a prevalence of 60-95% amongst *Alligator mississippiensis* throughout the hosts' range (Tellez 2014). This study investigates how habitat preference of the host affects the parasitic intensity of the pentastomid, *Sebekia mississippiensis.* As alligators tend to limit their dispersal and stay within a particular home range (Rosenblatt 2011, Goodwin 1979), location of an animal can often be a good indicator of the habitat in which it lives. The parasitic intensity of *S. mississippiensis* found in the lungs of *Alligator mississippiensis* across the St. Johns River and its distributary channels were analyzed through lung and trachea dissections within 48 hours of mortality. Overall parasite intensity was $\bar{x}$= 64.846 per alligator host, and samples gathered from *A. mississippiensis* indicate that those living in large lakes or in wider regions of the St. Johns River (n=8) showed a significantly higher parasitic load (46-181, $\bar{x}$= 89.375) compared to alligators collected from small creeks or in narrow parts of St. Johns river (n= 5), which illustrated a lower parasitic intensity (20-31, $\bar{x}$= 25.6). This variation in *S. mississippiensis* intensity could reflect the increased encounter rate of infected intermediate hosts or free swimming pentastomes in larger habitats relative to small creeks. Additionally, we discovered biological site preferences between male and female pentastomids within the alligator host. Female parasites aggregated in the viscera whereas males were exclusively found between the two membranes of the pleura or in the trachea, with a few alligators having no infection of male pentastomes. This could suggest that female *S. mississippiensis* are the true parasite, whereas males are free swimming and "infect" the alligator solely to reproduce as males were found traveling through the tracheas of 4 alligator hosts. The number of pentastomids discovered in an individual *A. mississippiensis* from a brackish water ecosystem (n= 7) could support this theory. Although more samples are needed from brackish environments, it is possible high salinity hinders the physiology, physical locomotion, or chemical cues of male *S. mississippiensis* in finding an alligator host (to eventually mate with a female). By furthering our understanding of the life history of *S. mississippiensis*, researchers, managers, and care-takers of crocodilians become better equipped to understand this parasites' mysterious life cycle, which can mitigate parasite-induced morbidity or mortality, particularly amongst captive crocodilians.

Differential Evolution of Complement Genes in Crocodilians

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The complement system plays an important role in the innate immune response in vertebrate and invertebrate taxa. It consists of about thirty proteins grouped in five major gene families. These encode for distinct plasma proteins that react with each other forming three activation cascades (alternative, lectin and classical) which converge in a single terminal pathway. Research of this system in crocodilians has focused on detecting serum activity but there is a knowledge gap about what genes are present and what evolutionary mechanism maintain the diversity in these taxa. To address this, we identified and investigated 20 complement system genes in the genomes of three crocodilian species (*Alligator mississippiensis, Crocodylus porosus* and *Gavialis gangeticus*). We evaluated what gene evolved faster than the rest of complement genes. Individual and concatenated alignments were generated using ClustalW and Maximum likelihood method. Also, total tree length (TL) were calculated. For comparative purposes, we retrieve other immune loci from the three crocodilian genomes which include cytokines type I (INFA, INFE, INFK), Viperin and MHC class II genes. In addition, 15 exons representing 6 genes were selected and survey the diversity from additional 20 species of Alligatoridae and Crocodylidae. Phylogenetic analyses of the key complement components genes have shown a considerable level of sequence conservation among species. C1s and C3 had the highest TL, 0.26 and 0.13 respectively. Genetic distances between groups and within groups show that alligators have more variation than crocodiles. Finally, from orthologous clusters observed that crocodilian complement genes grouped within the five gene families, indicating that they have evolved independently from each other after speciation. Overall, our results suggest that crocodilians have the necessary gene repertoire for the potential activation of the three complement system pathways and there is some level of genetic diversity across species. This provides the foundations to conduct future studies on the diversity innate immune system of wild and captive populations to understand the immune response to diseases and inform management and conservation programs.

Keywords: genome, complement, immunology, evolution

Standardization and Determination of Basal Values of Chromosomal Aberrations (CA) and Mitotic Index (MI) on *Caiman latirostris* (Broad-snouted Caiman) Lymphocytes as Markers of Genotoxicity and Cytotoxicity

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Chromosomal Aberrations (CA) and Mitotic Index (MI) are short-term tests that enable the early detection of effects produced by agents that cause chromosomal damage and / or alterations in cell kinetics, giving great information about their mechanisms of action. The aim of this study was to standardize and determine basal values of CA and MI on *Caiman latirostris* (broad-snouted caiman) lymphocytes, for their use as markers of genotoxicity and cytotoxicity in order to include them into the battery of tests applied routinely in this and other native reptile species. Blood samples were obtained from the spinal vein of three juveniles of *C. latirostris* and whole blood cultures were made under the optimal conditions previously determined for *C. latirostris,* to obtain the appropriate lymphocyte stimulation rate and metaphases quality and quantity necessary for the analysis of CA and MI. In order to induce CA, whole blood was exposed *in vitro* to the known alkylating agent Methyl methanesulfonate (MMS) at 10 and 20 µM, and a negative control (NC) without exposure was used as reference. All treatments were performed in duplicate. With the aim to obtain more information regarding genotoxic effects of MMS, the determination of the frequency of micronuclei (FMN) was included as a complementary biomarker to the induction of CA. The results indicated significant differences in the MI ($p<0.01$) and the FMN ($p<0.05$) in the groups exposed to both concentration of MMS, respect to the NC. It was observed that, as the concentration of the MMS increases, a decrease in the number of metaphases occurs (IM_{NC}= 103.40 ± 5.70 vs $IM_{10\mu M}$= 55.80 ± 6.93 and $IM_{20\mu M}$= 18.40 ± 4.08), while the number of cells with MN increases (FMN_{NC}= 4.20 ± 0.50 vs $FMN_{10\mu M}$= 6.80 ± 0.60 and $FMN_{20\mu M}$= 7.80 ± 1.10). CA types identified mainly in treated groups were: chromosome and chromatid breaks, double diminutive chromosomes, chromosomal rearrangements, and absence of complete pairs. In the NC we identified only chromosome and chromatid breaks and absence of complete pairs. This study provide the first report on the application of the CA technique in *C. latirostris* and in all crocodilian species, as well as the type of CA inducted by a known genotoxic agent, MMS. In addition, it allows the incorporation of this technique as a new biomarker of genotoxicity to be included into the battery of tests applied routinely for the evaluation of the genotoxic effect produced by different agents in this and other native reptile species.

Keywords: *in vitro* exposure, reptile, genetic damage

Human-Crocodile Conflicts with American and Morelet's Crocodiles

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Human-crocodile conflict (HCC) with American and Morelet's crocodile have been updated to 2014 with focus on Mexico. Here we analyze information of both species along their distribution up to September 2017, including the most common factors that influences HCC os both species. American crocodile accumulated 344 cases in 13 countries since late 1950s, being the fifth more conflictive species in the world, and the second in the American continent, after the American alligator, the fourth in the world.

Three countries accumulate the highest percentage (87.5%) of *C. acutus* in the 13 countries, Mexico the highest (62.9%), Costa Rica (17.6%) and Panama (7.0%). Morelet's crocodile have 111 cases recorded in three countries since early 1970s and Mexico accumulate 76.6% of total cases.

Keywords: HCC, American crocodile, Morelets crocodile, an analysis

Status Update on the *C. porosus* and *C. novaeguineae* Populations in Papua New Guinea, 1981-2018

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Abstract: The results derived from the 2018 survey indicated that habitats were critically being degraded due to the unknown biological reproductive nature of the herbivores invasive fish species. In addition, degradation as mentioned in earlier reports is further acerbated by the traditional practises of burnings during extended dry spells by communities. Even with the degraded habitats in most lagoons and lake fringes, positive identification was possible of all nests reported during each survey period for both species.

Analysis was conducted with two subsets for *C. porosus* (N= 12 primary) from 1982 to 2018 and again for (N= 12 primary and N= 12 secondary) from 1991 to 2018. The data was analysed using with and without the 1998 and 2010 data. The results for both were not significant indicating a stable to increasing trend at 2% p.a. Again the primary and secondary sets from 1991 exhibited a 0.03% p.a. and 11.4% p.a. respectively. In *C. novaeguineae* the regression exhibited for N= 20 and N= 43) from 1981 to 2018 a staple increases for the primary sets including the whole subsets when analysed.

The annual skins export trade figures for the period 2005 to 2015 (n= 13 years) indicated a large variation between years. The annual skins turnovers were between 25,000 and 30,000 annually. However, in 2016 and 2017 the total skin export figures are lower than usually expected for the whole year. There is possible lapse in recording keeping for both *C. porosus* and *C. novaeguineae* export permits. The ranched *C. porosus* maintained stable trade figures usually at 8000-9000 skins annually except when there is specific request for large quantities which showed an increase in culls.

Wild eggs harvested annually by Mainland Holdings (MHL) in 2017 is (n= 11,382) 8434 viable with 46.8% hatchability, 2017 is (n= 272 nests) 13,841 viable eggs with 62.8% hatchability. In 2018 (n= 207 nests) harvested with 10,155 viable eggs, the hatchability is yet to be determined with all eggs currently in the incubator. These are mostly wild eggs and are predictable due to environmental factors having <80% hatchability rate. No records of captive breed eggs from Mainland Holdings Farm are reported for the corresponding period.

1. Introduction

This report reflects Papua New Guinea's management efforts since the last reporting at the 23 meeting in Lake Charles, Lousiana in 2014, on activities that have been undertaken. It includes results on the monitoring efforts for the *C. porosus* in 2018 and *C. novaeguineae* in 2015, the annual wild eggs harvesting program and the export figures since 2005. This report emphasized the importance and urgency of regular

surveys and continuous monitoring efforts, especially for species of high conservation status in which crocodiles is one of these species (e.g. rare or threatened) or species of special concern (e.g. sustainable use programs). This is important for PNG especially when habitats are critically degraded by invasive species and the wild population is being hunted for skins by land owners managed under the current PNG Crocodile Trade (Protection) Act.

Moreover surveys are planned to be conducted biennially, unfortunately due to funding constraints; the 2016, C. porosus survey was not implemented. This year's *C. porosus* survey was conducted through contract by the Conservation and Environment Protection Authority to Mainland Holdings Limited, which provided full funding support to conduct and conclude the survey. The two surveys *C. porosus* and *C. novaeguineae* were conducted during different mating seasons, and were not set out to estimate the total population of PNG. This would have required a far more extensive survey across segments of habitats in the three regions with a much longer period. Because of the habitats suitability for this technique, this survey has been designed to provide presumptive information as to the status of both species wild breeding populations within selected representative habitats in the middle upper Sepik river of PNG (Solmu *et al*. 2011, 2013; Solmu 2004b).

Interesting views were expressed during this survey by one community, indicating that 50% of nests harvested and field graded from that village prior to being flown to the hatchery for ranching were infertile. Assumptions can be inferred that either the lagoons or channel systems are too shallow during the extended dry periods for normal mating or that there was younger nesting female's recruitment into the populations. It was also observed across the survey area that the population was strongly skewed towards animals 3.0-3.5± metres for salties and 2.5± for freshies basking or on nests (for which size was estimated). This is specifically important for determining nesting effort between species, within sites for both in the longer term for repopulation effort. However this view can be considered as useful public attitude to their observations and is subjective.

A record of interviews was also conducted with villages and the Sepik Wetlands Management Team (SWMI) on human crocodile conflict. There were reports and eye witness accounts of four (4) incidences that occurred over a period of one month during December 2017. The first two (2) attacks occurred on the 15 December, the third was on 28 December and the final victim was reported also in December with no date. All four attacked victims were local women. They were hospitalized with major injuries to their arms and legs with two of this needing assistance of wheel chairs. These attacks are not concentrated in one specific location but are separated by large distances and different villages. Three of the attacks are near Ambunti whilst the other is from Kubkain village. These attacks are presumed to be occurring with the onset of wet seasons and peak *C. porosus* mating season and women are vulnerably fishing in the open lake systems that have in the past having large habitats cover.

2. Crocodile monitoring - Aerial surveys

2.1. *Crocodylus porosus*

A regression analysis was conducted on the observations and assessments of three subsets (N= 12 Primary, 1982-2018), (N= 27 & N= 39, 1991-2018), and (N= 12 Primary & N= 12 Secondary, 1991-2018). Subsets of these data were analysed

indicating no significant variation between sets, and the subset dealt with and reported here is the N= 12 primary 1982-2018.

The data extrapolated was used to regress both with and without, 1998 and 2010 survey years based on the information provided below. This is because observational studies, independent variables are not manipulated and no treatments are assigned. This survey is conducted through this approach as it is conducted in a natural system. Thus, statistical analysis reveals the relationships among variables and that causality cannot be inferred because other unstudied variables may affect the measured variables.

- 1998. The data sets for that year was considered aberrant based on environmental effects of the previous year (1997) extensive El Nino, the counts were lower thereby could presumably interact with other variables in the model.
- 2010. The data sets for each set for the years were considered ambiguous; the predictor is significant with higher number of nests than usual.

The primary (N= 12), between 1982 and 2018, nesting effort has been noted to be fluctuating (n= 28 years) period. For instance where some sites were burned or drier this year would most likely be able to have higher number of nests the following year. It was also observed that the nesting effort is assumed to be dependent on habitat conditions, human accessibility and disturbances, and proximity to other non-monitoring sites within the area. However, this hypothesis can be tested to assertion the assumption in future survey work were funding permits e.g. through the CSG/SARS initiative.

The initial data regressed is the Primary sites since the survey inception (N= 12) including 1998 and 2010 (Fig. 1).

- Where N= 12 (1982-2018), with 1998 and 2010 data (red).

Y= 1.1160X - 2165.4, r^2= 0.4770, p= 0.000047. The mean nesting effort for this set is 63.75% annually (n= 28 years), (SD of 16.90 and a range of 30-97).

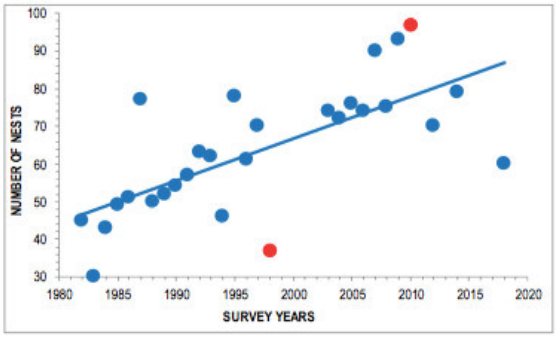

Figure 1. Primary data. N = 12, surveyed from 1982 to 2018 with 1998 and 2010 data. (n =28yrs)

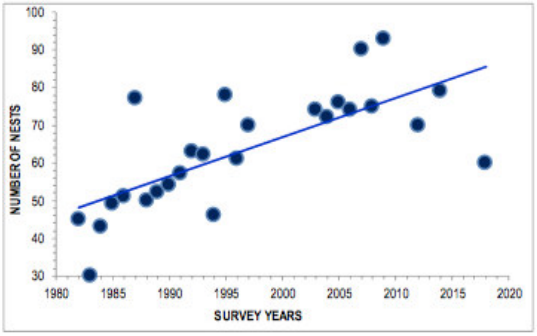

Figure 2. Primary data. N = 12, surveyed from 1982 to 2018 without 1998 and 2010 data. (n =26yrs)

Analysis was again conducted for the same set N= 12, (1982-2018), without 1998 and 2010 data (Fig 2), in considering that both sets had counts which are low following the 1997 El Niño effect and the 2010 counts unusually high.

- Where N = 12. (1982 – 2018), **without** 1998 and 2010 data.
  Y= 1.0371X - 2007.5, r^2= 0.5088, p= 0.000043 with a mean of 63.5% annually (n = 26 years), (SD of 15.36 and a range of 30-93).

The dependent variables is significantly correlated with variables A, p values = < 0.05 in both sets. The results exhibited a statistically upward trend of 2% annually for both sets over the longer term both with and without 1998 and 2010 sets.

We also examined the results for (N= 12 primary and N= 12 secondary) from 1991-2018, with and without 1998 and 2010. The secondary sites added later during the period (1991) as the survey progresses which are considered as smaller sets i.e. Primary N= 12 from 1982 to 2018 and the Secondary N= 12, 1991 to 2018. However, to ensure that the variables are compatible the analyses were conducted for both sets from 1991 to 2018 (Figs. 3 and 4).

- Figure 3, N= 12 Primary (1991-2018) with 1998 and 2010 data (blue).
  Y= 0.9286X -1789.5, r^2= 0.25, p= 0.028
  The mean is 70 (SD of 14.95, range of 37-96)

- Again in Figure 3, N= 12 Secondary (1991-2018) with 1998 and 2010 data (green).
  Y= 1.4214X - 2813.3, r^2= 0.59, P= 0.0001
  Mean of 33.47 (SD of 14.94, Range of 14-64)

The results for both sets exhibited a statistical variation of between 25% and 59% respectively, p= <0.05).

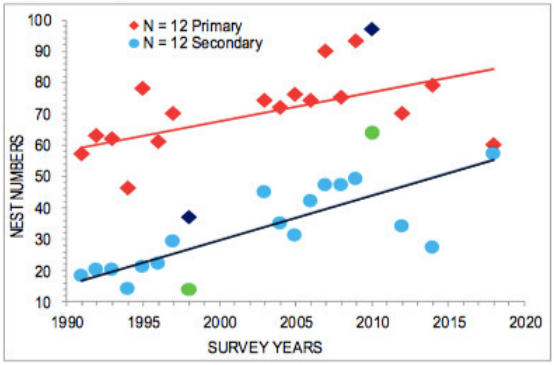

Figure 3. N = 12 Primary and Secondary (1991 to 2018), with 1998 and 2010 sets.

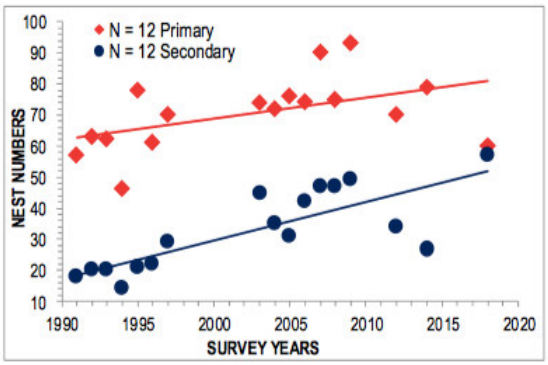

Figure 4. N = 12 Primary and Secondary (1991 – 2018), without 1998 and 2010 sets.

Similarly, data in Figure 4 were again regressed excluding the 1998 and 2010 data.

- In Figure 4, N= 12 Primary (1991-2018) **without** 1998 and 2010 data
  Y = 0.6757X -1282.6, r^2= 0.23, p= 0.051.
  A mean of 72 (SD of 11.74, Range of 46-93).

- Again Figure 4, N= 12 Secondary (1991-2018) **without** the 1998 and 2010 data
  Y= 1.2339X - 2438.2, r^2= 0.63, p= 0.0002
  A mean of 34.6 (SD of 12.7, Range of 14-57).

Statistically in observing the trend over the size effect (n= 19 years and n= 17 years), there is a significant difference between sites. e.g. N= 12 primary sites exhibiting a 0.03% increase p.a. in contrast to N= 12 secondary a 11.4% p.a. With eye fit observations the secondary sets exhibited an upward to increasing trend whilst the primary set exhibits a trend towards horizontal. The exhibition of both indices could be useful when inferring or contrasting different sets of data which are being considered temporally and spatially.

2.2. *Crocodylus novaeguineae*

The nesting survey results for this species conducted in 2015 were analyzed using two (2) subsets to ensure a general nesting trend was observed since 1981 for N= 20, and 1992 for N= 43 (Fig. 5). We could have presented results for all the subsets, however considering from eye fit observations for all subsets, would apparently be similar across all sets. This is irrespective of which sets are used (with or without 2007) as that year had significantly higher nests counts.

The results exhibited a similar trend as in previous other reports (Solmu *et al*. 2013). Even with the 2015 survey there was no significant increase due to the longer period of the El Niño effect. The raw counts for the whole set in 2015 indicated 88 nests from 105 counts recorded in 2013 exhibiting a 4% reduction than the previous year survey. With eye fit observations represented in Figure 5 the following observations is made;

- Between 1992 and 2015 nests counts for the (N= 43 sites), there was a significant increase from 1992 to 1995.
- With the same subset there was a reduction in 1996 to 1999 preceding the 1997 El Niño, and a moderate increase in 2005 with a significant increase in 2007.
- The 2011 survey observed a fluctuation trend in which there was a reduction in nests counts observed with an increase in 2013 and a reduction again in 2015.

It will be difficult to critically say if this phenomenon of extended dry weather will affect the nesting effort in later years as observations indicate that the 1997 El Niño effect, after some years did not affect the subsequent nests increases in 2003, 2005, 2007 and 2015 (Fig. 6).

The survey observations over a number of years indicated a fluctuating trend between each period, where there may be specific events e.g. long dry periods with extensive burnings or El Niño events.

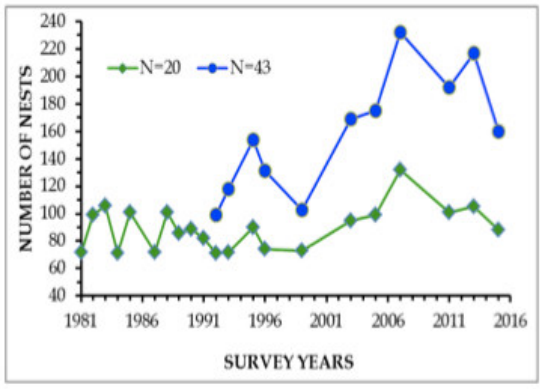

Figure 5. A fluctuating trend for N=20, 1981 to 2015 and N=43, 1992 to 2015.

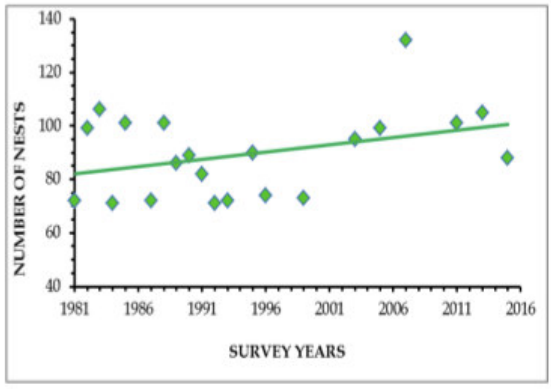

Figure. 6. Regression analysis for N=20 since 1981

- Regression for (N= 20) Primary set, 1981-2015, [Y= 0.543X - 995.0, r^2= 0.129, p= 0.139].
  A Mean of 89.47 (SD= 16.0, Range of 71-132). Although there is a stable increase, no significant difference for all years across the (N= 20) primary sites (Fig. 6).

In these notable events, it would be pre-emptive to conclude that nesting effort between both sets (N= 20 and N= 43) are an indication of nesting decline, because both trends generally indicate that nests do increase in subsequent years where in previous years they are at low (Manolis 1995). This is evident as we observe fluctuating trend for N= 20 from 1981 to 1988 and again from 2003 to 2015. The N= 40 set also reflected its fluctuating trend between 1993 and 1996 and again from 2007 to 2015.

3. Wild egg harvesting

In PNG only *C. porosus* nests are harvested from the wild for ranching (Cox *et al*. 2006). The status of nests observed during the conduct of the 2018 survey is recorded for all *C. porosus* sites (N= 39) and are categorized as numbers i.e. active, flooded, harvested/raided or successful hatch. In Table 1. we contrast the nesting status of all nests recorded. The nests that are harvested or raided by humans and predator lizards are considered as nests being removed from the nesting site and do not enhance re-population effort.

However, these nests are still counted to indicate the nesting effort for each index and the relative abundance of nesting females for the population during the survey period. The observations are again categorized into sites (N= 12, N= 14, N= 27 and N= 39). The degree to which nest count results have been extrapolated to infer the status of local populations is unknown, but an indicative relationship is assumed to exist, and local information suggests that *C. porosus* in the upper, and to a lesser extent, the middle Sepik has indeed increased substantially (Cox *et al*. 2006).

From the total nests counted within the targeted survey area, only 17.7% were observed from the air to be harvested by landowners a week earlier to the survey conduct. Most other nests harvested were from non-surveyed areas and communities participating in the program. The total nests harvested from the wild in 2016/17 and 2017/2018 harvest season are in Table 2.

Table 1. Status of nests spotted and harvested in surveyed areas.

NESTS STATUS	Primary sites			Secondary sites	Status of total nests	Percentage
	N =12	N = 14	N = 27	N = 12	N = 39	
Active	37	41	111	40	151	76.3%
Flooded	0	0	0	0	0	0
Harvested/Raided	22	22	27	8	35	17.7%
Successful Hatch	1	2	3	9	12	6.0%
TOTAL POSSIBLE COUNTS PER INDEX	60	65	141	57	198	100
Percentage calculated is based on N = 39, total sites surveyed in 2018						
Numbers of females physically sighted on nests or were basking were ± (est. sizes 2.5m – 3m) at various sites during the survey.						14?

The recent harvested eggs are currently being incubated (no hatchling numbers and percentage hatchability for 2018). In this program alone Mainland Holdings Limited over the years delivered cash outlays equivalent to about $US50,000-70,000 to the local communities. In addition to the payments for eggs harvested additional costs not included is paid to communities for services provided (canoes, outboard engines, accommodation, fuel and hire of local personal) to deliver the harvest program. This is value adding towards the program so that communities realize the importance they contribute to conserve habitats and practices of sustainable harvesting.

Table 2. Total nests harvested in 2017 and 2018 harvesting season.

HARVEST YEAR	NESTS HARVESTED	% OF NESTS SURVEYED	TOTAL No OF EGGS	VIABLE EGGS	EGG PAYMENTS TO FARMERS	INFERTIILE / DEAD EGGS	% VIABLE EGGS	AVG CLUTCH SIZE	HATCHLINGS	HATCHABILITY
2017	272	22.00	16363	13841	K185371.00	2522	84.59%	60.16	8691	62.8%
2018	207	17.00	12007	10155	K136329.00	1852	84.58%	58.00		0.0%

The program has been delivered for a number of years and communities have very much embraced this program to be maintained for the long term. Mainland Holdings has taken a positive role and has continued to support the efforts of egg harvesting and reviewing its operations to increasing prices of each viable egg.

4. PNG total skins exports

The records in Table 3 have been provided by the implementing agency, the Conservation and Environment Protection Authority (CEPA). The total annual exports is provided for the longer-term period from 2005 (n= 13 years). The average export

figure for farmed *C. porosus* is 8259, wild *C. porosus* is 3558 and average for total skins exports (both species wild and farmed) are 15,407.

Table 3. PNG Export Figures

YEAR	FARM POROSUS	WILD POROSUS	WILD NOVAEG	TOTAL EXPORTS
2005	6549	3852	17726	28127
2006	6453	3762	20773	30988
2007	7629	4128	15904	27661
2008	9211	4683	16955	30849
2009	9434	3893	21548	34875
2010	13139	5526	17605	36270
2011	8921	3399	11365	23685
2012	8500	2949	12364	23813
2013	4836	3335	15189	23360
2014	7324	3550	13750	24624
2015	8095	3361	21262	32718
2016	10057	2263	9728	22048
2017	12279	1559	6132	19970

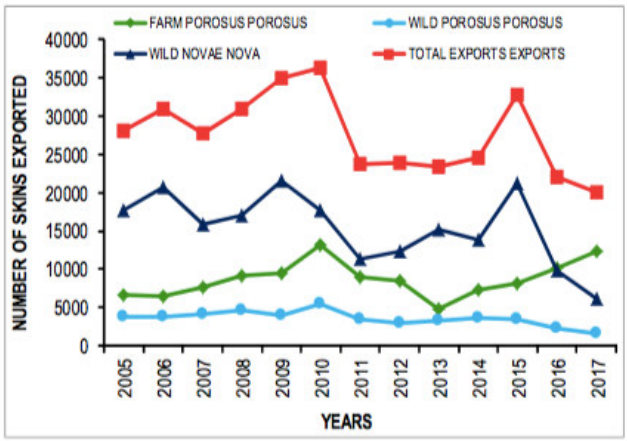

Figure 7. Skins exported from 2005 to 2017

In Table 3, the export figures for individual periods are considered stable at 25,000 to 30,000 skins per year. However, the table exhibited the consecutive years running in 2016 and 2017; *C. novaeguineae* figures were undoubtedly lower than preceding recorded periods. Numbers in Table 3, showed that the *C. porosus* and *C. novaeguineae* wild skins are lower for both periods. This could not be used as evidence that the number of wild skins are shrinking. There could be other contributing issues e.g. seasonal variations or record-keeping conducted by the management authority (CEPA). CEPA in the longer term should be aware of its obligation under national regulations for quarterly and monthly returns to be safely collated in a systematic approach for easier retrieval. This powerful enforcement tool could be more efficiently used to ensure better control and compliance by crocodile farms and skin industry throughout PNG.

5. Summary

Crocodile monitoring of which the aerial survey data is extrapolated from the representative sites in the Sepik is presumptive for Papua New Guinea. This is because the data let alone without the harvest figures and size structures from the other three regions is in complete to have an accurate estimate of the wild populations through the country. However, the approach is still relevant and quantifiable for the national situation because the Sepik alone supplies almost 50% of the wild skins for the export market hence it is imperative that the monitoring regime through the helicopter surveys is justifiable.

Since the inception of this program we were able to provide results that were analyzed from data tabulated for the last twenty-eight years (n= 28 years). Surveys was conducted each season pending funds over all *C porosus* sites (N= 39 sites) and *C. novaeguineae* (N= 43 sites) from the primary to secondary with data extrapolated and analyzed depending on years they were added. In general the current indications of regressed sets for both species over the periods are stable but for how long that remains a hypothetical issue. This is in consideration of all the imminent threats (forestry, agriculture, mining and invasive) that are currently occurring within the whole of Sepik catchment.

During the survey conduct this year we also received reports of four (4) humans being attacked and hospitalised by crocodiles, thereby fuelling human-wildlife conflict. This crocodile-human conflict (Aust *et al*. 2009) is assumed to be exacerbated by the increasing degradation of prime herbaceous habitats for nesting and additionally the day-to-day survival for humans e.g. fishing for food (Lamarque *et al*. 2009). It has been suggested that crocodile densities exhibit a negative correlation with human densities and development patterns (Aust *et al.* 2009), hence increased human concentration and developments near lagoon systems will likely result in higher risks of crocodiles attacks and *C. porosus*, regardless of size, is classed as a problem animal.

It is suggested that the future use of helicopter surveys will depend not only on its value in terms of accuracy and precision, but also on its costs and benefits in relation to stated objectives and to the relative merits of alternative techniques e.g. using drones. Importantly a trial use of UAV as an option for future crocodile nests monitoring is significant, because this practice is being used and implemented elsewhere and found to be an acceptable approach given that funding difficulties currently existed. It will be a positive direction to significant reduces costs by about 75% from the current $US75,000 per survey so that savings can be used for extrapolating skin sizes of export trade data from each region to ascertain the population structures of given areas being exploited.

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A Status Assessment and Long-term Conservation Plan for Siamese Crocodiles in the Xe Champhone Ramsar Site, Savannakhet Province, Lao PDR

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Abstract: The Siamese crocodile (*Crocodylus siamensis*) is regarded as one of the most critically endangered crocodilians in the world. In Lao PDR, potentially viable, albeit fragmented populations of wild Siamese crocodiles persist in Attapu, Salavan, and Savannakhet provinces. In 2008, the Wildlife Conservation Society–Lao Program working in collaboration with the Lao Government designed and implemented a long-term recovery plan for Siamese crocodiles in Savannakhet Province. Wetlands along the Champhone and Xangxoy rivers – now encompassed within the Xe Champhone Ramsar Site - were included in a community-based conservation project and designated Crocodile Conservation Areas (CCAs). Village Crocodile Conservation Teams (VCCT) were organized and tasked with monitoring crocodile populations and enforcing conservation regulations. An egg collection and head-starting program was initiated and ultimately 65 subadult crocodiles were successfully reared and then released in 2013-14. Despite meeting objectives, donor funding was unexpectedly cancelled and conservation efforts were suspended in early 2014. In late 2017, WCS conducted a rapid assessment of former CCAs to evaluate past conservation actions, and determine if renewed efforts are warranted. We visited CCAs (n = 7) from 4-11 January 2018 and conducted interviews of former VCCT members. We questioned participants about recent crocodile sightings (location and approximate date), perceptions of abundance, and potential threats. Of particular interest was evidence of recent crocodile nesting activity (e.g., eggshells, nests with eggs, and observations of small juveniles). According to these interviews, at least some crocodiles are now present in every CCA. Evidence of reproduction was reported at four of the seven (57%) CCAs and two other CCAs (where nesting was not reported) appear important as wet-season foraging habitat for crocodiles. The release of 65 head-started crocodiles in 2013-14 has

successfully augmented an existing population at the largest CCA (Kout Mark Peo). The reintroduced crocodiles (large subadults in 2018) will probably disperse into neighboring wetlands using the Champhone River as an immigration corridor. In anticipation of future population recovery, efforts should be made to identify additional wetland habitat amenable to protection, and expand the network of designated CCAs. Particular effort should be made to identify villages that afford protection to crocodiles on the basis of spiritual-religious beliefs. Our long-term conservation objective is to restore a viable metapopulation of Siamese crocodiles in wetlands of the Xe Champhone Ramsar Site.

Keywords: *Crocodylus siamensis*, Ramsar Site, reintroduction, Siamese crocodile.

Introduction

The Siamese crocodile (*Crocodylus siamensis*) is regarded as one of the most critically endangered crocodilians in the world (Simpson and Bezuijen, 2010). During the past 50 years, wild populations of *C. siamensis* throughout Southeast Asia have been decimated by illegal hunting for skins and meat, wanton killing, government sponsored extermination programs, habitat loss, and over-collecting to stock commercial crocodile farms (Platt and Tri, 2000; Platt et al., 2004; Simpson et al., 2006; Simpson and Bezuijen, 2010; Platt et al., 2011; Kanwatanakid-Savini, et al. 2012; Guérin, 2013). Furthermore, although hundreds of thousands of *C. siamensis* are now held on commercial crocodile farms in Southeast Asia, the genetic integrity of this burgeoning captive population has been compromised by widespread hybridization with estuarine crocodiles (*Crocodylus porosus*) (Fitzsimmons et al., 2002; Starr et al., 2009).

In Lao PDR, potentially viable, albeit fragmented populations of wild *C. siamensis* are confined to wetlands in Attapu, Salavan, and Savannakhet provinces (Stuart and Platt, 2000; Thorbjarnarson et al., 2004; Bezuijen et al., 2013). Despite being legally protected as a "Prohibited Category I Species" (hunting and trade strictly prohibited) in Laos, Siamese crocodiles are threatened by deliberate killing for food and to protect people and livestock, collection of eggs for domestic consumption and medicinal purposes, incidental take in fishing gear, and habitat loss (Simpson and Bezuijen, 2010; Platt, 2012; Bezuijen et al., 2013). The latter threat is particularly acute in Savannakhet Province, which not only harbors some of the largest remaining crocodile populations, but also supports the greatest rural population density in Laos (Bezuijen et al., 2006, 2013).

Recognizing that *C. siamensis* faced near-certain extinction in Laos unless immediate action was undertaken, the Wildlife Conservation Society−Lao Program working in collaboration with the Lao Government designed and implemented a long-term crocodile recovery plan in 2008 (Hedemark et al., 2009). Surveys first identified a number of small *C. siamensis* populations in Savannakhet Province that would likely benefit from conservation efforts (Bezuijen et al., 2006). These populations already received some degree of protection from the local belief that crocodiles embody the spirits of dead ancestors (Platt, 2012; Bezuijen et al., 2013). Six wetlands in the Champhone (Kout Kaen, Xelat Kadan, Nong Maehang, and the Kout Mark Peo–Phai Cheo Reservoir Complex) and Xangxoy (Kout Kouang and Kout Koke) river systems were then selected for inclusion in a community-based conservation project and

designated Crocodile Conservation Areas (CCAs). CCAs were thought to harbor 50–70 crocodiles (Table 1).

Table 1: Estimated size of Siamese crocodile populations at Crocodile Conservation Areas (CCA) in Savannakhet Province, Lao, PDR. Population estimates are based on sightings reported by villagers, interpretation of tracks and signs, and nesting activity. Data from Hedemark et al. (2009).

CCA	Population size	Comments
Kout Kouang	2 adults	Nest containing non-viable eggs found in 2008.
Kout Koke	1 adult	Hatchings observed in 2008.
Kout Kaen	1 adult	Juvenile (TL = 47 cm) captured by fishing net in 2008 and released; one adult and a juvenile observed in 2006.
Kout Xelat Kadan	10-12	Size classes not stated; remains of an apparently successful nest found in 2008.
Kout Mark Peo	50	Population contains at least one pair of adults; six juveniles and an adult observed in spotlight survey in 2005. Site could harbor largest remaining population in Champhone and Xangxoy river systems.

The crocodiles in these wetlands probably function as a single interacting metapopulation linked by riverine corridors (Platt, 2012). Additional wetlands near Naonua Village were later incorporated into the project. Crocodiles and other wildlife in these wetlands have long been protected by local animist beliefs (Baird, 2001).

Village-level discussions were held in area communities during 2008-09 to solicit local input for site-specific management plans designed to insure crocodile recovery, protect critical wetland habitats, and establish conservation zones with accompanying regulations (Hedemark et al., 2009). Later efforts (2010-12) focused on developing wetland management guidelines (especially critical during the dry season when farmers require large quantities of water for irrigation), designing crocodile-friendly fishing regulations, and conducting conservation awareness programs in key communities.

In 2010-11, Village Crocodile Conservation Teams (VCCT) were organized in rural communities adjacent to CCAs. VCCT cadres attended training workshops held during November-December 2011 where they learned basic monitoring and patrolling techniques, and each was issued a Lao-language edition of Simpson (2006). Cadres were subsequently tasked with monitoring crocodile populations, enforcing conservation regulations, and searching for crocodile nests, and received a small monthly stipend for their participation in the project. In addition to monitoring wild populations, WCS in collaboration with the Lao Zoo and area villages initiated an egg collection and head-starting program; eggs were collected from nests, incubated in specially-designed facilities, and hatchlings then reared to a size (total length [TL] ca. 70-90 cm) considered immune to predation (techniques described by Platt et al., 2014b). Augmenting these efforts, a conservation-breeding program using crocodiles genetically confirmed as *C. siamensis* was initiated at the Lao Zoo. Together these programs ultimately provided 65 head-started juveniles that were later released into Phai Cheo Reservoir near Tansoum in 2013 and 2014. Despite demonstrated success in meeting objectives, donor funding was unexpectedly cancelled in early 2014, conservation efforts were suspended, and no subsequent monitoring or evaluation was conducted.

In late 2017, WCS received funding from several zoos in the United States (see Acknowledgements) to conduct a rapid assessment of former Crocodile Conservation Areas, evaluate past conservation actions, and determine if renewed efforts are warranted. To this end, we conducted a rapid assessment of the former Crocodile Conservation Areas in Savannakhet Province during January 2018. Herein we present the results of this assessment, describe a long-term conservation strategy, and provide specific conservation recommendations.

Physical Setting

Crocodile Conservation Areas selected by WCS during the initial project (Hedemark et al., 2009) are located Champhone, Xonnabouli, Songkhon, and Xiabouli districts of Savannakhet Province in central Lao PDR (Fig. 1). The region is characterized by a tropical monsoonal climate with a prolonged wet season extending from late May through late October with peak rainfall and flooding in September and October. Little precipitation occurs outside of the annual wet season and water levels fall dramatically during this period. Wetlands in Savannakhet Province are found in the Mekong Plain physiographic province which is generally <200 m in elevation and encompasses much of southwestern Lao (Bezuijen et al., 2006). The Mekong Plain supports the highest human population densities within Laos, and contains most of the nation's productive agricultural lands (Bezuijen et al., 2006).

Rice is the principal crop produced in the lowlands of Savannakhet Province, where 8% of the land is occupied by paddy fields and approximately 80% of rural households are engaged in rice cultivation (Kosaka et al., 2006). The wet season rice cropping cycle begins with plowing in May, followed by transplanting in June, and harvesting in October. After harvesting, water buffalo and cattle are allowed to graze the fallow fields and fertilize the soil with their dung. In wet paddy rice fields (*na beung*) cultivation is often year-round with a second crop planted in late November and early December, which is then harvested in late April through early June. Water to irrigate dry season cropping is often obtained by diverting or pumping water from natural wetlands (Kosaka

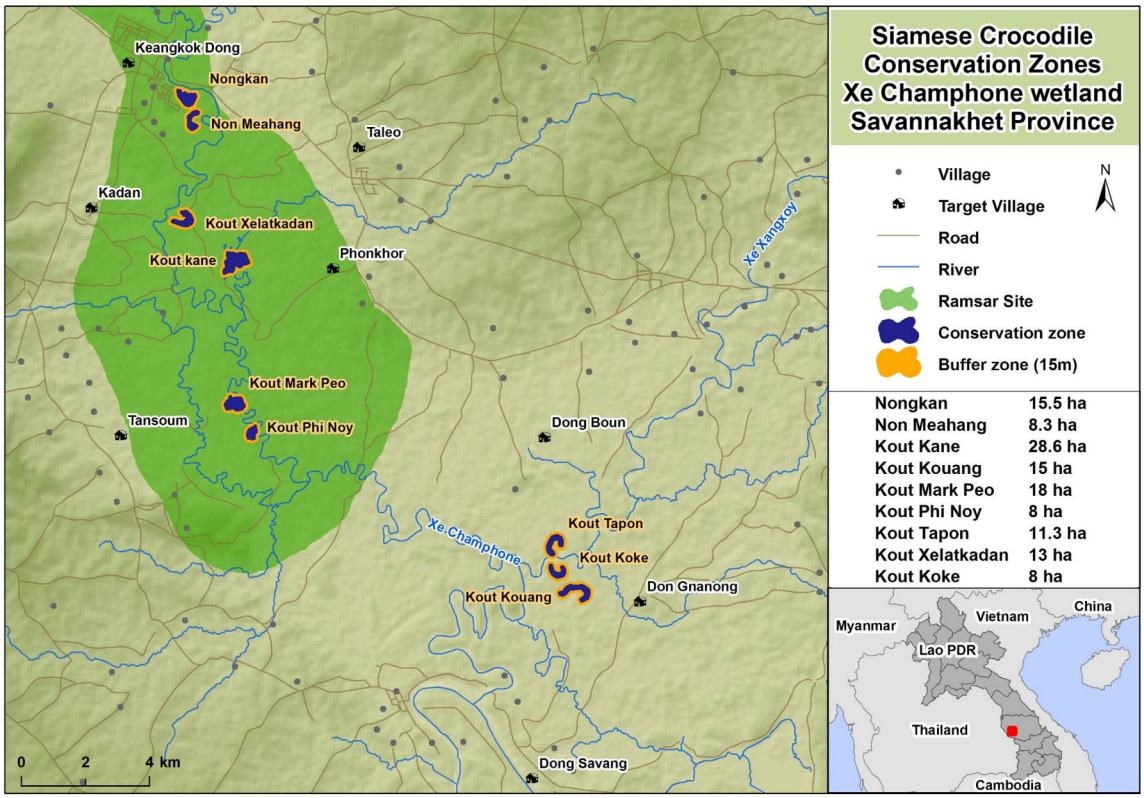

Figure 1. Map showing the location of Crocodile Conservation Areas (CCAs) in Savannakhet Province, Lao PDR. CCAs adjacent to Naonua Village (Beung Hor and Beung Bua) are not shown on this map (see text). Inset at lower right shows location of project area within Lao PDR.

et al., 2006). Many natural wetlands in Savannakhet Province have been either converted to rice-fields or are heavily impacted by rice cultivation (Bezuijen et al., 2006).

With the exception of Beung Hor and Beung Bua, CCAs are located in the floodplain of the Champhone and Xangxoy rivers, tributaries of the Banghiang River, which in turn debouches into the Mekong River. CCAs along the Champhone and Xangxoy rivers are permanent oxbow lakes that experience extreme seasonal fluctuations in water level. During the wet season, oxbow lakes are subject to overbank and backwater flooding from the Champhone and Xangxoy Rivers with floodwaters receding at the onset of the dry season. Barring excessive extraction for irrigation, most oxbow lakes retain water throughout the dry season and undoubtedly serve as important refugia for many aquatic organisms during this period. Mats of floating vegetation are found on most oxbow lakes, often precluding access by boat and making fishing difficult. Although the floristic composition of these mats remains poorly known, vegetation consists of various grasses, ferns, shrubs, and even small trees rooted in a base of floating organic matter. Observations made during our previous work (Platt, 2012) suggest floating mats are dynamic systems that appear to rise and fall in accordance with fluctuating water levels.

Crocodile Conservation Areas within the Champhone River floodplain are currently encompassed within the boundaries of the Xe Champhone Ramsar Site (Fig. 2). The existing Ramsar Site comprises 12,400 ha of natural and anthropogenic wetlands, agricultural ecosystems, scrublands, and forest, and includes two core areas totaling 2,550 ha (IUCN, 2011). Siamese crocodiles are

considered a focal keystone species within the Ramsar Site and critical crocodile habitat was taken into consideration when designating the core areas (IUCN, 2011). A proposed expansion to encompass the Nong Louang Wetland Complex would increase the area encompassed by the Ramsar site to 45,000 ha and include Crocodile Conservation Areas along the Xangxoy River (Fig. 2). Additional information on biodiversity of the Xe Champhone Ramsar Site is available in Timmins (2013). A management plan is currently being prepared for the Ramsar Site by IUCN.

Survey Methodology

We visited CCAs from 4-11 January 2018 and conducted open-ended, semi-structured interviews (Martin, 1995; Gilchrist et al., 2005) with former VCCT cadres and other knowledgeable persons (e.g., farmers dwelling adjacent to wetlands, fishers, and snail collectors). Such individuals are typically an excellent source of information regarding the local occurrence of wildlife, especially charismatic fauna, and culturally or economically important species (Fogerty, 2001). In accordance with the format of a semi-structured interview, we asked participants a series of closed– and open–ended questions that included standard questions prepared in advance and others that arose during the course of the interview. We guided the discussion, but the direction and scope of each interview was allowed to follow the participants' train of thought (Huntington 1998). In practice, semi-directed interviews are more of an informal conversation than a typical question and answer session, and rather than rigidly adhering to a set of prepared questions, the interview provides an opportunity for collecting and discussing unsolicited and often unanticipated information (Huntington, 2000; Gilchrist et al., 2005).

We conducted interviews of groups ranging in size from 2 to 11 people; these groups consisted primarily of men, reflecting the composition of former VCCTs, although some women dwelling near crocodile habitat were also questioned. We began each interview session by explaining the objectives of our survey and then questioned participants about recent crocodile sightings (location and approximate date), estimated TL of any crocodiles observed, perceptions of abundance, and potential threats (e.g., illegal killing, incidental take in fishing gear). Of particular interest was evidence of recent crocodile nesting activity (nests and eggshells), descriptions of nest sites and habitat, and observations of hatchlings and small juveniles (described as "gecko-sized" crocodiles during interviews). At several sites we accompanied informants to locations where nest mounds were reported and examined nesting habitat. Additionally, we queried informants about traditional ecological knowledge (*sensu* Berkes et al., 2000) and local animist beliefs concerning crocodiles. Given the difficulty that even scientifically-trained investigators experience in estimating the TL of crocodilians (Magnusson, 1983), we used caution when interpreting the size estimates given by our informants, particularly for large adult crocodiles. Moreover, rural Lao villagers often describe body size in terms of girth rather than length and estimates of girth must then be converted to

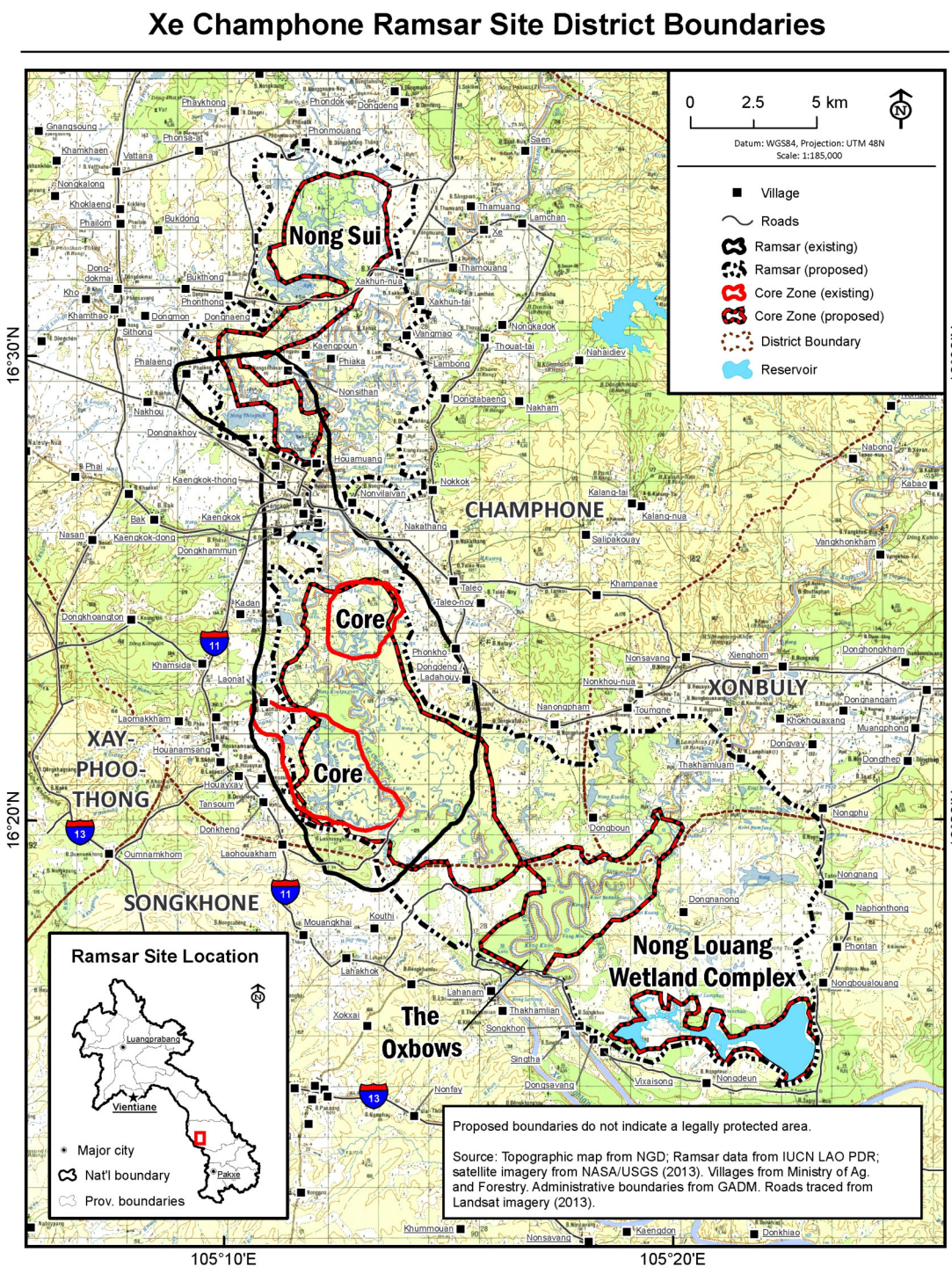

Figure 2. Existing boundaries, core areas, and proposed expansion of Xe Champhone Ramsar Site in Savannakhet Province, Lao PDR. Inset shows location of Ramsar Site within Lao PDR.

TL, introducing another possible error into the estimates (see also Platt, 2012). Our interviews were conducted by two native-Lao speakers also fluent in English (Oudomxay Thongsavath and Chanthone Phothitay), translated into English, and transcribed by SGP. Interview transcripts are contained in field notes are archived in the Campbell Museum, Clemson University, Clemson, South Carolina, USA.

Site Assessments

Below we provide a brief physical description of each CCA and then summarize our findings for each site. CCAs are arranged alphabetically. Asterisks denote CCA's where crocodile reproduction was reported during interviews. Place names follow Hedemark et al. (2009) and are largely in accordance with topographical maps issued by Service Geographique d'Etat.

Beung Hor and Beung Bua

In contrast to most CCAs, Beung Hor and Beung Bua are non-alluvial wet depressions near Naonua Village in Xiabouli District. Beung Hor (= "Lotus Lake") is an open lake surrounded by forest immediately north of Naonua. Beung Bua is an expansive marsh to the south of Naonua and surrounded by small terraced rice-fields. Dense stands of *Eleocharis* spp. are found in shallow water and rooted emergents (e.g., *Nymphaea* and *Nelumbo*) occur in deeper water. Several isolated patches of dense scrub occur some distance (>100m) from the shoreline of Beung Hor where crocodile nesting was documented in previous years (Platt, 2012). These nests however, contained only non-viable eggs, most likely owing to the lack of a resident male. Suggestions to translocate a male crocodile to the site were rebuffed by villagers who feared the action would bode ill for their community by angering the spirits associated with the wetland. Neither Beung Hor nor Beung Bua is located in a riverine floodplain and water levels are maintained solely by rainfall. According to Baird (2001), crocodiles, birds, and other animals are protected by especially strong animistic religious beliefs that include proscriptions against harvesting wildlife or destroying habitat.

We visited Naonua on 11 January 2018 and accompanied two former VCCT cadres (including a 61-year-old individual considered a "Crocodile Whisperer" reputedly able to communicate directly with spirits protecting the crocodiles) to Beung Hor and Beung Bua. According to these informants, only a single large female crocodile dwells in Beung Bua and Beung Hor, often moving between the two wetlands. Most recently the female moved from Beung Bua to Beung Hor where she is now in residence. We hiked the shoreline of Beung Hor, hoping to observe the crocodile and searching for tracks, drag marks, and other sign. Although we failed to observe the crocodile, fresh crocodile sign was abundant and included 1) dragmarks and body-form impressions in moist soil along the lake margin, and 2) three clusters of fresh crocodile dung. The average diameter of the dung was about 35 mm, suggesting it was deposited by a large adult. Interestingly, most of the body-form impression and dung occurred in close proximity to old campfires. According to our accompanying informants, local villagers visit the lake and hoping to view the crocodile, construct small fires and burn hair-covered pigskin and roast chicken carcasses. Odors emanating from the fire are said to attract the crocodile which is then fed the cooked items. This positive reinforcement has been so effective that merely igniting a small fire along the shoreline is now sufficient to lure the crocodile into view. The resident crocodile is also said to relish fresh watermelon. Although infrequently observed, frugivory has been reported among many species of crocodilians (Platt et al., 2013).

Kout Kaen*

Kout Kaen was formerly a meandering, isolated oxbow in the Champhone River floodplain. The main channel reportedly contained water (1.5 to 2.0 m deep) throughout the year, although the adjacent wetlands were dry by late May or early June (Hedemark et al., 2009). During the wet season this area is deeply inundated (> 3 m) by overflow from the Champhone River. An earthen dam approximately 1.6 km long was constructed in 2011 with the dual objective of

creating crocodile habitat and supplying irrigation water for area farmers during the dry season. The dam altered the hydrology of Kout Kaen and adjacent wetlands by making the main channel considerably deeper, retaining water in the adjacent wetlands throughout much of the year, and creating an open water body extending 2.4 km upstream from the earthen dam. Segments of the dam collapsed during the wet season in 2011 and again in 2012. Repairs were undertaken in both years, but following withdrawal of donor support annual upkeep was terminated. Villagers continue to repair the dam after floodwaters subside every year. Extensive floating mats supporting grass, ferns, shrubs, and small trees occur in Kout Kaen, along with water hyacinth and patches of *Mimosa pigra*; the latter is especially abundant in low-lying areas of the floodplain around Kout Kaen. Dense aquatic vegetation precludes the use of nets by fishermen in this lake. Shoreline vegetation is dominated by floodplain bamboo forest and scrub interspersed with numerous small rice-fields.

We conducted a reconnaissance of Kout Kaen on 8 January 2018 with three former VCCT cadre. We also interviewed farmers (n = 12) tending rice paddies around the wetland. During our visit we found a 30 m section of the dam had been washed out during the 2017 wet season and not yet repaired. Additionally, we noted rice farmers were encroaching on the crocodile conservation zone previously established around Kout Kaen. Water was being pumped from Kout Kaen to irrigate adjacent rice fields, although farmers assured us the lake retains water (ca. 1.5 to 2.0 m deep) through the dry season, albeit at reduced levels.
According to our informants, crocodiles are present throughout the year in Kout Kaen. Encounters are frequent with most sightings occurring under flooded conditions in the late wet season. The most recent sighting occurred in December 2017 when about 10 small juveniles were observed in company of a large adult. Several of the juveniles were reportedly perched on the dorsum of the adult. A subadult crocodile was observed scavenging dead fish discarded by a fisherman in shallow water along the bank. Scavenging behavior has rarely been reported for Siamese crocodiles (see also Sam et al., 2015). Two to three crocodiles are thought to be permanent residents of the oxbow, including an adult with a TL > 3.0 m. Nesting has not been reported, although the presence of an adult in company with small juveniles suggests reproduction is occurring at Kout Kaen. The extensive and heavily vegetated floating mats seemingly offer excellent nesting habitat for crocodiles.

Kout Kouang and Kout Koke*

Kout Kouang and Kout Koke are oxbow lakes in the floodplain of the Xangxoy River linked by a shallow slough which retains water for most of the dry season. Given this linkage, we treat Kout Kouang and Kout Koke as a single site in this report. These lakes are considered the most remote and least disturbed of all CCAs. During the wet season, floodwaters from the Xangxoy River completely inundate both lakes and much of the adjacent floodplain. Both oxbow lakes are characterized by a matrix of open water interspersed among large floating peat mats supporting grasses, ferns, shrubs, and small trees. Water is extracted from Kout Kouang by three families from Dongsavan Village for dry season rice cultivation. According to villagers, irrigation water is not extracted from Kout Koke which has a depth of at least 3.0 m at the end of the dry season. Limited fishing occurs in both lakes. Kout Kouang is surrounded by bamboo thickets, secondary forest, and open rice fields. The vegetation surrounding Kout Koke is less disturbed; rice fields are absent, the banks are covered in dense clumps of timber bamboo, and the secondary forest contains several remnant dipterocarp trees of an impressive size. A concrete dam

impounded a slough was constructed near Ban Dongyanong in 2011 to provide irrigation water for dry season farming thereby reducing pressure on Kout Kouang.

We surveyed Kouang and Kout Koke on 10 January 2018 with a group of former VCCT members from Ban Dongyanong. The irrigation dam constructed in 2011 has apparently been successful in reducing pumping from Kout Kouang. We noted extensive clearance in the vicinity of the dam and according to our informants, 30-40 families are now farming near the village. We found no dry season rice cultivation adjacent to Kout Kouang. Crocodiles are observed frequently and throughout the year at both Kout Kouang and Kout Koke suggesting the existence of a resident population. Local villagers regard Kout Koke as particularly suitable crocodile habitat. Informants from Ban Dongyanong and Ban Dongboun stated that during the dry season, crocodiles frequently emigrate from Kout Tapong to Kout Koke. Evidence of crocodile reproduction (nests with eggs and observations of small juveniles) was reported during multiple years at Kout Kouang and Kout Koke (Table 2). Likewise, nests with eggs were found during previous work at this site (Bezuijen et al., 2006, 2013; Platt et al. 2014a, 2014b). Two oxbow lakes (Kout Payoun Doung and Nong Or) peripheral to Kout Kouang but not encompassed within the conservation area also reportedly harbor crocodiles.

Kout Mark Peo*

The Kout Mark Peo Crocodile Conservation Area is a complex of wetlands consisting of Kout Mark Peo, Kout Pinoy, and Phai Cheo Reservoir in the Champhone River floodplain. Kout Mark Peo and Pinoy are oxbow lakes adjacent to the main river channel, subject to overbank flooding during the annual wet season, and contain water (depth > 2.0 m) throughout the year. Extensive mats of floating vegetation cover the surface of both oxbow lakes and dense woodland and floodplain bamboo forest occur along the shoreline. Phai Cheo Reservoir was constructed during the mid-1980s to provide irrigation water for rice-fields near Tansoum Village (Platt, 2012). The reservoir is approximately 3,200 ha, although the surface area varies depending on season; backwater flooding from the Champhone River can substantially increase the area of the

Table 2: Summary of Siamese crocodile nesting activity at Kout Kouang and Kout Koke (2016 and 2017). Information provided by former VCCT cadre.

Year	Observations
2016	Three nests found on floating mats of vegetation in July contained clutches of 16, 26, and 36 eggs. Eventual fate of these nests is unknown.
2017	Small crocodiles (about 10) observed in company of a large adult in September. Informant observed crocodiles at night with in the aid of a head-lamp. Group was swimming in a flooded rice field immediately adjacent to Kout Kouang.

reservoir and inundation of peripheral wetlands and rice-fields is common. Phai Cheo Reservoir is characterized by a mosaic of open water, interspersed with dense stands of emergent aquatic vegetation, and floating peat mats supporting grass, shrubs, and trees. Numerous woody snags occur in the reservoir. The shoreline is dominated by floodplain bamboo forest, scrub, and thickets of

Mimosa pigra, with seasonal rice-fields hewn from the scrub in some areas. Phai Cheo Reservoir serves as a major source of irrigation water during the dry season. Construction of a concrete-lined aqueduct that is currently underway will no doubt increase the dry season off-take of water.

Fishing with monofilament nets and traps occurs throughout the wetland, but owing to dense vegetation and floating mats, most such activity is confined to open water in Phai Cheo Reservoir. Other aquatic resources harvested by villagers include apple snails, frogs, and large sedges (*Scirpus* sp.), the latter being used to manufacture mats. Aquatic resources are harvested by villagers from Tansoum and at least one other community. Siamese crocodiles and large wading birds are the focal species in a nascent ecotourism project underway in Tansoum. A total of 65 head-started crocodiles were released into Phai Cheo Reservoir near Tansoum in 2013 and 2014.

We visited Tansoum and Kout Mark Peo Crocodile Conservation Area on 5 and 6 January 2018. Two concrete grow-out pens were constructed in Tansoum (one on the grounds of the village monastery, the other adjacent to the school) during 2013 and 2014 for rearing young crocodiles as part of the head-starting program (Platt et al., 2014b). Each grow-out pen (4 m wide × 8 m long × 1.1 m high) is subdivided into four rearing chambers (2.0 m wide × 4.0 m long × 1.1 m high) containing a shallow pool and land surface for basking, and topped by a hinged covering of heavy gauge chain-link mesh welded to a metal frame (Platt et al., 2014b). During our visit we inspected both grow-out pens. One pen (adjacent to school) is empty, the chain-link mesh covering having been damaged by village urchins, but otherwise intact. The second pen (on monastery grounds) is operational and contains four subadult crocodiles (TL ranging from ca. 130 to 150 cm). According to the village headman, these crocodiles (almost certainly head-started individuals) were removed from the reservoir after closely approaching people on numerous occasions, possibly having learned to associate humans with food during the head-starting process. The water is clean and the crocodiles appear in excellent physical condition, being fed (2-3 times/week) an appropriate diet of locally collected fish, crabs, eels, and apple snails. Tourists are shown the crocodiles during visits to Tansoum. The headman stated the crocodiles will eventually be returned to the wild, although the particulars were ambiguous. That said, the unstated rationale for keeping the four crocodiles appears at least in part, to meet the perceived expectations of visiting eco-tourists.

Our interviews of former VCCT cadre and rank-and-file villagers at Tansoum strongly suggest that survival of the head-started juveniles released in 2013-14 has been high and moreover, a robust crocodile population now inhabits the Kout Mark Peo Wetland Complex. In contrast to earlier years (e.g., Platt, 2012; Platt et al., 2014a), villagers now regard crocodiles as common, especially in Phai Cheo Reservoir where encounters are reportedly frequent. Several interviewees stated that crocodiles are now “everywhere” in the reservoir. Most importantly, villagers appear enthusiastic about the recovering crocodile population and if funding becomes available, wish to continue participating in conservation efforts. Villagers in Tansoum seem to view crocodiles as a unique resource that sets their village apart from others. Furthermore, the village leadership recognizes the potential role crocodiles could play in developing village-based ecotourism.

As previously recognized (Platt et al., 2014a), the Kout Mark Peo wetlands are an important site for crocodile reproduction and hence play a vital role in the continued viability of the Xe Champhone metapopulation. According to

interviewees, 1-2 crocodile nests are opportunistically found each year by villagers, and at least some of these nests have successfully produced hatchlings (Table 3). As in other crocodile populations (Grigg and Kirshner 2015), the principal causes of nest loss are not unexpectedly flooding and predation. Our informants considered rats (*Rattus* spp.) and monitor lizards (*Varanus* sp.) to be the most important predators of crocodile eggs.

Kout Tapong

Kout Tapong is an oxbow located in the floodplain of the Xangxoy River and as such, is not encompassed within the current boundaries of the Xe Champhone Ramsar Site. The surface of Kout Tapong is covered by a floating mat supporting mostly grasses with scattered shrubs with some *Mimosa pigra*. The oxbow is surrounded by degraded dipterocarp forest interspersed with rice-fields. Farmers living around the oxbow extract water for irrigation; water depth is about 1.0 m by the end of the dry season. Fishing activity at Kout Tapong is minimal owing to the extensive floating mat and shallow water.

We visited Kout Tapong on 9 January 2018 accompanied by former VCCT members from Dongboun Village. During our visit we also interviewed 11 farmers dwelling in temporary dry season agricultural encampments bordering the wetland. One farmer reported finding the fresh trackway of a crocodile during either September or October 2017. The trackway exited Kout Tapong and entered the Xangxoy River. The overwhelming consensus of former VCCT cadres and local farmers is that crocodiles are present in Kout Tapong during the late wet season (August through late September). As

Table 3: Summary of recent (2015-17) Siamese crocodile nesting activity at Kout Mark Peo Wetland Complex near Tansoum. Based on information provided by former Village Crocodile Conservation Team cadre.

Year	Nesting activity
2015	Single nest found in June. Eggshells were found scattered about the nest mound several months later, although it was unclear if the clutch had been destroyed by predators or successfully hatched. A group of small juveniles were subsequently observed by fishermen in same area, suggesting the latter.
2016	Eggshells found scattered around a nest constructed among grass on a floating mat; the nest appeared to have been opened by rats (*Rattus* sp.) which consumed the eggs. Badly decomposed eggs were recovered from a second nest mound also constructed on a floating mat; this clutch was lost when nest was inundated during a rapid water level rise in the wet season.
2017	Nest mound containing decomposing eggs found in late July, presumably lost to flooding that occurred in late June or early July. Eggs in a second nest constructed among grass and low shrubs (< 1.0 high) on a floating mat appeared to have successfully hatched.

water levels recede, crocodiles leave Kout Tapong and move across the Xangxoy River to Kout Koke. Our informants were unaware of crocodile nesting activity at Kout Tapong.

Kout Xelat Kadan*

Kout Xelat Kadan is an oxbow lake in the Champhone River floodplain. Much of the lake is covered by floating peat mats supporting ferns, grasses, shrubs, and small trees. Extensive mats of water hyacinth are also present. Relatively little fishing is conducted in Xelat Kadan because the dense aquatic vegetation precludes the use of monofilament nets. Native vegetation has been cleared from one bank of the oxbow and the land converted to rice-fields. However, the opposite bank remains forested, and reportedly protected against further encroachment by village-level restrictions. Local farmers pump water from Xelat Kadan during the dry season to irrigate rice-fields and in the past this off-take severely threatened the biological integrity of the site (Hedemark et al., 2009). Village-level regulations were enacted in 2011 to prevent a complete dry season drawdown (Platt, 2012), although it remains unclear if farmers continue to adhere to these guidelines. Resident farmers stated that at least 2.0 m of water remain in Xelat Kadan at the end of the dry season. That said, the expansion of dry season rice production on adjacent lands could result in water extraction becoming a serious conservation issue in the near future.

We visited Kout Xelat Kadan on 7 January 2018 in company of a group of former VCCT cadres. According to our informants, one to three adult crocodiles are said to be resident in the oxbow. The most recent observations occurred in November and December 2017, and January 2018. Head-slapping and bellowing were heard in June 2017. In 2016, villagers found a recently constructed nest mound on a floating mat of vegetation in Xelat Kadan. Although the mound remained unopened and the presence of eggs was never confirmed, five small juveniles were observed the following year, possibly offspring from this nest. Xelat Kadan is located in close proximity to Kout Kaen and cadres stated that crocodiles frequently move between these wetlands.

Non Maehang

Nong Maehang consists of a complex of several interconnected oxbow lakes in the Champhone River floodplain adjacent to Kengkok on the outskirts of Champhone. Despite frequent manual removal, water hyacinths now occlude much of the surface and little open water remains in Nong Maehang. What effect these invasive plants have on crocodiles remains unknown. Extensive floating grass- and sedge-covered mats are also present in the oxbow and used by villagers as goat pasture. The land surrounding Nong Maehang has been largely denuded of forest cover and in many places rice fields extend to the water's edge. Water is extracted for dry season rice cultivation, although Nong Maehang apparently retains water throughout the dry season.

We visited Nong Maehang on 4 January 2018 and interviewed three former VCCT cadres together with several farmers who live adjacent to the wetland. According to these informants, crocodiles are observed in Nong Maehang during most years, usually in September. The most recent observations occurred in September 2017 when two subadult crocodiles (TL ca. 150 cm) were repeatedly seen over a period of four days. During this period, the crocodiles were said to have taken 10 domestic Muscovy Ducks at a farmhouse on the banks of Nong Maehang. Additionally, a woman collecting apple snails encountered a large adult (TL ca. 300 cm) in the Champhone River near Nong Maehang in May 2017, and "three or four" juveniles (TL ca. 60-70 cm) were observed by a fishermen "about three years ago" (circa 2014) in Nong Maehang. Our informants were unaware of any crocodile nesting activity in Nong Maehang. Information gleaned during interviews together with our previous observations

(Platt, 2012; Platt et al., 2014a), suggest that Nong Maehang is primarily used by crocodiles during the late wet season.

Summary of site assessments

According to information provided during interviews, at least some crocodiles are present in every CCA, although population estimates vary widely and some sites are inhabited only seasonally. Most importantly, our informants reported evidence of crocodile reproduction (eggshells, nests with eggs, and observations of small juveniles often in company of much larger adults) at four of the seven (57%) wetlands (Kout Kaen, Kout Kouang – Koke, Kout Mark Peo, and Kout Xelat Kadan). Two other CCAs (Kout Tapong and Non Maehang) where nesting was not reported appear to serve as wet season foraging habitat for crocodiles. Furthermore, the release of 65 head-started crocodiles in 2013-14 appears to have successfully augmented the small existing population in Kout Mark Peo. Most likely, the reintroduced crocodiles (now subadults measuring approximately 150 cm TL) will disperse into neighboring wetlands using the Champhone River as an immigration corridor. A single large adult crocodile remains at Beung Hor. Because males are absent, reproduction can no longer occur at Beung Hor. Given the reluctance of villagers to permit the release of a male crocodile at Beung Hor owing to entrenched animist beliefs, the site is of minimal conservation significance.

Threat assessment

For the most part, crocodiles in the CCAs are relatively safe and face few direct threats. However, some seemingly innocuous practices (e.g., water extraction for irrigation) could pose a threat to the long-term survival of crocodiles in the Xe Champhone Ramsar Site. Below we provide a prioritized list of potential threats, assess the risk posed by each threat, and suggest measures to mitigate any danger to crocodiles.

- **Water extraction** – removal of water for irrigating rice-fields during the dry season is probably the single greatest threat to wetland integrity, and hence the long-term viability of crocodile populations in the Xe Champhone Ramsar Site. At present sufficient water appears to remain in the wetlands we visited during the dry season to support resident crocodile populations. However, this favorable situation could change as dry season rice cultivation increases with concordant demands for irrigation water. In the past, water management agreements were designed in collaboration with local villages to insure sufficient water remained in wetlands hosting crocodiles. These regulations will also safeguard local fisheries.

- **Incidental take by fishers** – certain fishing gear (particularly monofilament gill nets) represent a potential threat to crocodiles. Although we are unaware of any recent mortality, in past years crocodiles have become entangled in gill nets and drowned (Platt, 2012). Given the relatively small mesh size of most nets deployed in area wetlands, smaller crocodiles (juveniles and subadults) are the ones most at risk. Well-designed fisheries regulations can dramatically reduce the likelihood of inadvertently capturing crocodiles and at the same time boost local fish stocks. Electro-fishing poses a related threat, but this illegal practice seems frowned upon by most villagers. Our past experience suggests electro-fishers are viewed as “cheaters” who harvest more than their share of the resource. Moreover, electro-fishers often hail from outside of the local community.

- **Illegal killing** – many villagers believe crocodiles are the embodiment of dead ancestors and as such, respect them as living talismans imbued with great powers. These animist beliefs appear widespread in villages within the Xe Champhone Ramsar Site and provide a high degree of de facto protection to crocodiles. Notably, these beliefs seem especially strong in villages adjacent to wetlands where crocodile reproduction was confirmed. Indeed, some have attributed the continued survival of crocodiles in this heavily populated agricultural landscape to the existence of these powerful beliefs. Nonetheless, these beliefs are not universal among rural villagers; illegal killing of crocodiles has occurred in the past (Platt, 2012) and must be guarded against in the future.

Long-term crocodile conservation strategy

Crocodiles inhabiting wetlands in Xe Champhone Ramsar Site were historically part of a larger metapopulation linked by riverine corridors. However, the remaining demes are now much reduced and somewhat isolated, and as such, each is subject to the extinction risks inherent in any small population (Gilpin and Soule, 1986). Our ultimate conservation objective should therefore be not simply to restore multiple small populations, each of tenuous viability, but to restore a viable metapopulation of Siamese crocodiles in the Xe Champhone Ramsar Site that functions in the manner of a source-sink system (Hanski and Simberloff, 1997). To achieve this objective, individual populations must first be recovered to the point of viability. To this end, our future efforts should be focused on those populations with the greatest likelihood of eventually functioning as source populations (e.g., Kout Mark Peo).

Connectivity among wetlands in the Ramsar Site already exists in the form of 1) Champhone and Xangxoy river corridors, and 2) widespread annual wet season flooding that inundates the landscape. Thus, once recovery is achieved, offspring dispersing from these populations can be expected to colonize other wetlands in the Ramsar Site (the expanding inkblot analogy of counterinsurgency warfare theorists; Kaplan, 2013; Mansoor, 2013). In anticipation of future population recovery, increasing emphasis in the near-term should be placed on identifying suitable habitat amenable to protection, and expanding the network of designated CCAs. Particular effort should be made to identify villages that afford protection to crocodiles on the basis of spiritual-religious beliefs. Conservation measures grounded in local belief systems often have a greater likelihood of success than legalistic or economic approaches to resource management (e.g., Platt et al., 2003).

Conservation Recommendations

The following recommendations are designed to achieve the objectives of the long-term conservation strategy as out-lined above:

1. WCS developed a community-based conservation program that proved highly successful in beginning the recovery of Siamese crocodile populations within the area now designated as the Xe Champhone Ramsar Site. Given the initial success of this project coupled with the results of the recent reconnaissance mission, renewed crocodile conservation efforts seem warranted. To this end, we recommend re-engaging at those CCAs most likely to support relatively robust crocodile populations. Without a doubt Kout Mark Peo (Tansoum Village) is the most important CCA in the Ramsar Site. Other high

priority sites include Kout Kaen, Kout Kouang and Kout Koke, and Kout Xelat Kadan. Non Maehang and Kout Tapong are used seasonally by crocodiles and therefore considered less important (but not unimportant) for conservation efforts. Beung Bua and Beung Hor should be removed from consideration as a CCA because 1) these wetlands are not encompassed by the boundaries of the Ramsar Site, 2) the "population" consists of a single female crocodile, and 3) village custodians of the wetland rebuffed proposals to release one or more males, fearing this intervention would bode ill for their community by angering the spirits.

2. Regular population monitoring should be undertaken at each designated CCA. Evidence of crocodile reproduction (e.g., active nests, old nests, hatchlings, eggshells, etc.) appears to be the most effective metric to evaluate population trends (see also Platt et al., 2014a).

3. As in the past, conservation efforts at priority sites should focus on recruiting VCCTs to monitor local crocodile populations, search for nests, assist with egg collection and incubation, and enforce conservation regulations agreed upon by the community.

4. Head-starting young crocodiles for eventual release will be the cornerstone of conservation efforts at priority sites for the foreseeable future. Head-starting is a demonstrated means to quickly boost recovery trajectories of crocodile populations. Head-starting should be conducted in Tansoum and Ban Dongyanong villages. Rearing facilities are available in Tansoum (minor repairs will be required). An additional facility should be constructed in Ban Dongyanong and villagers trained in basic crocodile husbandry. Our past experience indicates that incubating eggs and rearing hatchlings in villages imparts a sense of community ownership in the project (Platt et al., 2014b).

5. Post-release monitoring of future reintroductions with radio telemetry is strongly recommended. Monitoring will determine post-release dispersal and survival of reintroduced crocodiles and provide the scientific rigor necessary to quantify conservation success, evaluate reintroduction protocols, and make changes if necessary (i.e., adaptive management).

6. A GIS-based inventory of potential crocodile habitat for the Xe Champhone Ramsar Site is urgently needed. Additionally, a classification system ranking the suitability of wetlands as crocodile habitat should be developed. These measures are crucial for conservation decision-making and developing management guidelines. Inventory results will also help identify potentially valuable crocodile habitat not currently designated as a CCA (see below).

7. Surveys of other wetlands in the Xe Champhone Ramsar Site likely to harbor crocodiles are warranted. If heretofore unknown populations are identified, the existing community-based conservation program should be expanded to include villages adjacent to wetlands inhabited by crocodiles. Particular effort should be made to identify villages that afford protection to crocodiles on the basis of spiritual-religious beliefs.

8. A conservation-breeding program should be developed at the Lao Zoo to provide offspring for head-starting and release in suitable wetlands

within the Xe Champhone Ramsar Site. Crocodiles selected for the breeding program should be screened to insure the genetic integrity of the breeding population. Additionally, the importation of captive-reared offspring from an on-going captive-breeding program in the United States warrants serious consideration. Releasing these animals will boost numbers and genetically diversify the existing wild population.

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Stay a long while, crocodile: mitigating human-crocodylian conflicts

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Introduction

This paper provides a definition of human wildlife conflict which disaggregates direct impacts of wild animals on humans and vice versa, from conflicts over direct impacts. It summarises current guidance on HCC drawing on the literature on *Crocodilus niloticus*, and current CSG guidance. The second section of this paper explains the aims of the new IUCN Task Force on Human Wildlife Conflict, and proposes that CSG members have much to contribute. With this in mind, the paper outlines a draft outline for a guidance document on HCC, as a basis for discussion between CSG members. The paper concludes with a summary of the discussions on HCC at a breakout meeting convened during the Santa Fe CSG Working Group meeting, and includes a few notes on developments following the conference.

Human wildlife conflicts

Human wildlife conflicts (HWC) emerge from situations where actions by humans or wild animals have negative impacts on the other (Conover 2001). However, it is useful to divide this into two components (Redpath et al. 2015):

1. Human-wildlife interactions: impacts of direct interactions between humans and wildlife;
2. Human-human interactions: conflicts between conservationists and those with other, apparently incompatible goals.

The Crocodile Specialist Group (CSG 2018b) defines human crocodile conflict (HCC) as: 'Any interaction which results in negative effects on human social, economic or cultural life, on conservation of the species or on the environment.' This fits the broad definition of conflict covered by points 1 and 2 above, but the CSG guidance continues as follows: 'However, HCC more commonly refers to interactions between crocodiles and humans where they or their livestock are threatened, injured or killed, or livelihoods are affected.'

For the CSG, as for most conservation initiatives until recently (Pooley et al. 2017), the focus of HCC work has been on direct impacts rather than human-human interactions. This is obvious when looking at the main causes (table 1) given for HCC involving Nile crocodiles (*Crocodilus niloticus*), summarised in table 1.

Table 1: main attributed causes of HCC involving *C. niloticus*

Causes	details
Competition	for fish/food; livelihood implications
Damage to property & structures	fishing nets, earth dams and weirs; economic and social costs

Attacks on humans and domestic animals	Personal trauma; economic and social costs; opportunity costs
Increasing human and croc populations	The latter resulting from conservation efforts
Habitat loss	encroachment, conversion and destruction
Risky behaviour	resulting from traditional beliefs, poverty and lack of resources, ignorance of croc behaviour and biology, alcohol and sometimes general idiocy

The focus on direct impacts is also clear from the management interventions recommended or implemented for Nile crocodiles (the literature as summarised in Pooley 2018), as summarised in tables 2 and 3.

Table 2: Main management interventions applied or recommended by conservation authorities relating to HCC for Nile crocodiles (*C. niloticus*)

Local authorities	Where applied (e.g.s)
Pay compensation	Namibia, Botswana
Provide economic benefits	Zimbabwe
Raise awareness/educate	South Africa
Control problem animals	Botswana, Mozambique, S. Africa

Table 3: Main management interventions or recommendations applied by locals relating to HCC for Nile crocodiles (*C. niloticus*)

Locals	Where applied (e.g.s)
Kill problem animals	Kenya
Use fences / enclosures	Mozambique, Zambia
Report problem to authorities	All countries
Use protective charms	S Africa, Mozambique

Make a noise when entering waterways	Mozambique

Based on these attributed causes, and steps taken to deal with crocodile attacks, the recommended interventions for HCC focus on direct impacts, as summarised in table 4.

Table 4: main recommendations for dealing with HCC involving *C. niloticus*

Recommendation	Rationale
Provide economic benefits & pay compensation	Increase tolerance/offset losses
Lethal control or relocation	Remove problem / impact
Protective structures & safe water points	Separate humans/crocs
Raise awareness	Change behaviour / reduce exposure
Education on ecological role of crocs	Improve tolerance
Keep database of attacks	Identify trends, hotspots, vulnerable demographic, dangerous activities
Survey crocodile populations	Identify potential problem areas, inform better land use planning / zoning
Understand locals' attitudes to crocs	Change behaviour, inform effective mitigation
Control fisheries	Reduce competition / exposure

CSG Guidance on HCC

Current CSG guidance, as it appears on the website, is included in three main places: the 'human-crocodile conflict' page (CSG 2018b), the 'croc attacks page,' (CSG 2018a) and in the *Crocodilian Capacity Building Manual* (CSG 2018c) The human-crocodile conflict page notes two mitigation strategies: harvesting to reduce croc populations (not regarded as a long term solution) and crocodile exclusion enclosures. On the Croc Attacks page, two mitigation strategies are mentioned: problem animal removal or destruction (translocation is not recommended as crocodiles have a strong homing

instinct); and collecting data to better understand what leads to croc attacks and so guide mitigation measures (CrocBITE). The Capacity Building Manual has a section on problem croc control by Alan Woodward, which has sections on:

- Education and awareness raising
- Selective removal of problem crocodilians
- Harvest (killing problem crocs), sometimes with incentives
- Population reduction through general harvesting (includes increasing wariness of crocs).

The *Manual* also has useful sections on 'human/community livelihoods' by Alejandro Larriera, and the 'Ecological value of crocs' by James Nifong.

Draft HCC Guidelines

The second section of this paper gave an overview of the aims of the IUCN Task Force on Human Wildlife Conflict, chaired by Alexandra Zimmermann, and of which I am a member. The aims are: to support the IUCN SSC network and conservation community in addressing human-wildlife conflict, by providing interdisciplinary expert guidance and assistance**.** I used a slide to show the resources on offer on the Task Force website (hwctf 2018). In this part of the paper I went on to outline draft guidelines on HCC, adapted in part from work by IUCN Task Force chair Alexandra Zimmermann.

My proposed guidelines would begin with an introductory section including the following headings:
What is HCC?
About crocodiles (biology and behaviour)
Principles of HCC management
Hotspots and incidence worldwide.

Part One of the guidelines could focus on impacts, providing summary advice for the public and managers, with sections on: avoiding attacks; responding to attacks (generic, basic advice); on how to escape / rescue victims, and first aid and medical treatment.
Part Two of the guidelines could cover the process of managing HCC, focusing on conflicts, with sections on: analysis of HCC including social, cultural and political issues; designing research to assess these issues; ecological assessments; croc movement patterns and feeding behaviour. Other sections could cover:

1. designing HCC interventions, focusing on good project design, and thinking through underlying assumptions;
2. participatory and collaborative approaches, including facilitating stakeholder dialogues, building rapport with communities and working with local politicians;
3. Project implementation, including working with the media, and within national strategies and policies;
4. Monitoring, evaluation and learning;
5. Conflict mitigation approaches (options & resources) from technical solutions (enclosures etc.) and culling or removal to financial instruments, and approaches focussing on human behaviour, culture, wellbeing and livelihoods.

Part 3 of the Guide could include a section on regionally specific HCC resources and contacts, including outreach/educational materials, contacts and details of conservation authorities, and references.

The third and final section of this paper provided a summary of the lunchtime meeting I convened at the Working Group meeting in Santa Fe to discuss ways forward for working on HCC. Some of these have since been put into action, and communications are being maintained and knowledge exchange encouraged through a members-only

Facebook Group on HCC. Some key action points included circulating and commenting on the draft HCC guidance resource presented in this paper, and standardising HCC data collection – with the input of Adam Britton and Brandon Sideleau of CrocBITE. We also resolved to work on translating and collating the questionnaires on HCC devised by myself and translated and circulated to the CSG Caribbean, Central and South America network by Pablo Siroski.

The aim of this paper and discussions during and subsequent to the CSG Working Group Meeting in Santa Fe, Argentina, is to set up and maintain and active community of CSG members working on HCC around the world, and develop resources that will be genuinely useful to CSG members, managers and locals dealing with HCC.

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