Born to be Bad: Agonistic Conflict in Hatchling Saltwater Crocodiles (*Crocodylus porosus*)

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Abstract

Saltwater Crocodiles (*Crocodylus porosus*) are considered one of the least tolerant of conspecifics of all crocodilians. Yet they begin their life living in groups (hatchling crèches) for around two months, suggesting ontogenetic changes in social behaviour may underpin the growing intolerance with increasing age and, or size. In this study, detailed observations on groups of captive *C. porosus* hatchlings, particularly in the first 6 weeks of life, demonstrated they exist with high levels of close contact and little aggression (tolerance). Yet this quiescent existence is interspersed with sporadic periods of agonistic events (signalling intolerance), with highly distinctive behaviours (N= 12), particularly in the morning (0600-0800 h) and evening (1700-2000 h). Of these behaviours, 5 were postures involving no movement, with 2 non-contact movements, and five contact movements that were considered either discrete (stereotypic; N= 4) or graded (not stereotypic; N= 8), based on whether the form or intensity of the display varied. Ontogenetic shifts in agonistic behaviour were quantified by examining 18 groups of hatchlings, 6 groups each at 1, 13 and 40 weeks of age. Agonistic events between hatchlings at 1 week of age varied in intensity (low, medium, high) and involved one (dominant) or both (combat) individuals. Almost all encounters involved actual contact, with a high number resulting in the instigator losing. At 13 and 40 weeks, a more formalized, hierarchal dominance relationship had established, based primarily on aggression-submission interactions. Conflict was high intensity and more frequent, with the subordinate individual fleeing in response to an approach by a dominant animal that often did not make contact. Social hierarchies among hatchling *C. porosus* may well underpin the high variability reported in individual growth rates, while the similarity of agonistic behaviours displayed by hatchlings and adults suggests a 'juvenile structured' pattern of behavioural ontogeny for this species.

Isolation and Characterization of Antimicrobial Peptides from the Leukocytes of the American Alligator (*Alligator mississippiensis*)

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Abstract

We have isolated a 4746 Da peptide (39 amino acids), with antimicrobial activities, from the leukocytes of the American Alligator. The peptide was isolated by acid ultracentrifugation, reverse phase high performance liquid chromatography, and ion mobility chromatography. The mass was determined using matrix-assisted laser desorption ionization. The peptide has a net positive charge (+8), an isoelectric point of 9.5, an arginine and lysine content of 36%, and is extremely amphipathic. Another peptide of 4.9 kDa (43 amino acids) was isolated, and had a net charge of +13 with an arginine and lysine content of 32%. Both of these peptides have several cysteine residues, and show sequence homologies to mammalian b-defensins.

Host-Parasite Interaction of the Order Crocodylia

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It is possible that parasites of crocodilians are highly host specific, the results of a relationship that began over 65 million years ago. Records of parasitism in crocodilians dates back to the early 1800s, distributed among published and unpublished manuscripts, and international parasite catalogs. Previously published checklists of vertebrate or reptilian parasites have included crocodilians, however many did not include all crocodilian species, or all crocodilian parasite species that had been found up to that date. Additionally, various species of crocodilians and their parasites have been through extensive taxonomic evaluation and past sources of data may not have identified host or parasite with its new systematic name. This can be confusing for scientists, researchers, students, or parasitologists and herpetologists interested in crocodilian parasitology. To decrease any ambiguity I have created a crocodilian parasite database to bring an up-to-date document of this particular host-parasite relationship in order to assist those pursuing investigations on the ecological, biological and veterinary significance of crocodilian parasites. In addition I have analyzed parasite distribution among crocodilian taxa, postulating host-parasite evolutionary and ecological interactions.

Crocodilians appear to be parasitized by a diverse array of parasitic phyla and species. Patterns of parasitism among crocodilian species can be described in two categories: generalist and host specialists. Generalists parasitize various crocodilian species, whereas host specialists or host-specific parasites infect a single host species. Overall, there are fewer host specific (~95) than generalist (~310) parasites (categorization did not include unidentified parasite species or parasites only identified to genus). Species-specific parasitism can be attributed to the lack of sympatry among crocodilian species and/or populations (Brooks 1979a,b; Brooks and O'Grady 1989; Huchzermeyer 2003). Many crocodilian species are isolated either due to geographic or anthropogenic barriers (Huchzermeyer 2003). Therefore a parasite must evolve strategies or life cycle patterns that minimizes or removes obstacles posed by vicariant barriers, enabling the capability of the parasitic species to infect multiple hosts throughout a geographic range. Three propositions are suggested that can assist in explaining the evolution of generalist parasites in crocodilian species: host dispersal, similar life-history traits and diets, and broad range of intermediate hosts. In evaluating the association of generalist parasites to geographic region, a pattern emerged reflecting three geographic regions: the Americas (includes North, Central, and South America), Africa, and Indo-Australia. The majority of generalist parasites shared amongst crocodilians within these regions was nematodes from the family Ascarididae.

Crocodilians are parasitized by several parasitic phyla, and are briefly discussed: Acanthocephala: Acanthocephalans infecting crocodilians are under the order Polyacanthorhynchida, represented by one species. *Polyacanthorhynchus rhopalorhynchus* parasitizes the intestines of all members of the genera *Caiman*, *Melanosuchus* and *Paleosuchus*. Although Bush *et al*. (2001) state that only acanthocephalans from the order Polyacanthorhynchida parasitize crocodilians, two other orders are documented to infect crocodilians. *Gorgorhynchoides* sp. from the order Echinorhynchida was found in *Crocodylus acutus* in Mexico, and *Polymorphus mutabilis* in the order Polymorphida was discovered in the small intestine of *Crocodylus rhombifer* in Cuba.

<u>Apicomplexa</u>: Seventeen (17) known species of coccidia (not including unidentified species) from four families under the order Eucoccidiorida parasitize crocodilians worldwide. Route of infection is most parsimoniously explained via horizontal transmission through contaminated water or food (Bush *et al*. 2001; Combes 2001; Huchzermeyer 2003). The majority of these protozoans (13 known species, 4 unidentified species) are from the family Eimeridae infecting 13 crocodilians.

<u>Arthropoda</u>: The order Porocephalida includes a group of parasites commonly known as tongue worms. This order includes the families Subtriquetridae, Sebekidae and Porocephalidae, that contain crocodilian specific parasites. The majority of these described pentastomids are adults found in the lungs and trachea of their host, but some are known to parasitize other parts of the body such as the nasal cavity and intestine.

<u>Nematoda</u>: Nematodes are the second largest group parasitizing crocodilians. Adult nematodes parasitize various organs and tissue in crocodilians, but most documentation of parasitism is described from the stomach. Besides inflammation caused by stomach nematodes (Huchzermeyer 2003) or scarring such as by *Paratrichosoma*, there are no ill effects unless the host is immunocompromised.

<u>Platyhelminthes</u>: Platyhelminthes is the most diverse and largest phylum parasitizing crocodilians, comprising of 5 orders, 16 families (not including one superfamily) and 125 species. Three orders of Platyhelminthes appear to have an ecological and/or evolutionary relationship with crocodilians. Parasites of the order Echinostomida are a diverse intestinal parasitic

group of reptiles that are considered not to be host specific (Bush *et al*. 2001), yet the majority recorded in crocodilians are found in only one crocodilian taxon. Furthermore, most documentation of echinostomes are described from South American caimans, principally in the region of Matto Grosso, Brazil. Plagiorchiida is the second largest order of crocodilian platyhelminthes. Almost all plagiorchiids are described as intestinal parasites, yet few are found in other organs such as *Pseudotelorchis caimanis* discovered near the oviduct of *Caiman yacare*, and *Renivermis crocodyli* from the kidneys of *Crocodylus porosus*. The third largest order of platyhelminthes parasitizing crocodilians is also the most diverse in location of parasitism. Species of the order Strigeidida are documented from the buccal cavities, cloaca, major organs (including the brain), yet majority are found in the intestinal tract. Sarcomastigophora: Trypanosomes have an ancient evolutionary relationship with crocodilians, dating back to the late Cretaceous (Viola *et al*. 2009). Two species have been described from two crocodilians, *Trypanosoma cecili* from *Caiman crocodilus crocodilus*, and *Trypanosoma grayi* from *Crocodylus niloticus*. There are other parasites that have been documented in crocodilians, but considering these phyla are represented only by one parasite and were found in only one host within the crocodilian assemblage, it is hypothesized that these reports are examples of opportunistic parasitism.

It is probable that a unique mutualistic or commensal relationship between crocodilians and their parasites has developed over evolutionary time, allowing them to adapt to changing environments and novel pathogens. However, human conflict, climate change, and habitat loss, pose a threat to this dynamic, resulting in one of two negative outcomes. First, anthropogenic interactions can decrease parasitic prevalence and abundance in a host population by external factors hindering parasitic transmission or killing free-living stages of the parasite (Bush *et al*. 2001). As this may seem beneficial, parasites that are host-specific for crocodilians may have developed a commensal relationship over evolutionary time with their archosaurian host, contributing to crocodilians' ability to eradicate a broad spectrum of invasive pathogens over evolutionary time. Therefore the alteration of a beneficial coevolved dynamic may contribute to crocodilian populations inability to adjust to anthropogenic disturbances or novel pathogens. In contrast, ecosystem perturbations may enhance parasitic prevalence, intensity and abundance of a host population (Combes 2001; Lafferty and Holt 2003; Lafferty and Kuris 2008). Increase stress due to ecosystem disturbance, or accumulation of toxic metals can interfere with hosts' immune function (Sures 2006; Arkoosh *et al*. 2008; Lafferty and Kuris 2008; Rohr *et al*. 2008). Immunosuppression allows viral, bacterial and parasitic infections to proliferate, ultimately causing extreme morbidity, or mortality, of the host. Either alternative illustrates that a disruption in this reptilian-parasite system will have a detrimental outcome for the individual, population, or crocodilian species under investigation, resulting in a negative trophic cascade due to their role as keystone predators.

The purpose of this crocodilian-parasite database is to provide a foundation for future research on crocodilian parasitism. Data extrapolated from this study can be utilized to investigate coevolution and host phylogeny, as well as the role of crocodilian parasites in food webs, and ecosystems, and how external stressors may alter host-parasite dynamics. Previous ecological parasitology studies have linked predator reduction to reduced presence of trophically transmitted parasites, and an increase in other types of parasites of abundant hosts at lower trophic levels (Lafferty and Kuris 1999; Combes 2001; Bush *et al*. 2001; Lafferty *et al*. 2008). Moreover, the fundamental dependence of parasites on both host and environment make them biological indicators of the stability of the environment. Therefore, quantifying parasites of keystone predators, such as crocodilians, may enable analysis of ecosystem function. In a rapidly changing environment (due to climate change, land use practices, and direct exploitation of the environment), knowledge on the significance of crocodiles and their parasites is necessary to propose proper action for conservation and responsible stewardship of their environment.

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The Role of the American Alligator (*Alligator mississippiensis*) and American Crocodile (*Crocodylus acutus*) as Indicators of Ecological Change in Everglades Ecosystems

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Abstract

The system-wide monitoring and assessment plan for the Comprehensive Everglades Restoration Plan identified indicators and established performance measures to monitor system responses and track progress toward meeting restoration goals. The crocodilian indicator uses monitoring parameters (performance measures) that have been shown to be both effective and efficient in tracking trends. The alligator component uses relative density (reported as an encounter rate), body condition, and occupancy rates of alligator holes; the crocodile component uses juvenile growth and hatchling survival. We hypothesize that these parameters are correlated with hydrologic conditions. Alligators and crocodiles are keystone and flagship species to which the public can relate. Additionally, the parameters used to track trends are easy to understand. These relationships are easy to communicate and mean something to managers, decisionmakers, and the public.

Using Critter-Cams to Compare Prey Capture and Success Rates of American Alligators (*Alligator mississippiensis*) from Two Florida Estuaries

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Abstract

Recent advances in video processing and remote imaging have forged a new era in the study of animal behavior. The fast evolving field of Animal Born Imagery utilizes these technological advances to collect video data from the point-of-view (POV) of the animal. This non-intrusive, POV approach offers researchers a novel opportunity to observe and quantify natural, unobstructed patterns in animal behavior. Videographers in National Geographic Remote Imagining (NGRI) department are pioneers within this field and have developed a number of multifaceted video systems, collectively known as Crittercams. In 2010 and 2011, we partnered with NGRI to deploy Critter-cam systems on a wild American Alligators. Here we present findings from analyses of video data retrieved after deploying Critter-cams on 15 adult alligators (~90 hours of video) within the Merritt Island National Wildlife Refuge Cape Canaveral and Guana Lake Wildlife Management Area Florida. Video data collected has provided an intimate view of the foraging habits and behaviors alligator's exhibit in Florida estuaries. A common pattern revealed was that alligators primarily forage along the benthic substrate and often forage fully submerged. Additionally, the frequency of foraging events far surpassed our initial expectations and demonstrates alligators frequently attempt capture of smaller prey items.

Assessment of Nest Attendance of the American Alligator (*Alligator mississippiensis*) Using a Modified Motion-Sensitive Camera Trap

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Abstract

Previous data from our laboratory has shown that motion-sensitive, infrared (IR)-based camera traps are not reliable for the quantitative capture of images of alligators. Therefore, we designed a camera trigger mechanism which included an electrical circuit board, coupled to a camera, which powered an IR Led light. The circuit was designed to turn the IR LED on for 2 seconds every 5 minutes. In the field, the IR LED was positioned such that the light was pointing directly into the IR detector of the camera. Therefore, the cameras were stimulated to take photos every five minutes, throughout the entire nesting period. The data revealed that alligators attend and maintain their nests more frequently during the first four to seven days after egg deposition, and then attendance is decreased. Nest attendance increased toward the end of the incubation period as eggs neared the hatching stage. In addition, 87.3% of alligator nest attendance occurred during the nighttime hours, between 8 pm and 6 am. In addition to nest visitation data, we also gained information concerning nest predation.

Ultrasound, a Powerful Tool for Health Assessment in Crocodilians

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Abstract

Because of their peculiar and poorly understood anatomy, physiology and behavior, assessing the health of crocodilian is not straightforward. Observation, palpation, auscultation and blood analysis are not sufficient to form an accurate picture of the health status. Ultrasound allows visualization and recognition of size, shape and appearance of the visceral organs. This study presents in detail the ultrasonic approach and appearance of the various internal organs. Images are correlated with post mortem gross and microscopic appearance. The authors conclude that ultrasound is a useful and powerful tool that should be part of the health assessment of crocodilian individuals or populations.

Sexual Maturity in Male American Alligators in Southwest Louisiana

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Abstract

Very little is known about the attainment of puberty in reptiles. In the American Alligator (*Alligator mississippiensis*) males are assumed to be sexually mature at about 1.8 m total length, but it is not clear at what size they produce spermatozoa and mate successfully. The minimum size for sexual maturity is thought to be around 1.8 m, but social hierarchy favors breeding by male alligators over 2.2 m. We decided to re-examine this question by studying plasma testosterone levels in blood samples from a large sample of alligators (around 1500) collected in every month of the year and ranging in size from approximately 61 cm (2') to 360 cm (11.5'). In addition a number of testicular samples were taken for histology from alligators (close to, and equal to 1.8 m TL) during the mating season to assess degree of spermatogenesis and testicular maturation. Plasma testosterone values ranged from 0.05 ng/ml to 115.41 ng/ml. All size classes of alligators exhibited a seasonal cycle in testosterone levels, but the concentrations were size-dependent: the larger the alligator the higher the testosterone. In all alligators sampled testosterone reached a peak in the breeding season (March-May). Mean testosterone in the largest size class during breeding was 75 ng/ml whereas in the smallest size class peak testosterone was less than 3 ng/ml. The smallest size class (61-89 cm) showed an additional rise in testosterone in late summer. The attainment of sexual maturity in alligators appears to be closely associated with growth, and is a gradual process lasting several years. Sexually immature alligators show a seasonal pattern of testosterone secretion similar to that of adults, but the values are significantly lower.

Head-Starting as a Tool for Crocodile Conservation

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Abstract

The critically endangered *Crocodylus mindorensis* is a freshwater species endemic to the Philippines. The wild population size is <250 adult individuals. In 1999 a conservation project started in the municipality of San Mariano, Isabela, targeting a very small population. Hatchling mortality is high, preventing a quick population recovery. Most suitable hatchling habitat has been converted into agricultural lands, consequently the crocodiles nest next to fast flowing rivers and hatchlings are swept downstream. In 2005 we started a head-start program to increase hatchling survival. We collect hatchlings from the wild and rear them in captivity for 18-24 months before releasing them back into the wild. Between 2007 and 2011, 162 hatchlings were collected and raised in a rearing station. 91 juveniles have been released into the wild and 39 individuals are at this time still at the rearing station, to be released in 2012 and 2013. Rearing strategies are evaluated and improved and growth rates are increasing. Survival rates of crocodiles in the rearing station are high and crocodiles adapt to wild conditions. Survival of released crocodiles is difficult to assess but indications are that survival rates after one year in the wild are at least 50%.

Influence of Natural and Artificial Light on Broad-Snouted Caiman (*Caiman latirostris*)

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Introduction

The UV spectrum irradiation on the planet surface is regulated by temporal, geographical and meteorological factors. Ultraviolet radiation (UVR) plays an important role to determinate the natural balance of the planet and also is an important factor in many physiological functions of organisms. The UVR are part of the electromagnetic spectrum and appear in three frequencies: A, B and C. (Diffey 1991). These radiations act in many biological processes. Ectothermic vertebrates use sunlight as a thermoregulatory mechanism (Johnson *et al*. 2008). Under natural conditions in the wild, many reptiles synthesize their own vitamin D3 from the UV component of sunlight. Certain wavelengths in the UV spectrum (290-320 nm) react with sterols (provitamina D) in the skin to produce pre-vitamin D3 that then is transformed in cholecalciferol, a previous form of the vitamin D3. This is in turn converted into vitamin D3 itself, based on a process which also depends upon heat. Reptiles get a high proportion of their vitamin D3 requirement from their food. Vitamin D3 is controlling the absorption, transport and deposit of calcium, and in minor proportion, phosphorus.

Objectives

To determine the effects of different time periods of natural and artificial light UV exposure on the growth of *Caiman latirostris* yearlings.

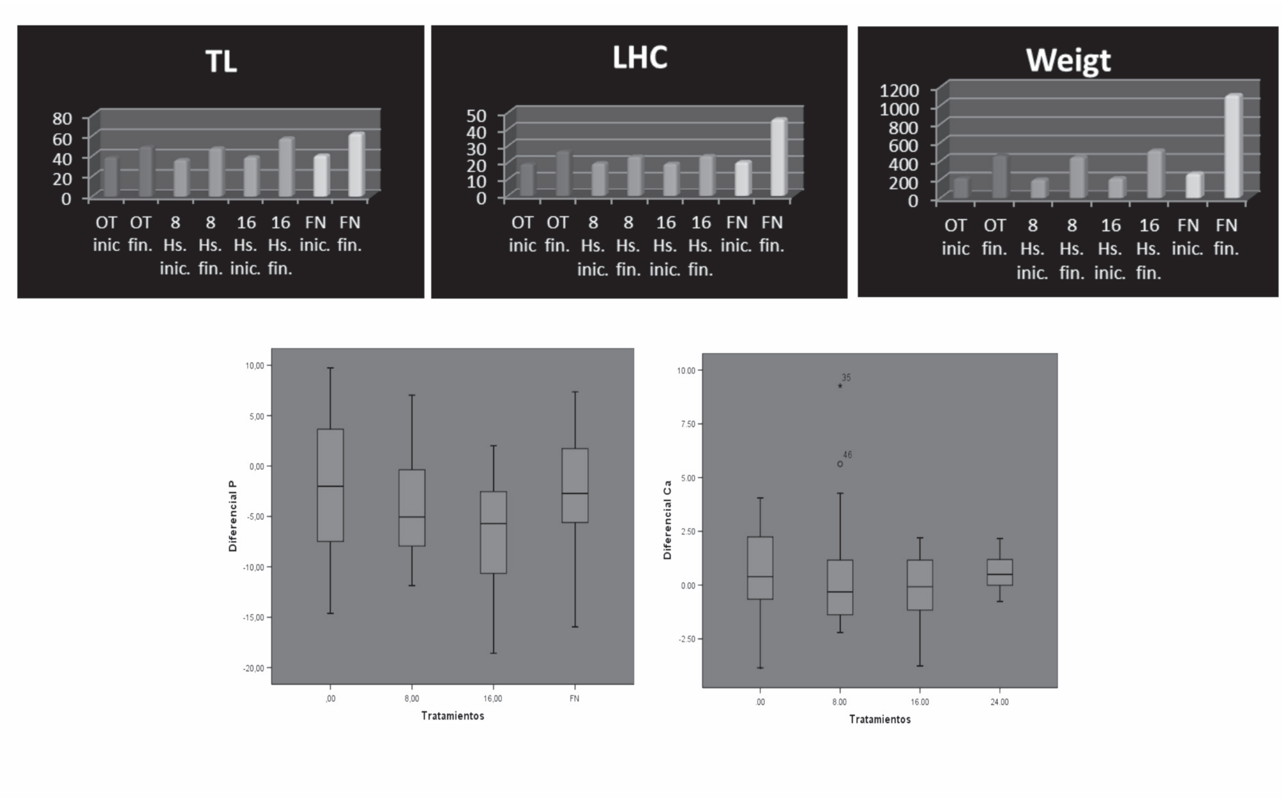
To determine the effects of different time periods of natural and artificial light UV exposure on calcium and phosphorus plasmatic concentrations in *Caiman latirostris* yearlings.

Materials and Methods

We used 96 4-month-old Broad-snouted Caiman (*Caiman latirostris*) from 4 wild-harvested and artifically incubated clutches. The animals were randomly separated in four duplicate groups of 12 individuals. Each animal was identified with a tag numbered (Natl. Band and Tag Co., Newport, Kentucky). At the beginning and at the end of the study, total length (TL, precision 0.5 cm), weight (BM, precision 0.1 g) and snout-vent length (SVL) were measured. Blood samples were taken from the spinal vein (Tourn *et al*. 1994; Zippel *et al*. 2003) using 25G x 5/8" needle and 5 ml syringe, and transferred to serum tubes. Animals were exposed to 4 treatments: total darkness (td); 8 hours of UVR (uva-uvb) (8 h); 16 hours of UVR (uva-uvb) (16 h); normal photoperiod of natural light (NP), in plastic pens over 90 days. Temperature was 31 ± 2°C, recorded with HOBO Dates Logger (ONSET Computer Corp., Pocasset, MA, USA). Food was offered three times a week ad libitum with a mixture of 60% minced chicken heads and 40% dry pellets. Cleanup was done the following day. Calcium (Ca) concentrations were determined by colorimetric method; and phosphorous (P) concentrations were determined by direct UV method. Changes in BM, SVL, TL, Ca and P concentrations were analyzed by ANOVA, with UV-exposed period and clutch as grouping variable.

Results

The animals exposed to FN showed the highest increases in TL, LHC and BM ($p<0.05$). There was no significant difference in calcium concentration in any of the treatments. However, P concentration in animals exposed to UV radiation (16 hours) ($p<0.05$) dropped significantly. Finally, the effect of nest-of-origin was considered, and resulted in significant differences for all initial differential variables, in growth and Ca and P concentrations. All animals survived in all treatments and none presented external injuries.



Discussion

The results showed that a FN would be ideal for the captive organisms to increase their growth. As it was demonstrated by Ferguson *et al*. (2005) in *Anolis lineotopus merope* and *Anolis sagrei*, and Karsten *et al*. (2009) *Furcifer pardalis*, sun radiation would provide the necessary quantity and quality of UV radiation so as the organisms effectively develop life cycles. Calcium concentrations (final results), were similar to those reported for *C. latirostris* but they were higher in sub-adult animals exposed to FN (Barboza *et al*. 2008) and in other crocodile species captive in farms (*C. niloticus*, Watson 1990; *A. mississippiensis*, Schoeb *et al*. 2002; *C. porosus,* Millan *et al*. 1997; *C. moreletii*, Sigler 1991; *T. schlegelii*, Siruntawineti and Ratanakorn 1994; *C. yacare*, Barboza 2006). In the same species and other captive crocodile species the P values were higher than in Barboza *et al*. (2008). *C. porosus*, Millan *et al*. 1997; *C. moreletii*, Sigler 1991; *T. schlegelii*; Siruntawineti and Ratanakorn 1994; *C. yacare*, Barboza *et al*. 2006). But in others, the concentrations were higher than in our own research. (*A. mississippiensis*, Schoeb *et al*. 2002). The organisms may have been under a stressful situation influencing on the final serum values of P and Ca, and a as result provoking a remarkable fall. Regardless the kind of diet, temperature, etc. provided, the absorption of minerals will not be optimum (Brames 2007) if UV radiation is not properly provided. Taking into account that all the animals are exposed to natural UV radiation, and based on the results of the research, the concentration values could show some deficiency or difference in the minerals included in the diet (Coppo 2001). Based on the importance of UV radiation for phosphorous metabolism, we can assume that the periods of exposure were not enough for presenting a change in such metabolism. However, it was enough to notice a less growth in the animals that could be related to alteration in the absorption of minerals related by an inappropriate synthesis of vitamin D.

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Deficiencies of Crocodilian Husbandry in Large Head-Starting Facilities and a Proposal for an Alternative Concept

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Abstract

The supplementation of depleted populations of wild crocodilians is frequently initiated in large head-starting facilities or breeding centres based on a farming model of husbandry. This paper discusses conditioning of crocodilians in zoos and farms and how it may produce crocodiles that have limited survival skills when released. This conditioning process is exacerbated by deficiencies in pen design. A typical nursery pen in a large (unnamed) breeding centre is discussed, and how its deficiencies contribute to high mortality rates. An alternative proposal based on developing local or villager based aquaculture skills is proposed as a solution to producing crocodiles that are better equipped for survival after release, and as part of a solution to addressing significant socio-economic pressures on freshwater ecosystems for which crocodiles are frequently an icon.

Introduction

This paper is based on personal experience of crocodile husbandry in both zoos and farms, with some observation of various methods of head-starting crocodilians in South and Southeast Asia. Head-starting is the most commonly used method for supplementing or reinforcing depleted populations of wild crocodilians. It is often perceived as a simple process but in reality is very complex, often including species-specific issues associated with husbandry of endangered crocodilians, while habitat health and complicated socio-economic pressures impact on survival rates of released animals. Various methods have evolved to overcome these issues including paying villagers to guard hatchlings in village ponds, guarding natural nests and collecting hatchlings for captive rearing, or incubation of eggs from wild or captive breeders with captive rearing and release of suitably sized juveniles. This latter process usually occurs in larger rearing facilities or breeding centres similar to crocodile farms and is the subject of this paper.

Chabreck *et al*. (1998) state that survival rates of farm-raised or hand-reared animals is generally lower than wild conspecifics, and refer to Blake and Loveridge (1975) who report very low survival rates of farm-raised Nile Crocodile hatchlings. In Zimbabwe Ferguson (1998) found that released farm-raised Nile Crocodiles had better body condition than wild crocodiles at the time of release but this situation deteriorated to inferior condition after their first non-growing season, and that survival of released crocodiles was lower than wild conspecifics. Released farmed American Alligators in Louisiana at 1.2 m total length are subject to higher rates of cannibalism than wild conspecifics, and are easier to approach during recapture (Chabreck *et al*. 1998).

This is not to say that supplementation programs are failures. The IUCN Re-Introduction Specialist Group rates supplementation programs on a scale from highly successful to successful, partially successful, or as failure. Soorae (2008) notes that van Weerd and van der Ploeg (2008) had 'partial success' in supplementing Philippines Crocodiles in Luzon, and Rao states 'partial success' in supplementing wild Gharial in the Chambal River in India. Elsey and Kinler (2011) found that a reintroduction program using farm raised alligators in Louisiana is 'highly successful' with released animals attaining adulthood and reproducing.

Thus only one of these programs is rated as highly successful. As a result of these issues and personal experience this paper is critical of large head-starting facilities based on a farming model, and proposes a simpler alternative particularly suitable for lesser developed countries. In this context the term 'husbandry' includes both management (eg daily routines, diet, and collecting data) and pen design, while conditioning is defined as simple learning. The relevant issues discussed below are divided into three sections:

1. Conditioning of captive-reared hatchling and juvenile stock

   The behaviour of various species of wild crocodilians is noted by their similarities rather than by their differences. However their degree of tolerance, as shown in Figure 1 by Lang (1987) varies, and has a marked influence on captive husbandry. In captivity tolerance also varies between individuals and is subject to conditioning. To give an example with supposedly intolerant saltwater crocodiles (*C. porosus*): In a farming situation it is common practice for one keeper to be responsible for the daily routines of a given group of hatchlings. Hatchlings become conditioned to a particular routine and a particular keeper, to the extent that they may be imprinted on him/her and will even hand feed if the keeper is silly enough to attempt it. On one occasion a change of keeper caused a reduction in food consumption by up to a third despite the same standard feeding routine being adhered to.

Some of these hatchlings were maintained as future breeding stock. At a total length of approximately 3 m they were fed from a wheel barrow (trolley), and on two occasions some of them crawled under the barrow and stopped within a few centimetres of the keeper's feet waiting for their dinner. They showed no aggression and simply waited to be fed.

This conditioning process can vary between zoos and crocodile farms because they have different objectives which impact on behaviour and thus have direct implications for conservation programs that use captive crocodilians to supplement wild populations.

Zoos are contemporary Noah's Arks. Zoo-keepers value their animals by an educational value for presentation and interpretative initiatives. Their husbandry is often anthropomorphic, by caring for the welfare and maintenance of individual animals in perpetuity. This type of care and conditioning rarely promotes those survival skills that are necessary for release into the wild and if animals are raised in isolation they may even lack social skills for attaining compatibility which (at least) in captivity is a prerequisite of reproduction.

Crocodile farmers place an economic value on their stock as skins and meat, and develop intensive systems of husbandry based on productivity, caring for the welfare of animals in homogeneous groups rather than as individuals. It is also possible that hatchlings are imprinted on keepers (note example above), and that the accepted method of rearing young stock in densities that promote tolerance by removing personal space to limit physical damage to skins is reinforcing a form of conditioning that is not conducive to survival if released into the wild. Some large head start facilities also use stocking density to condition crocodiles for tolerance as a means of avoiding injuries that may preclude survival after release. It is possible that this form of management is counter-productive to the survival of all individuals in the cohort. It is also probable that crocodiles raised in groups of homogeneous size are unable to recognise dominant animals as a threat.

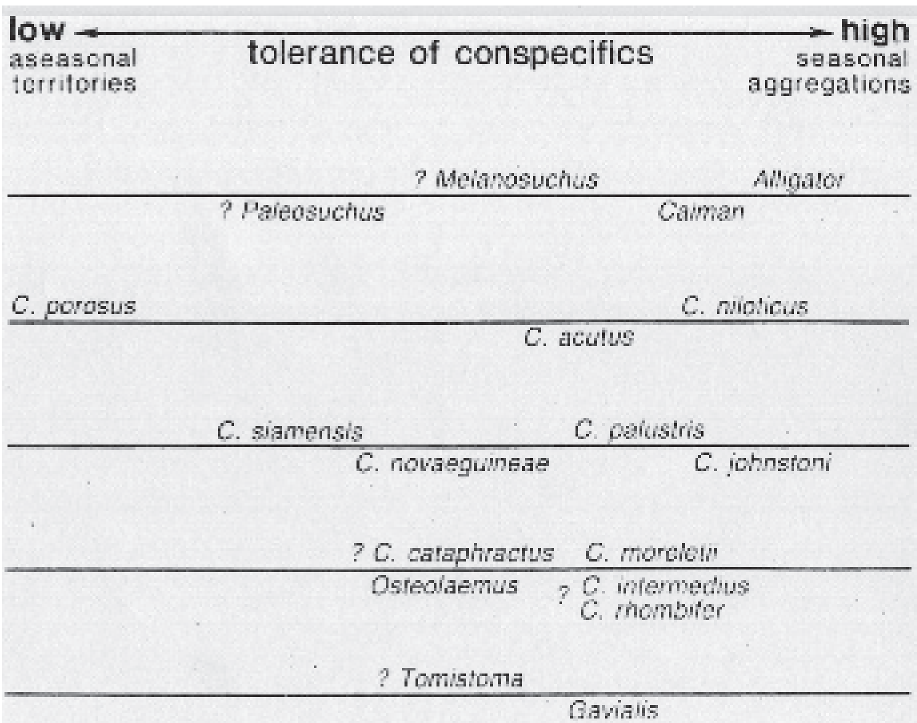

Figure 1. Tolerance of conspecifics by wild crocodilians (Lang 1987). This diagram may be used as a basis for husbandry, and in particular for designing breeding pens.

Many wild hatchling and juvenile crocodilians (eg Gharials) cohabit with their parents and parental guidance may be important. The author has observed a captive juvenile Saltwater Crocodile (NB: intolerant species) coexisting with its parents in a relatively small 1:1 breeding pen, while Brueggen (2002) has observed parental guidance with captive Siamese Crocodiles over a two-year period and suggests that we should not be dismissive of poorly understood crocodilian behaviour because they are often considered as primitive. Care should be taken in citing these observations because captive behaviour may not necessarily be the same as that in the wild.

2. Inherent problems in the design of head start facilities

Figure 2 shows a typical hatchery pen in a large head start facility or breeding centre. Presentation is very good - it is clean and generally well maintained. This is an indication of keeper's respect for their livestock.

On crocodile farms the contribution of a pen to overall productivity is measured by what comes out of it. Unfortunately in this pen (Fig. 2) fungal infection causes mortality rates up to 60% or more so it contributes very little to productivity. On small closed-cycle crocodile farms mortality rates should be approximately 5%, and marginally higher on large farms. The very high mortality rates in this pen are rationalised by management by comparing them to wild mortality rates. This approach can not be justified. Problems with livestock in captivity are managerial problems and should not be attributed to deficiencies of animals, particularly when critically endangered species of high biological value are concerned. Below are some aspects of the pen in Figure 2 which detract from high productivity:

- Wild hatchlings use micro-climate (eg mud and rocks) to thermoregulate but this building does not collect or store heat. This can easily be achieved by orientating the building to early morning sun, installing a plastic cover on the roof, using building materials with high thermal mass, passing pond refill water through small diameter poly pipe, using a 10-20% flush rather than feed and drop 100% of pond water (water exchange), and incorporating a heat box into hideboards. Most of these ideas could be incorporated after construction.
- Porous building materials such as timber and sand substrate may harbour high pathogen loads. Initially river sand may be relatively clean but over time pathogen loads will increase. Therefore it is important that sand used in artificial incubation or as a pen substrate is changed regularly. Timber is porous and also harbours pathogens, particularly when used for hide boards. In this instance it should at least be painted.
- Controls for all plumbing should be external to the pen to reduce disturbance.

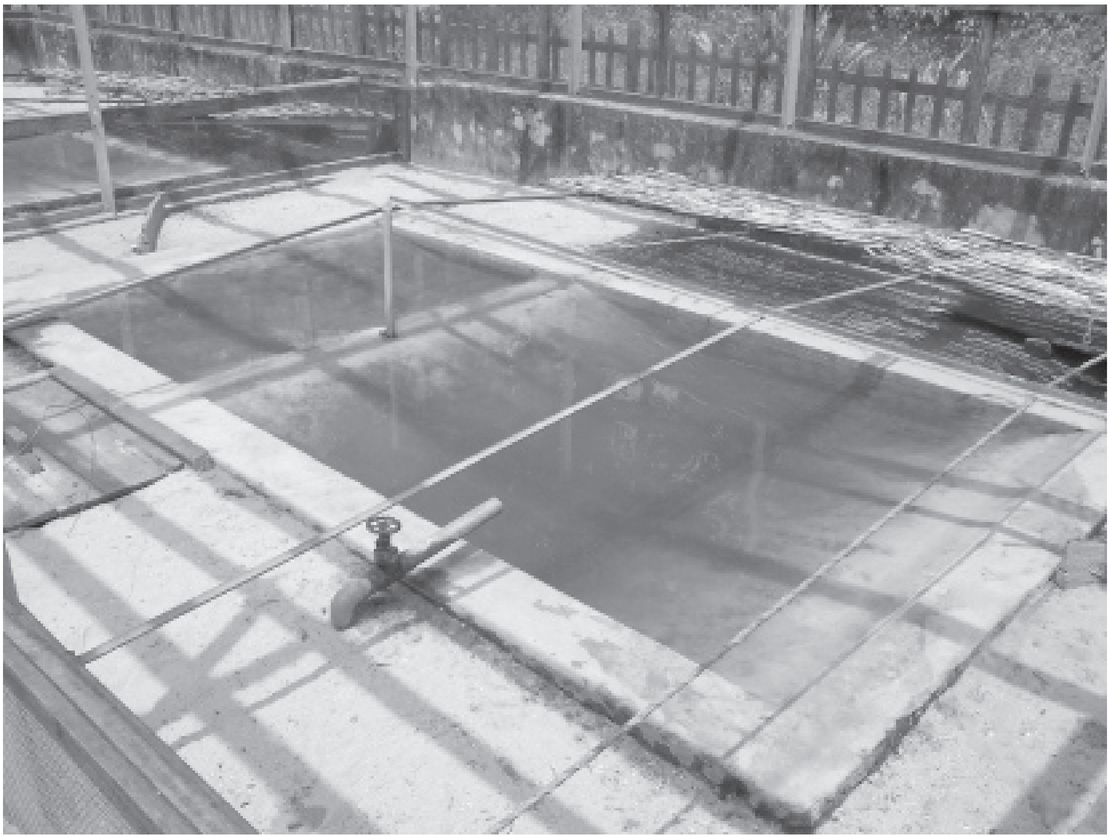

Figure 2. An example of a hatchery/nursery pen built in 2006 in a head-start facility which uses husbandry techniques based on farm husbandry. This system of husbandry and poor pen design creates a number of issues including a compromised ability to thermoregulate and increased pathogen loads in porous building materials such as sand substrate and timber in walls and hideboards. Hatchling mortality frequently reaches 60% or more. Note: Crocodiles have been edited out to maintain confidentiality.

High mortality rates in the pen shown in Figure 2 are the result of a sequence of events during incubation and predetermined by pen design. This sequence is as follows:

- Possible infection during incubation. Captive and wild-laid eggs are collected and incubated in artificial sand banks.

Thomas (2001) found that *Fusarium* and *Paecilimyces* spp. of fungi occur naturally in sand and nesting media in Queensland (Australia) and caused the most fatalities of embryos and hatchlings. These fungi may invade eggs and infect embryos during incubation. Infected hatchlings may die three or more months later (Thomas 2004) from massive growth of fungus in the liver. It is possible that hatchlings at this facility are infected during incubation but pathogen loads in sand banks have not been assessed.

- Falling temperature favours fungi and causes thermal stress. Crocodilian immune systems and metabolism are temperature dependent. Crocodiles have a preferred body temperature of 32 to 33°C (Huchzermeyer 2003) while Johnson *et al*. in 1976 (cited in Mayer 1995) found that Saltwater and Australian Freshwater Crocodiles have a Preferred Optimum Temperature Zone (POTZ) of 25.5 to 35°C. Hatchlings released into this pen have a compromised ability to thermoregulate as winter water temperatures eventually fall to 17°C. Thermal stress and the onset of cooler weather favour fungus - for example *Fusarium* and *Paecilimyces* spp. of fungi prefer an optimal temperature range of 26°C (most favourable temperature) to 31°C (Thomas 2004).
- Failure to initiate feeding. In this pen the majority of hatchlings fail to initiate feeding and are subjected to further stress from force feeding - it should be noted that the stimulus to feed in many species is poorly understood but this pen does not provide flexibility for trialling various methods.
- Depletion of antibodies and onset of infection. Within a few weeks an inability to thermoregulate coincides with a depletion of yolk sack antibodies and more stress caused by individual medication for fungal infection.
- Pathogen loads. Because of pathogen loads in sand and timber any crocodiles washed or medicated for disease such as fungus are reinfected.

Further to the above the importance of collecting and collating data in any facility can not be over emphasised - 'you can't manage what you don't measure'. Professional managers collate appropriate data to reveal deficiencies in husbandry and act accordingly, while those managers that do not are generally ignorant of deficiencies in their husbandry. They are letting the crocodiles manage the facility and rationalise their deficiencies in husbandry by blaming their animals.

3. General logistics associated with large head start facilities

Crocodiles are an icon or keystone species for freshwater ecosystems so many head starting initiatives in lesser developed countries are part of a far more complex program addressing socio-economic issues related to the wider community and particularly overfishing by local villagers. Generally larger facilities or breeding centres create larger problems, including: -

- They are cost and labour intensive compared to other methods.
- They need considerably more managerial skill.
- In the absence of refrigeration the practicalities of supplying large quantities of fresh fish are difficult. This may contribute to dietary disease which can have long-term implications.
- They may be seen as competing with local people for limited supplies of protein.
- They tend to release large numbers of juveniles of similar size class in a limited area. This may exceed habitat carrying capacity, distort the social balance of wild populations, and may increase cannibalism.
- They may discharge relatively higher loads of nutrients.

Developing an Alternative Concept

In an attempt to solve some of the issues noted above an alternative system of head starting crocodilians is proposed (see Fig. 3). This proposal has evolved from a Captive Breeding Project at Pagasa Farms, which is a joint venture with the Protected Areas and Wildlife Bureau (PAWB-DENR) and the Silliman University in Dumaguete City, Philippines. Three male and four female (3:4) Philippine Crocodiles (*C. mindorensis*) are accommodated in a small fenced lagoon approximately 28 m x 39 m. No supplementary food has been offered since March 2007 and production to date is three juveniles and 36 hatchlings.

This concept has been further enhanced as a conceptual drawing in Figure 3. There are four components shown in the diagram:

Pond A. This pond is a dedicated aquaculture pond for the production of food for adult crocodiles and their progeny. It is important that selected species should be endemic to local freshwater ecosystems so captive-bred crocodiles will become conditioned to hunting them. Undesirable species should be avoided (eg *Tilapia* and *Gambusia* spp.) because there will be escapees. Excess production can be used by local villagers and thus reduce socio-economic pressure on freshwater ecosystems. At the very least this pond could be used as a training facility with the objective of establishing local aquaculture. Ponds are separated by a series of suitably sized mesh screens as drop gates to preclude the movement of larger fish and crocodiles. Pond B is included to demonstrate that any number of ponds may be incorporated to specifically cultivate small fish, crustaceans, endangered species of turtles, juvenile crocodiles of mixed ages, or future breeding stock. Pond C is a crocodile breeding pond/s. The design should be species and climate specific, and must include ample shallow water for hatchling security and feeding. Pond D is a trap to assist in measuring production, and assessing suitability of stock for release - eg biosecurity issues such as deformity and disease.

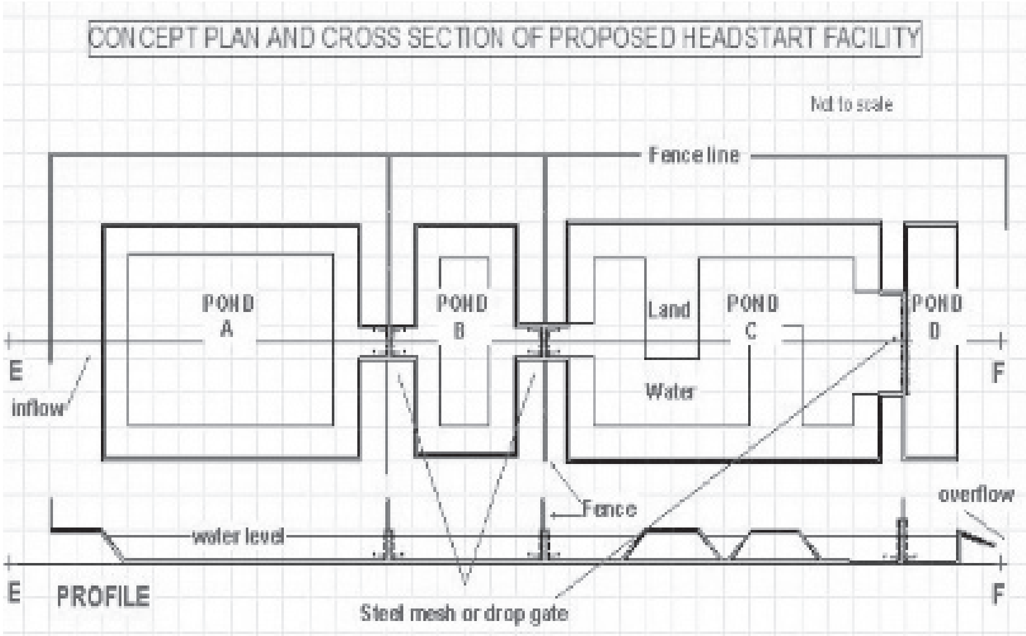

Figure 3. Concept plan for an alternative head-starting facility or breeding centre. In lesser developed countries this system should produce protein for local villagers and contribute to reducing socio-economic pressure on freshwater ecosystems. Juvenile crocodiles for release should have improved survival skills.

Some general notes:

- The site must have impermeable soil. Initially the only machinery needed is a pump to lift river water.
- The site should be in proximity of good juvenile crocodile habitat.
- A food pyramid should be established in each pond. It will need to be created over a period of months starting with hay or straw, followed by endemic aquatic plants and smaller prey species of fish.
- This facility will remove nutrients and not dump excessive nutrient loads.
- As a breeding centre this system should be cost-effective and could possibly be used for integrated farming.
- Local people should be involved in planning and construction. This will help develop a sense of ownership and is a prerequisite to their commitment and success of the project.
- A number of smaller breeding groups are considered superior to one large breeding centre. This avoids release of large numbers of juveniles in one place, will be beneficial if site fidelity is an issue, improves biosecurity, and will reach a broader community of people.

Where there is life there is death but at least in this proposed system mortality will occur as part of the process of learning survival techniques rather than poor husbandry such as that discussed above.

Conclusions

Releasing a few crocodiles is simple, but head-starting crocodilians deals with many complex issues that vary between species, catchments and cultures. It is often a necessary and expensive bandaid which remains important not only because of the intrinsic value of the species, but because they are iconic to many freshwater ecosystems that are among the most threatened in the world. These threats will be exacerbated by increasing socio-economic pressures and climatic influences in forthcoming decades.

Conservationists are competing for limited sponsorship that demands success. They need to think in business terms such as ‘do we have a product to sell?’ and develop a sense of cost benefit analysis by asking ‘is crocodile conservation getting a bang for its buck?’ Essentially this involves a professional approach to improving productivity. Managers of head-start facilities should be able to define what they want to produce as an objective, and then set about adopting a system that will achieve their objectives.

One of the areas in which this may occur is by taking the next step in pen design. It is no longer satisfactory to design a pen with a surrounding fence and central pond and then add a substrate. Certainly crocodile husbandry is part of a young emerging industry but this type of thinking is a relic of the past. Professional farmers have realised the benefit of experience and consultation, and the use of collated data to reveal deficiencies in husbandry and lateral thinking to provide solutions. Conservationists that are head-starting crocodiles of high biological value need to follow suite.

Acknowledgements

I would like to thank Vic Mercado and Michael Cruz of Pagasa Farms for their assistance.

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Research Influences a Conservation Breeding Program - The European StudBook for African Dwarf Crocodiles (*Osteolaemus tetraspis*)

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Abstract

The endangered African Dwarf Crocodile (*Osteolaemus tetraspis*) is distributed in central and Western Africa. Traditionally two subspecies are described (*tetraspis* and *osborni*), which can be distinguished only on minor morphological differences. Recent molecular studies suggest the existence of thee allopatric taxa which occur in the Congo Basin, the Ogooue Basin and West Africa. African Dwarf Crocodiles are regularly kept in European Zoos. The collection is managed by a European Studbook coordinated at Leipzig Zoo since 2007. For conservation reasons it is important to know the provenance of these animals registered. Hence in cooperation with the University of Leipzig a genetic screening of the studbook population was conducted The results confirmed the existence of at least three different lineages of *Osteolaemus* as recently postulated, but also revealed hints of the existence of a fourth evolutionary lineage. A majority of the animals originate from the Ogooue basin. Unfortunately also hybrids between these lineages were detected, all of them bred in zoological institutions. This case study shows the importance to reflect results from research in breeding programs to ensure the survival of genetically viable and taxonomically pure *ex-situ* populations.

Community Conservation Initiative by the Sepik Wetland Management Initiative

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Abstract

The Sepik Wetland Management Initiative (SWMI) was set up locally as a consultative Community-based organisation (CBO) in Ambunti, East Sepik Province, Papua New Guinea. As a result of the changing dynamics of the Sepik River System, the remnant prime nesting habitats for the two PNG crocodile species, *Crocodylus porosus* and *C. novaeguineae*, are being afforded the protection to enhance wild population sustainability. SWMI's vision is to ensure that both crocodiles are used as flagship species to augment better appreciation and community based management/conservation for the wetlands as a result of sustainable harvest of eggs, juveniles and skins. Sustainable use in this area provides all important school fees, fuel for river transport and other goods and services otherwise unobtainable. SWMI, in collaboration with MHL, also noted that terrestrial and aquatic wetlands are managed holistically, providing a benchmark for conservation of inland wetlands. Through consultation with government and other local stakeholders the ultimate aim is to influence the legislative process to improve socio-economic development in these very remote communities as well as improving the management of the entire Upper Sepik Wetlands system.

Gastric Nematode Community of *Crocodylus acutus*, *Crocodylus moreletii* and *Caiman crocodilus chiapsius* from Southern Mexico

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Abstract

Stomach nematode prevalence, intensity, abundance and species richness were evaluated and identified in *Caiman crocodilus chiapsius* (n= 3), *Crocodylus moreletii* (n= 12), and *C. acutus* (n= 1) from Lagoon Illusion (Tabasco), Rio Hondo (Chetumal) and and Puerta de Arisa and Boca del Cielo (Chiapas), in Mexico. Stomach nematodes were collected via stomach flushing during night surveys, stunned with hot alcohol, and put in labeled vials of 70% glycerin alcohol. Three nematode genera from the family Ascarididae made up the parasite species richness: *Dujardinascaris helicina* (n= 26), larval *Dujardinascaris* sp. (n= 6), *Terranova lanceolata* (n= 1), *Brevimulticaecum* sp. (n= 1). *Dujardinascaris helicina* was the most abundant stomach nematode species found, particularly in *C. moreletii*. This study records the first host record of *Terranova lanceolata* parasitizing *C. moreletii*, and documents the first parasite ever recorded in *C. c. chiapsius*. The parasite discovered in *C. c. chiapsius* was identified to the genus *Brevimulticaecum*, but was not identified to species as it has characteristics unique from its congenerics parasitizing other crocodilians. To date, we consider this specimen as a new species, but more parasite specimens are needed for verification.

Statistical analysis of parasitic prevalence (44%), intensity (2.1) and abundance (2.1) was performed via a Wilcox t-test. Comparative analysis of *C. moreletii* between urban and non-urban populations in Mexico illustrates a significant difference in nematode intensity (P= 0.016). A plausible explanation for the difference in nematode intensity among urban and non-urban areas could be crocodilian immunosuppression via anthropogenic impacts such as heavy metal pollution. Production, maturation, and function of monocytes, and the humoral immune response of vertebrates are hindered upon the disruption of internal heavy metal homeostasis (Rink and Gabriel 2000; Crossgrove and Zheng 2004; Kannan *et al*. 2006). This increases the susceptibility of an individual or population to pathogens that normally would be exacerbated by the immune system. Therefore, if a pollutant is interfering with the normal biological functionality of a crocodilian, parasites are more likely to succeed as a result of host immunosuppression (Morley *et al*. 2006). With future data, we intend to provide more information on how heavy metal pollution affects the crocodilian-nematode dynamic in order to analyze crocodilian health and response to anthropogenic impacts.

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Effects of *In Vivo* Exposure to Roundup® on the Immune System of *Caiman latirostris*

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Introduction

Caiman latirostris (Broad-snouted Caiman) is one of the two species of crocodilian in Argentina, distributed in the Provinces of Chaco, Corrientes, Formosa, Salta, Santa Fe, Entre Rios, Misiones, Santiago del Estero and Jujuy (Larriera *et al*. 2008). Since the introduction of transgenic soybean varieties resistant to glyphosate, this herbicide became the most widely used agrochemical in Argentina (Aizen *et al*. 2009). Among the possible effects that can result from *in vivo* exposure to glyphosate in *C. latirostris* are alterations on the immune system (IS), considering it is particularly vulnerable to xenobiotics.

Some of the alterations observed in the IS include variations in the number and type of leukocytes, resulting in reduced activity to trigger a defence against foreign organisms or removing damaged cells (Banerjee *et al*. 1996). Another possible disturbance is at the level of production of proteins that play an immune function, the most important fraction comprising antibodies (Song *et al*. 2000). The present study aimed to evaluate the effect of Roundup® (RU, glyphosate-based formulation) on some parameters of the IS and growth of *C. latirostris*.

Materials and Methods

Seventy-two 20-day-old caimans from three clutches collected by ProyectoYacaré (Gob. Santa Fe/MUPCN) during ranching activities were used. The animals of each clutch (N= 24) were randomly divided into three different groups: a control group (NC) and two treatments exposed to different concentrations of Roundup® (RU), each one with two replicates of 12 animals. Animals were measured (snout-vent length; SVL) and weighed at the beginning and end of the experiment to determine changes in size in each experimental group (final-initial values).

Exposure was performed during two months in plastic pens, tilted to offer a dry area and the other containing RU water solution. Water renewal was done every three days and RU concentration progressively reduced so that the concentration ranges were: treatment 1 (RU1): 11 mg/l (initial concentration) to 2.6 mg/l (final concentration) and treatment 2 (RU2): 21 mg/l (initial) to 5 mg/l (final), taking into account glyphosate metabolism in water, previously established by HPLC. At the end of the experiment, blood samples were taken from all specimens (Olson *et al*. 1977).

The total leukocyte count was performed in a Neubauer chamber. For the differential white blood cells (WBC) count, two smears were performed per animal, fixed with ethanol, and then stained with May Grunwald (50%) - Giemsa (10%) solutions. We determined the heterophil/lymphocyte index (H/L) as a marker of stress produced by exposure. From each treatment a subgroup of animals were used to determine plasma total protein (TP) and protein electrophoresis (Díaz Portillo *et al*. 1996).

Results

Results showed that total WBC count was lower in the groups of animals exposed to RU. Animals exposed to RU2 had the lowest leukocyte count (20282.61 ± 2302.65 WBC/mm^3), the difference being statistically significant with respect to the NC (29142.87 ± 1882.92 WBC/mm^3, $p= 0.011$) and to RU1 (28937.50 ± 1949.85 WBC/mm^3, $p= 0.01$; Fig. 1a). The results showed an increase in the population of heterophils in animals exposed to RU2 (28.24 ± 1.54%) compared with NC (19.46 ± 1.68%, $p<0.01$; Fig. 1b). In the case of lymphocytes, monocytes and eosinophils no significant differences were observed between experimental groups ($p>0.016$).

The H/L index showed a significant increase in RU1 (0.47 ± 0.05) and RU2 (0.45 ± 0.03) compared to the NC (0.30 ± 0.03, $p<0.016$), but no significant difference was observed between RU1 and RU2 ($p>0.016$; Fig. 1c). The total protein concentration was significantly higher in RU1 compared with NC ($p<0.016$), but there were no differences between the other groups ($p>0.016$). The analysis of protein fractions showed a significant difference between groups only in the case of F2 ($p<0.05$).

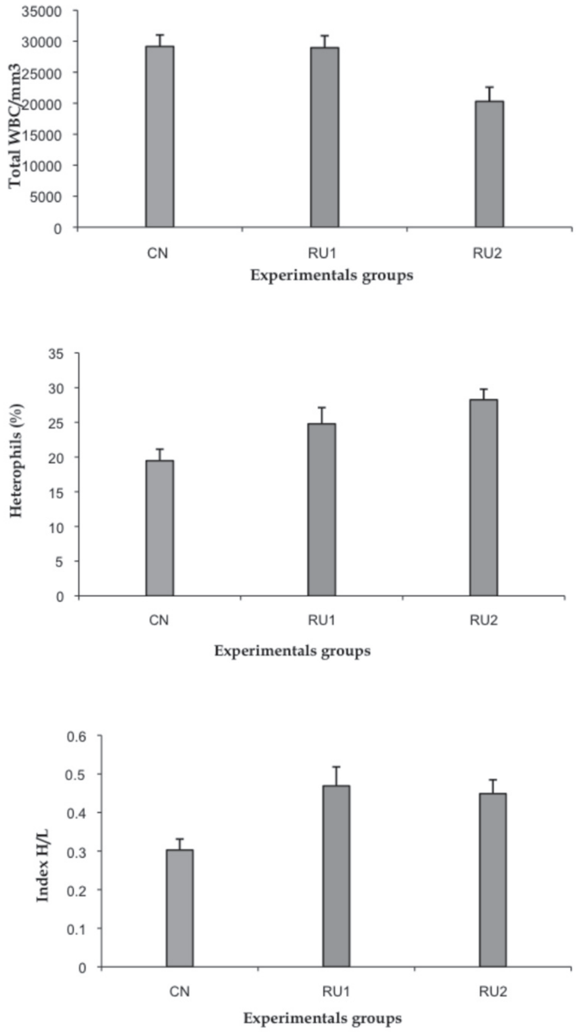

Figure 1. Mean (± standard error) for white blood cell count (top), Heterophile (middle) and heterophil/lymphocyte index (bottom). NC: negative control; RU1 and RU2: groups exposed to different concentrations of Roundup ®. *= statistically significant difference with respect to the NC (ANOVA-Tukey) for WBC and heterophil/lymphocyte index (Kruskal Wallis - Mann Whitney).

Besides, animals exposed to the highest concentration of RU (RU2) reported less growth in SVL and weight (2.80 ± 0.36 cm and 54.13 ± 6.80 g, respectively) than those of the NC (4.17 ± 0.29 cm and 69.44 ± 6.02 g, respectively) and RU1 (4.06 ± 0.19 cm and 82.16 ± 5.11 g, respectively); this difference was significant only in the case of SVL (Fig. 2).

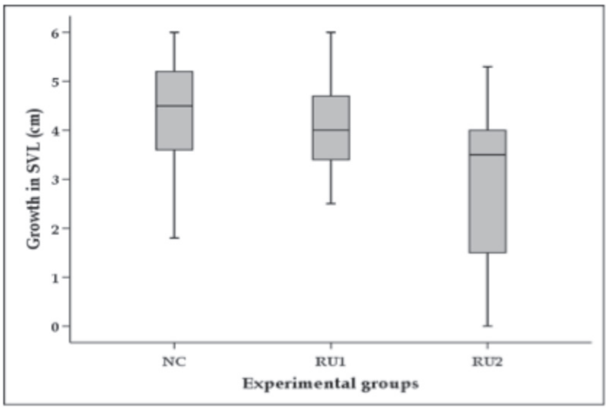

Figure 2. Increase in snout-vent length (SVL) in the different experimental groups.* = statistically significant difference with respect to the NC.

Discussion and Conclusions

Exposure to RU induced a decrease in the total WBC in caimans, with the lowest WBC count at the highest RU concentration (RU2). These values were higher than those found for captive sub-adult *C. latirostris* (Mussart *et al*. 2006; Barboza *et al*. 2008), which could be due to chronic stress state produced by captivity. The percentage of heterophils found in RU2 was higher than those reported by other authors for sub-adults of this species (Mussart *et al*. 2006; Barboza *et al*. 2008). The absolute values of total protein were similar to those reported by other studies (Coppo *et al*. 2006; Barboza *et al*. 2008). In our study, this value was higher in RU1 and analysis of protein fractions showed a significant difference between groups only in the case of F2, the fraction that includes $\alpha 1$ (Alfa-1-antitripsine), which is a component of IS and acts as an acute phase reactant, taking part in inflammatory processes or trauma, and stressful situations (Brandán *et al*. 2008). This means that pesticide exposure would generate a stressful situation, being also evident in the synthesis of proteins, especially those in the F2. The result of SVL and weight showed that exposure to RU has a negative effect on growth of animals, in agreement with previous studies made under in ovoexposure (Poletta *et al*. 2011).

This study indicate that exposure to Roundup® may cause alterations in the parameters of the IS and growth of caimans, so that the ability to respond to infectious agents could be diminished in caimans exposed in natural environments, especially hatchlings, where the IS is still immature. It is important to highlight that this is the first study reporting pesticides effects on the immune system of *C. latirostris*.

Acknowledgements

This study was supported by ProyectoYacaré and Yacarés Santafesinos (Gob. Sta. Fe/MUPCN), and was part of MAL's undergraduate thesis to obtain a Bachelor of Science degree. To Bioq. J.C. Collados, Laboratory of Central Analysis, Immunology Section, Hospital José Maria Cullen.

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Philippine Crocodile Attacks on Livestock: Implications for Conservation

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Abstract

Human-crocodile conflicts pose a serious threat to the conservation of crocodiles in the wild. This study examines conflicts between people and the critically endangered Philippine Crocodile (*Crocodylus mindorensis*). Interviews were conducted in 2010 to quantify the damage inflicted by Philippine crocodiles in the municipalities of San Mariano and Divilacan on the island of Luzon, Philippines. A total of 112 conflicts were recorded, mostly predation on livestock. These conflicts erode local support for the conservation of the species in the wild. Improving livestock husbandry, for example the construction of pig and chicken pens, offers the best prospects to prevent crocodile predation on livestock in the future.

Introduction

The Philippine Crocodile *Crocodylus mindorensis* is endemic to the Philippines. The species is listed as Critically Endangered on the IUCN Red list (IUCN 2012). The Mabuwaya Foundation aims to conserve the species in the wild with the consent and cooperation of rural communities and local governments in the northern Sierra Madre, northeast Luzon (van Weerd and van der Ploeg 2012). Most conservation efforts focus on the municipality of San Mariano where a remnant Philippine Crocodile population survives in the wild (van der Ploeg *et al*. 2008). In 2009, 50 captive-bred sub-adult Philippine Crocodiles were re-introduced in Dicatian Lake in the Northern Sierra Madre Natural Park (van Weerd *et al*. 2010). As a result of these conservation efforts, the Philippine Crocodile population is slowly recovering in the northern Sierra Madre. But predation on livestock increasingly causes frictions, and might erode local support for the conservation of the species in the wild.

Methods

Data collection

Between August and October 2010 we conducted 71 semi-structured interviews to record the damage inflicted by crocodiles in the Municipalities of San Mariano and Divilacan. Data were collected in all villages in the northern Sierra Madre where crocodiles are known to be present. Respondents were non-randomly selected by the head of the village (barangay captain). This resulted in a list of names of people who had negative experiences with crocodiles. Other respondents were identified using snowball sampling (in which respondents identify other people to be interviewed).

A structured questionnaire was used to gain information about household composition, livelihood strategies, and crocodile-livestock conflicts. In addition we had informal discussions with the respondents in which we focused on what happened, what the respondent did and felt at the time of the conflicting situation and what he/she thought would be an appropriate solution to the problem. Characteristics of the attack (distance to houses, distance to water, victim type, time of day and involved crocodile in terms of size and possible visible tags) were also recorded.

Thirty-five respondents were asked to rank pictures of 8 animal species (including the Philippine Crocodile) which could be potentially harmful to crops and livestock. By ranking pictures of these species, the relative damage caused by crocodiles was compared to other potentially pests.

Data analysis

The variables ‘time of day’, ‘distance to water’ and ‘distance to house’ were divided in classes (night/day, in water/<10 m from water/>10 m from water, <10 m from house/>10 m from house). These variables were tested for differences between observed and expected number of attacks per class using Chi-square tests where the expected number of attacks was equal for each of the classes.

In order to analyze the perceived relative threat of crocodiles compared to other potentially harmful species, each of the species was given a score according to the rank given by the respondents. The rank ranged from 'most harmful' (8 points) to 'least harmful' (1 point). All points were summed for the 35 respondents which resulted in one score for each species. These scores were analyzed using a Chi- square test to test for differences between the observed and expected rank, where the expected rank had equal scores for all species.

The costs associated to crocodile attacks were classified by the respondents: 'none', 'small' or 'big'. These classes were analyzed using a Chi- square test to test for differences between observed and expected classification, where the expected classification had equal scores for each level of financial loss.

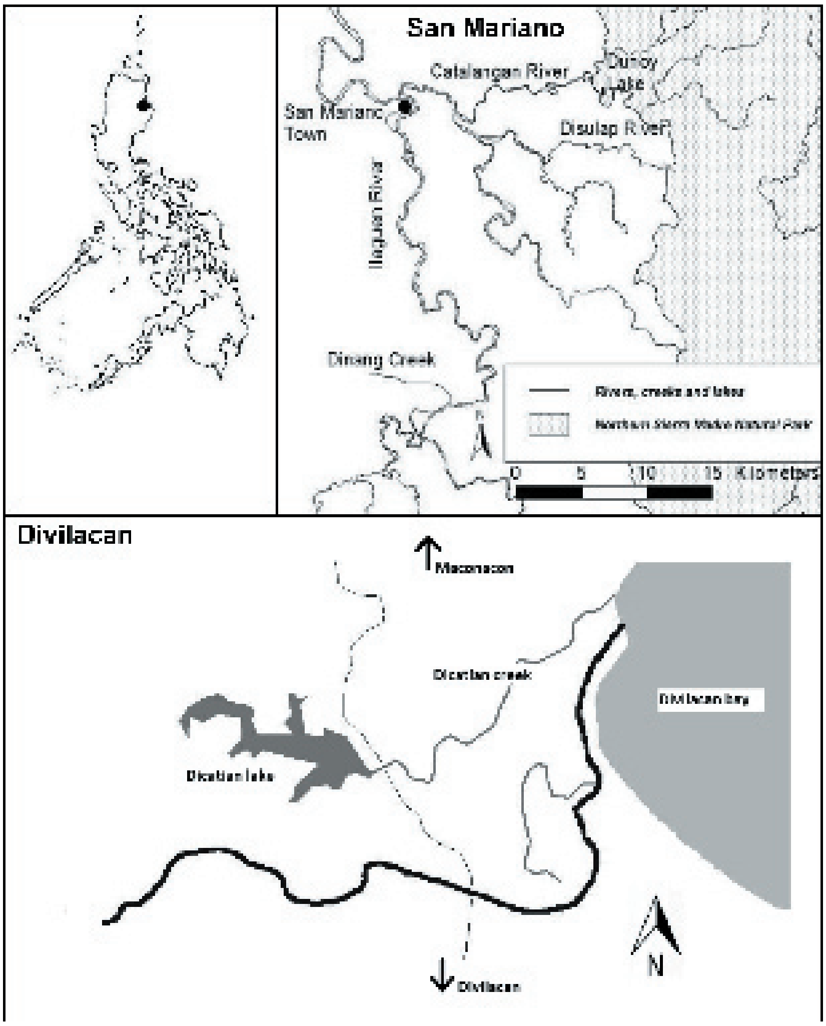

Figure 1. Location of the Municipalities San Mariano and Divilacan, Isabela Province, Luzon, Philippines.

Results

Conflicts

A total of 109 incidents involving predation on livestock or damage to fishnets by crocodiles were recorded, of which 106 took place between 2000 and 2010 (Table 1). The remaining three conflicts happened before that time. It is likely that there were more cases before 2000, but the respondents could no longer remember these. Most livestock predation cases involved chickens and ducks. Dogs and pigs were also attacked by crocodiles (Fig. 2). Fish was taken out of fishponds, and fishing gear (nets, fykes) was damaged (Fig. 3). In 13 incidents it was not clear whether the damage was caused by a crocodile, but respondents suspected that their animal was taken by a crocodile. In 7 cases animals had to be butchered after a crocodile attack. In these cases the owners could still eat or sell the meat and did not suffer direct financial loss (but could no longer generate income from these animals in the future).

Table 1. Philippine Crocodile attacks in San Mariano and Divilacan.

"Victim"	Killed/Destroyed	Injured/Damaged	Lost	Total
Pig	9	9		18
Carabao	1	2	1	4
Chicken	17	1		18
Dog	14	3	4	21
Fighting cock	1			1
Duck	24		4	28
Fishing net	10	3	4	17
Fish pond		2		2
Total	76	20	13	109

Locations and characteristics of crocodile attacks

Table 2 shows that there is a significant difference between the expected and observed number of attacks per class of the recorded characteristics of attacks. Most of the attacks took place at night, usually close to water and far from houses.

Table 2. Characteristics of Philippine Crocodile attacks.

Characteristic	Class	No. of Attacks	Chi-squared	df	P
Time of Day			14.7	1	<0.001
	Day	28			
	Night	65			
Distance to water			6.1	2	<0.05
	In water	47			
	<10 m from water	36			
	>10 m from water	25			
Distance to house			15.4	1	<0.001
	<10 m from house	32			
	>10 m from house	72			

Figure 2. (left) Piglet killed by a Philippine Crocodile in Dinang Creek, San Mariano (Telan 2011); (right) Dog killed by a Philippine Crocodile in San Mariano (Tinhout 2011).

Figure 3. (left) Fisherman shows his net damaged by a Philippine Crocodile (Telan 2011); (right) Fighting cock killed by a Philippine Crocodile in Dicatian (Rodriguez 2010).

Perceptions on possible causes

In the interviews the respondents explained what happened at the time of the attack, and what caused the incidents. Some people assume that crocodiles attack livestock because their natural prey is disappearing. The housing of livestock was often considered inappropriate: pigs often roam around at night and come in contact with crocodiles. According to some respondents in Divilacan, captive-bred crocodiles are less afraid of people and show more aggressive behavior. They suspect that these animals come closer to people and livestock since they are conditioned to be fed.

Costs suffered by people

Table 3 gives an indication of the prices of livestock animals and fishing gear, and the total financial costs of crocodile attacks.

Table 3. Financial costs of Philippine Crocodile attacks. Php= Philippines peso, EU= Euro.

"Victim"	No. Killed/ Destroyed	No. Injured/ Damaged	Price (Php)	Price (EU)	Total Damage (Php)	Total Damage (EU)
Pig	9	9	5000-7000	81-114	45,000-126,000	729-1026
Chicken	18	1	50-120	1-2	900-2280	18-36
Dog	18	3	1000	16	18,000	288
Duck	28	-	50-120	1-2	1400-3360	28-56
Fishing net	10	-	1000	16	18,000	160

The total costs associated to crocodile attacks are generally low, but the loss of livestock or fishing gear can have significant impact on the livelihood of individual households, especially in a remote and poor rural area such as the northern Sierra Madre. About 60 percent of the people in the study area live below the poverty line and have less than 40 pesos (0.73 Euro) to spend per person, per day (NSCB 2012).

For 94 out of the 109 attacks, a cost estimate was given by respondents in terms of big, small or no costs. The loss of ducks and chicken were usually categorized as small financial costs, with the notable exception of a fighting cock (Fig. 4). Loss of larger animals and fishing nets were generally considered big losses.

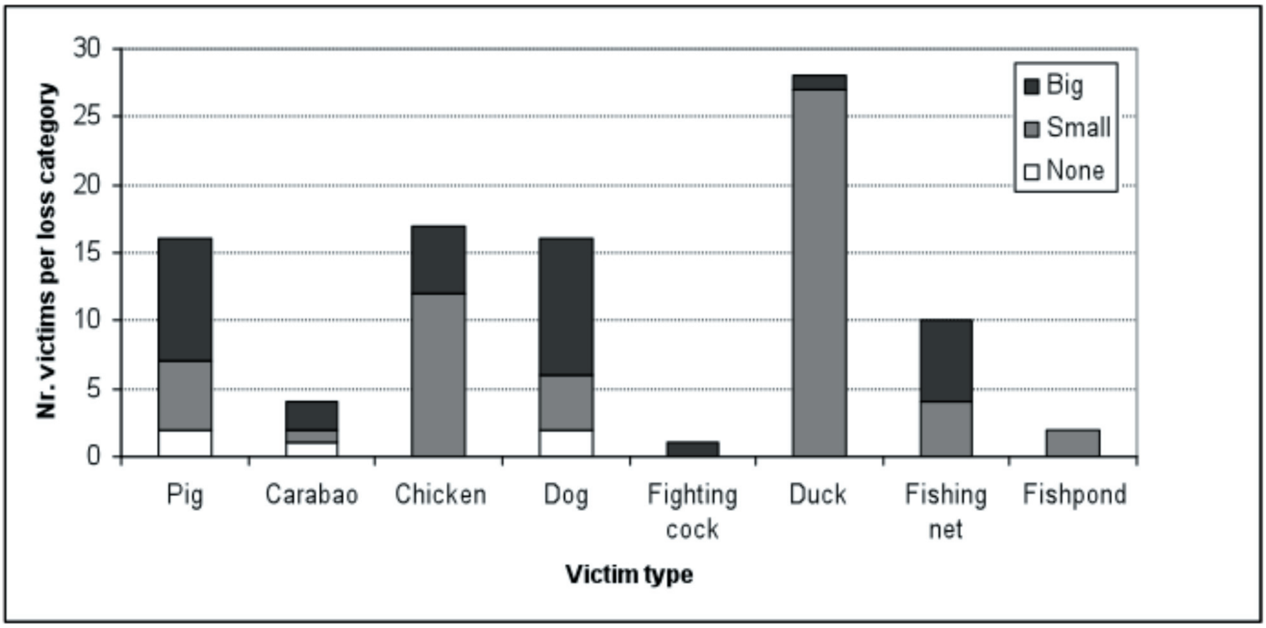

Figure 4. Perceived costs of crocodile attacks by respondents.

Remarkably, crocodiles are not perceived to be the most harmful species to people's income (Fig. 5). Damage to crops and livestock inflicted by brown rats (*Rattus norvegicus*), chestnut munias (*Lonchura malacca*) and Philippine wild pigs (*Sus philippensis*) was considered much worse than damage caused by crocodiles. The invasive cane toad (*Bufo marinus*) was ranked as least harmful to people's livelihood. The rank was significantly different from the expected rank (Chi square= 215.31, df= 7, p<0.001).

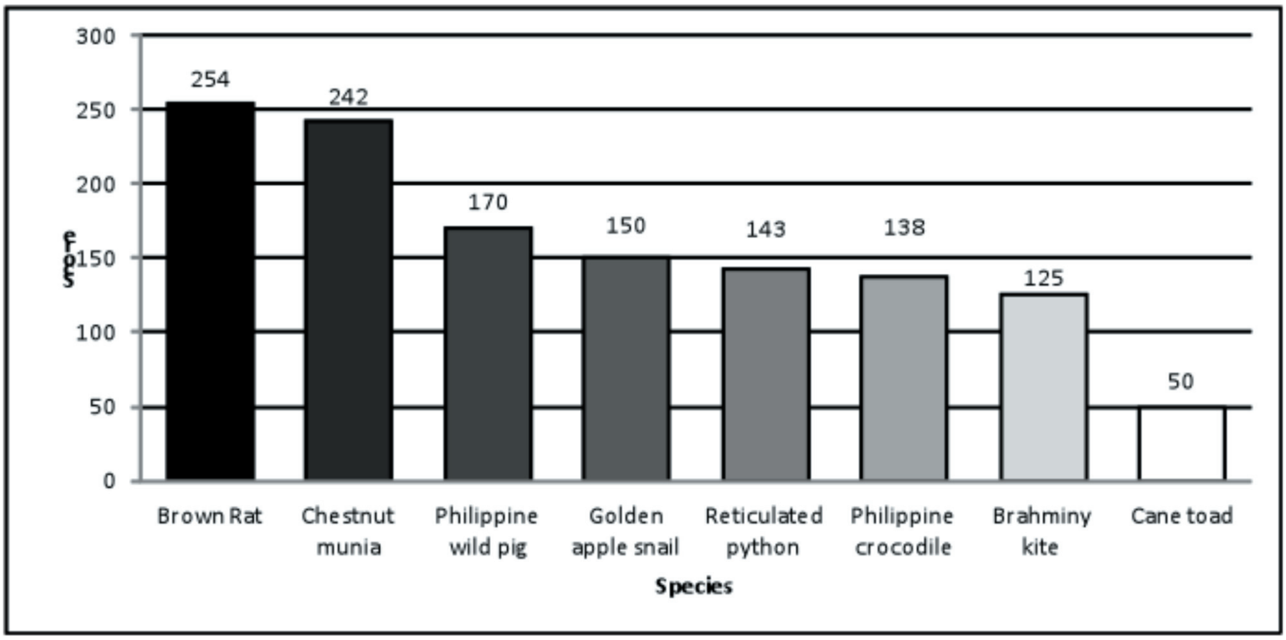

Figure 3. Ranking of harmful species to crops and livestock by respondents.

Solutions

All respondents gave suggestions how to solve livestock-crocodile conflicts. Multiple solutions were mentioned by most people. Placing fences around areas where crocodiles are known to be present was mentioned in 31 interviews, especially by people who live near Dicatian Lake and Dinang Creek where most attacks occured. Other solutions mentioned by the respondents were: financial and material compensation (mentioned 23 times), improvement of livestock housing (mentioned 21 times) and relocation or killing of crocodiles (mentioned 20 times). Financial compensation was mostly mentioned by people with big financial losses. Improved livestock housing was perceived to be a good solution by people who lost livestock to crocodiles.

The extension of buffer zones around rivers and lakes was generally regarded as ineffective. Respondents expressed that they would not like to move their houses or agricultural fields in order to increase space around river banks. Moreover, along Dinang Creek, where a reforestation project has already started, people say that it will take years before the planted trees mature and an actual buffer zone is formed. Some respondents liked the idea of an insurance program but others mentioned that this would be very difficult to accomplish and control.

Discussion

We recorded 112 human-Philippine Crocodile conflicts in the study area. But it can be expected that the actual total number of conflicts is higher. Most attacks on livestock took place at night, in close proximity to water and far from houses. This can be explained by the ecology of crocodiles and the behavior of livestock. Many crocodilian species feed mostly between dusk and dawn and mainly remain in the water during this time (Webb and Manolis 1989; Seebacher 1999). When not tied, pigs search for food and water in the early morning and late evening and ducks sleep in ponds or on river banks and thus become easy prey for crocodiles.

Crocodile abundance, density of natural prey species or vegetation cover might play a more prominent role in the explanation of attacks. Human population density and the associated level of disturbance in the area can be important factors related to vegetation density and thereby to the availability of habitat and natural prey species for crocodiles (Michalski *et al*. 2006). This can for example be the case in Dinang Creek, where the human population is rapidly growing.

Some respondents expressed fear of crocodiles living in their proximity. They are especially concerned about the safety of their children and said that they would kill the animals when their child would get attacked. They were aware of the fact that the killing of crocodiles is prohibited by law, but in such case they would ignore this. Although local people in the study area are afraid and suffered financial costs, only four Philippine Crocodiles have been deliberately killed in San Mariano since 2007. Traditional beliefs and practices determine to a large extend the relatively high level of tolerance towards crocodiles. Indigenous people in the research area revere crocodiles as the reincarnation of their ancestors, and refrain from killing the species (van der Ploeg *et al*. 2011a). Moreover, an intensive communication, education and public awareness campaign has been effective in mobilizing support for Philippine Crocodile conservation (van der Ploeg *et al*. 2011b).

Nonetheless, it is essential to reduce the number of conflicts and to alleviate the associated costs. As mentioned by respondents, the placement of fence lines along specific locations might reduce interaction between livestock and crocodiles. However, such crocodile-proof fences will be expensive and might easily wash away in rainy seasons. Another solution might be the use of compensation schemes. Although this seems an attractive concept, many programs where this was incorporated have difficulties related to claim assessments, long term viability and a lack of funding (Aust *et al*. 2009; Madhusudan 2003). Insurance programs can offer financial help for livestock mortalities to people who have taken the appropriate precautions to protect their livestock from predators (FAO 2009). It might however be difficult to proof attacks and validate claims, and the financial sustainability of such an approach seems problematic.

Improving livestock husbandry offers the best prospects to prevent crocodile predation on pigs, ducks and chicken (Odaga *et al*. 2003). Keeping livestock in proper enclosures has additional advantages, such as reducing crop raiding, preventing theft, providing manure and facilitating the control of diseases.

Conclusions

This study indicates that coexistence between people and the threatened Philippine Crocodile is challenging. Although the damage caused by the species is often much lower than the damage caused by other animals, crocodile predation on livestock needs to be effectively addressed as it erodes local support for the conservation of the species in the wild. Improving livestock husbandry, for example the construction of pig and chicken pens, offers the best prospects to prevent predation on livestock by crocodiles in the future. In areas where people and crocodiles live in close proximity, such as Dinang Creek and Dicatian Lake, additional measures such as fencing might be necessary.

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Human-Wildlife Conflict: Increasing Understanding Through Satellite Tracking, Education, and Resource Management

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Abstract

Sabah's Saltwater Crocodile (*Crocodylus porosus*) population has begun to recover after hunting was banned in the late 1980s. Consequently, the number of crocodile attacks has increased, especially in areas where the landscape is dominated by palm oil plantations. The primary focus of the project is to assess the medium to long-term movements of large crocodiles in 10 main rivers in Sabah and understand how the introduction of oil palm monoculture could have affected these movements. To date two male crocodiles have been tagged with satellite units. Early results show that, collared in well-forested areas, both individuals have relatively small home ranges and spend large amounts of time in flooded forest. We plan to collar up to 20 crocodiles in both forested and plantation areas. We will also carry out crocodile sampling in the 10 rivers, collecting tissue samples for DNA analysis, in order to ascertain population genetic health and inter-river migration. Finally, we will carry out nesting surveys in two rivers, Kinabatangan and Paitan, to assess how females select their nesting sites in a man-shaped landscape.

Caiman Surveys in Corrientes Province, Argentina

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Abstract

In this work we report relative densities (ind./km) of *Caiman latirostris* and *Caiman yacare* in Corrientes Province, Argentina, which is one of the Provinces where there is a ranching program going on. We studied 11 different sites. Four sites where nest harvesting occurs, three sites within Iberá Reserve where management is not allowed, and four places outside the reserve where populations are not yet managed. Since 2008 relative densities were highly variables, except in Yaguareté Corá, Galbán (harvesting), and Empedrado (not managed). The most variable number of caiman observed over time was in La Salada (not managed), Tabé, San Juan Poriahú (Iberá Reserve), and San Martín (managed). In 2009 and 2012 Corrientes had a drought period that increased relative densities. Present results do not indicate any tendency in population due to management, but in those sites where harvesting occurs Class IV (22.3 ± 6.8%), and Class III + IV (63.3 ± 17.6%), represent a higher percentage of animals (excluding Class I) than places where there is not management (Class IV= 2.8 ± 3.3%; Classes III+IV= 44.8 ± 23.9%), actually managed sites and Iberá Reserve sites presented similar values (Class IV= 15.2 ± 11.7%; Class III+IV= 62.3 ± 11.7%). In the managed sites we have found hatchlings indicating that harvesting does not include 100% of the nests.

Soft and Hard-Shelled Eggs of *Caiman latirostris*

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Abstract

We report the first record of abnormal eggshells (soft-shelled and hard-shelled eggs) in nests of the Broad-snouted caiman (*Caiman latirostris*). These eggs were collected in Santa Fe Province, Argentina, from wild and captive nests. Two types of abnormalities were identified: 1. hard-shelled eggs, among which can be distinguished (1a) eggs exhibiting a wrinkled texture that occurs in circles around one pole and (1b) aggregates of calcitic grains on the eggshell surface; and, 2. soft-shelled eggs. The inner portion of the shell units that comprise the eggshell is interrupted (2), producing gaps that weaken the shell. These soft-shelled eggs lack the hard and continuous shell wall present in normal eggs. Some nests containing recently laid clutches included eggs with most of the eggshell broken and detached from the flexible membrane. In *C. latirostris*, most hard-shelled eggs, those with the granular texture (1b) could be lost during incubation, even dissapear on the end of that process. Therefore, the granular texture presence in earlier stages of development does not directly affect the embryos survival. Soft *C. latirostris* eggs studied here (2), have mean hatching success of 8.9% (range 0% to 38%). Most of the loss of eggs is probably due to infection (fungi and bacteria).

Detectability of *Caiman latirostris* (Crocodylia, Alligatoridae) during Night Count Surveys in Argentina

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Abstract

Night count surveys are one of the most used methods to study distribution and determine some population parameters in crocodilians. However, there are some difficulties and biases during the sampling preventing the sighting of animals submerged or hidden among vegetation. We investigated the proportion of caiman available to be observed during a night count survey based on the positions of 8 adult female *Caiman latirostris* on which we had placed radio-transmitters (VHF, GPS and UHF). Fieldwork was carried out in a protected area with a natural stream and lagoon (30º11'26"S, 61º0'27"W) between January and March 2011. We only considered for analysis locations acquired at night (1800 to 0500 h) and those acquired inside vegetation were considered undetectable. Lagoons with greater availability of vegetation (cattail) can offer refuges and therefore more than 80% (60-100%) of the animals were hidden and not able to be sighted during surveys, on the other hand in the stream 100% of the caiman were located in an area where they could be observed. We did not observe a relationship between female body size and the probability of being sighted. The best time to do a survey appears to be between 2200 h and 0300 h.

Distribution of Tissue Enzymes in *Crocodylus porosus*

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Abstract

In domestic animals changes in serum enzymes are used to diagnose morbid organ changes, including liver, kidneys, muscle, heart. In crocodile medicine there are no studies correlating known diseases or lesions with changes in blood chemistry. For the most part, extrapolation and assumptions replace veracity and certainty. We have measured the concentration of the enzymes AST, ALT, LDH, ALP, CK, GGT and SDH from the fat, muscle, liver, lung, kidney and spleen of 10 apparently healthy, slaughter-size *C. prorosus*, as well as brains from 5 of these. Tissues were homogenized according to the techniques from Deborah *et al*. (2008). This is a starting point for studies wanting to relate blood pictures to actual morbid processes. Two animals in this study (BY27 and S5) were retrospectively found to have unusual distribution of enzyme activity, suggesting morbid process of the lung and liver respectively.

Literature Cited

Deborah, A.F., Mazet, J.A.K., Gulland, F.M.D., Spraker, T.R. and Christopher, M.M. (2008). Distribution of tissue enzymes in three species of pinnipeds. Journal of Zoo and Wildlife Medicine 39(1): 1-5.

Effect of Venipuncture Site on Hematologic and Serum Biochemical Parameters in the Chinese Alligator (*Alligator sinensis*)

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Abstract

Complete blood count and serum chemistry are commonly used to assess health status and clinical response. References values and validity, sensitivity or specificity of the various blood parameters have not been established in crocodilians. Blood values are affected by a number of extrinsic factors such as temperature, season, husbandry and, relevant to this study, venipuncture site. Understanding the effect of venipuncture site allows better interpretations of blood results. Blood is typically obtained from the tail (ventral coccygean vein) and the neck (supravertebral vein). In this study we also included a third site discovered by the author, and as yet unpublished, the mandibular shelf.

Delayed Hatching Moment on *Caiman latirostris* Under Experimental Conditions

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Abstract

The incubation period and its conditions are relevant issues to consider on the planning of the sustainable use programs based on the ranching technique, so to be able to manage the hatch moment of the hatchlings could be a valuable tool. The aim of this study was to evaluate the effect of low temperature during a part of the incubation period as a hatching delay factor. We utilized three nests of *Caiman latirostris* with known date of lay (102 eggs in total). Eggs were randomly distributed in two treatments: A) full incubation at standard conditions (30°C $\pm$ 1°C.); and, B) after a period of 8 weeks at standard incubation conditions, eggs were placed at 20°C ($\pm$ 0.5°C) for a period of 15 to 20 days, and after this they were placed back to the standard incubator to complete development. The delay in incubation period was on average 22.5 days. Significant differences were found with respect to size of hatchlings from the two treatments. Despite the fact that hatchlings from both treatments showed excellent body conditions, the hatching success for treatment A was 54.1% ($\pm$ 15.7%) and for treatment B it was 89.2% ($\pm$ 4.15%).

Usefulness of Homemade Camera Traps for Recording Activity Patterns in *Caiman latirostris* Nesting Areas

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Abstract

Camera traps are a useful tool for a variety of natural history and biology research lines, and one of the very few techniques that allow recording an activity pattern on many species. The aim of this study was to evaluate the effectiveness of homemade camera traps model, weight-activated on the surroundings of *Caiman latirostris* nests. The equipment is based on an electro mechanic activation system consistent in two sets, one of a camera and shooter, and the other one of an interrupter together with a retarder (0 to 4 minutes), with a sensitivity of about 300 g. Two traps were located on the nesting area at the Experimental Breeding Station "Granja La Esmeralda" in Santa Fe, Argentina, and were leaved activated during 10 days in December, 24 hours per day. We obtained 83 *C. latirostris* photos in that period. The breeders showed an intense activity around the nesting areas between 1100 and 0500 h, with two peaks, one at 1500-1600 h and the other at 2000-2100 h. No activity was registered between 0500 and 1100 h.

Status and Conservation of the American Crocodile in Jamaica

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Abstract

American Crocodiles are concentrated on the southern coast of Jamaica and present on the northern coast as individual sightings and at least one small breeding population. Presence of crocodiles on the northern coast has been facilitated by translocation of crocodiles to provide viewing opportunities for tourists. The American Crocodile in Jamaica inhabits a wide variety of natural and man-made wetland habitats, but is particularly fond of brackish water coastal wetlands including estuarine sections of rivers, coastal lagoons, ponds, and mangrove swamps. Man-made habitats include aquaculture ponds, water and sewage treatment ponds, and canals. Threats to the American Crocodile include habitat loss and increased persecution by humans and the situation appears dire. The solution to the conservation of crocodiles in Jamaica is the initiation of a systematic countrywide survey of crocodiles and their habitat, protection of existing habitat, development of a public education program and enforcement of the Wild Life Protection Act to protect against increasing human hunting pressure.

History

The Jamaican Coat of Arms was granted to Jamaica in 1661 under Royal Warrant. Designed by William Sancroft, then Archbishop of Canterbury, the Coat of Arms shows a male and female member of the Taino tribe standing on either side of a shield which bears a red cross with five golden pineapples. The Crest of the Coat of Arms is a Jamaican crocodile above a royal helmet. Taino Indians represented the original inhabitants of Jamaica, the pineapples and crocodile symbolized indigenous flora and fauna. The reverence for crocodiles expressed by placing them atop the Crest of the Coat of Arms did not appear in early writings about crocodiles in Jamaica.

Early accounts of the American Crocodile (*Crocodylus acutus*) provide little detailed information on their status or ecology but consistently indicated that crocodiles were abundant, if not too abundant. Hickeringhill (1661) described crocodiles as being "in Jamaica too great aplenty" and in 1740 Charles Leslie in his "New History of Jamaica" described crocodiles as being "terrible creatures" common in rivers and ponds where they "breed like toads" (Leslie 1740). Phillippo (1843) also observed that crocodiles were numerous and he mentions that they were found on the southern coast, an observation repeated by Caine (1908). Phillippo (1943) also provides the only mention of crocodile nesting by describing clutch sizes of 30-40 eggs. Additionally, Barbour (1910) notes that crocodile specimens "hideously stuffed" could be found in the tourist shops in Kingston and according to the local people crocodiles were abundant in some rivers flowing to the South and East.

Until the early 1960s crocodiles were considered plentiful in Jamaica (Garrick 1982). During the 1950s and 1960s there was considerable hunting for recreation and perhaps more importantly, for hides (C. Moody, pers. comm.). In addition, hundreds of crocodiles were removed from Jamaica during the 1960s, destined for commercial exhibits, primarily in the United States (Garrick 1982). By 1970 the number of crocodiles in Jamaica had dwindled to a level considered to threaten survival of the species, and by 1971, the species was added to the Third Schedule of the *Wild Life Protection Act of Jamaica*. The Third Schedule lists animals that are endangered and need protection for continued survival. Under Section 6 of the *Wild Life Protection Act*, it is an offence for anyone to kill or have in their possession the whole or any part of a crocodile, living or dead. The *Wild Life Protection Act* does not protect habitat and did not have any enforcement provisions. Removal of crocodiles continued, then in 1975 the American Crocodile was listed under Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and in the Protocol on Specially Protected Areas and Wildlife (SPAW). Species listed under Appendix I of CITES are subject to restrictions on international trade. Also in 1975 the United States declared the American Crocodile an endangered species throughout its range (USFWS 1975). By the end of the 1970s, public attention and a lack of an international market effectively brought an end to exploitation and export of crocodiles from Jamaica; but not an end to problems for crocodiles in Jamaica.

Status and Ecology

In "The Herpetology of Jamaica" Lynn and Grant (1940) noted that distribution of American Crocodiles in Jamaica was limited to the southern coast and emphasized their absence from the northern coast. Today, crocodiles are concentrated on the southern coast of Jamaica and present on the northern coast as individual sightings and at least one small breeding population. Presence of crocodiles on the northern coast has been facilitated by translocation of crocodiles to provide viewing opportunities for tourists. For example, a small breeding population near Falmouth, Trelawny Parish, escaped from an adjacent commercial exhibit.

Jamaica is a small island, about the same size as the historical Everglades (11,000 km^2). Only a small portion of the island provides habitat for crocodiles. The American Crocodile in Jamaica inhabits a wide variety of natural and manmade wetland habitats, but is particularly fond of brackish water coastal wetlands, including estuarine sections of rivers, coastal lagoons, ponds, and mangrove swamps. Although it is principally a coastal species, the American Crocodile is ecologically adaptable and is known to extend its distribution inland, especially along rivers and their associated wetlands habitat such as those found in the Black River Morass. Man-made habitats include aquaculture ponds, water and sewage treatment ponds, and canals and ponds in residential areas. American Crocodiles are proof of the concept that if you create their habitat, they will occupy it (Mazzotti *et al*. 2007).

Most of Jamaica's principal wetlands are distributed in patches along the coast (Table 1, Fig. 1) and offer potential crocodile habitat ranging from mangroves to brackish water lagoons and estuarine sections of rivers. Crocodiles are also known to inhabit three freshwater marsh ("morass") habitats. However, these areas are relatively small; the largest, Black River Lower Morass, is approximately 6000 ha and is the one best known for containing crocodiles (Garrick 1982, 1986).

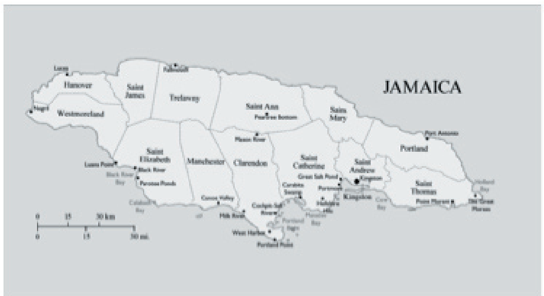

Figure 1. Map of Jamaica showing locations important to crocodiles.

Table 1. Major wetlands of Jamaica (source: Natural Resources Conservation Authority).

Location	Parish	Size (ha)	Description
Black River Lower Morass	St. Elizabeth	6000	Riverine/Estuarine; Marsh with Swamp, Forest & Mangrove
Negril Geat Morass	Hanover	2400	Estuarine; Marsh and Swamp Forest
Cabarita Swamp	St. Catherine	1600	Marine/Estuarine; Mangrove
The Great Morass	St. Thomas	1600	Marine; Mangrove
West Harbour	Clarendon	1600	Marine; Mangrove
Canoe Valley	Manchester	1200	Riverine/Estuarine; Marsh with Swamp, Forest & Mangrove
Falmouth and Saltmarsh	Trelawny	1070	Marine/Estuarine; Mangrove & Marsh
Amity Hall	St. Catherine	480	Marine; Mangrove
Great Salt Pond	St. Catherine	448	Marine/Estuarine; Mangrove
Manatee Bay	St. Catherine	370	Marine; Mangrove and Marsh
Luana Point, Fonthill	St. Elizabeth	400	Marine; Mangrove; Ponds
Carita	Westmoreland	240	Estuarine; Mangrove and Marsh
Kingston Harbor	Kingston & St. Andrew	200	Marine/Estuarine; Mangrove
Cockpit-Salt River	Clarendon	160	Riverine/Marine; Marsh and Mangrove
Cow Bay	St. Thomas	146	Estuarine; Marsh
Mason River	Clarendon	80	Palustrine; Marsh
Peartree Bottom	St. Ann	80	Riverine; Marsh

Supported by the Wildlife Conservation Society (WCS), Les Garrick conducted the most systematic study of crocodiles in Jamaica between 1975 and 1983. He was guided on many of his crocodile surveys by Mr. J. Charles Swaby, who has long been recognized as an expert on crocodiles in Jamaica. Other investigators visited Jamaica in the 1980s. However, other than brief observations of crocodile behavior and population structure, a general description of distribution of crocodiles (and nests), and importance of specific habitats for their conservation in Jamaica (Garrick 1982, 1986), little information is available on ecology of crocodiles in Jamaica.

By the end of the 1990s increased interactions between crocodiles and humans prompted the Natural Resources Conservation Authority (NRCA; now the National Environmental and Planning Agency, NEPA) to request assistance from the Crocodile Specialist Group (CSG). In 1995, 1997 and 2001, charrettes, field trips and training workshop sponsored by the NRCA

(NEPA), WCS, National Geographic, University of Florida and CSG, were conducted in Jamaica with diverse stakeholders to identify potential habitats for crocodiles, to summarize knowledge of occurrence of crocodiles and to provide training to government biologists in proper survey and capture techniques. WCS returned to Jamaica in 2001 when Dr. John Thorbjarnarson traveled to Jamaica at the invitation of Mr. Swaby. Mr. Swaby provided updated information on locations of crocodiles in Jamaica and guided Dr. Thorbjarnarson on field trips around St. Elizabeth Parish (Thorbjarnarson 2001). Results of these field trips and charrettes have never been published, but are summarized here and synthesized with observations of Les Garrick.

An immediate caveat is that these are impressions about distribution and abundance of crocodiles in Jamaica and are not based upon systematic surveys. However individuals involved in charrettes were recognized as experts on crocodiles in Jamaica. There was a strong consensus among experts at a charrette and a remarkable consistency in descriptions of distribution and abundance among different sources and over time.

A clear finding of all observations, charrettes, and field trips was that coastal wetland habitats along the southern coast are still the main locations for crocodiles in Jamaica. This supports Lynn and Grant's (1940) observation that American Crocodiles in Jamaica were distributed along the southern coast and absent from the northern coast. Today, exceptions include a mangrove wetland in Falmouth, Trelawny Parish (Fig. 1, Table 1), where a small population of crocodiles has become established adjacent to a commercial exhibit. Escaped crocodiles took up residence in the adjacent mangrove swamp and eventually began nesting on berms of a canal along the small swamp. The combination of human-aided movement to the north coast for a tourist attraction and creation of nesting habitat provided this new location for crocodiles in Jamaica.

Crocodiles are also occasionally observed on either end of the north coast near Port Antonio, Portland Parish; and Lucea, Hanover Parish (Fig. 1, Table 1). Whether those crocodiles were displaced by humans or dispersed naturally from source populations is not known. Permanent populations of crocodiles are found in the morasses on the east coast (Point Morant, Holland Bay, The Great Morass) and west coast (Negril Point, Negril Great Morass, Negril River). The impression from charrettes and field trips is that crocodiles are not uncommon in these areas but are in low density. The same description, that crocodiles are sighted frequently but not abundantly, characterizes most wetlands along the southern coast of Jamaica in St. Thomas, St. Andrew, and Westmoreland parishes. The main concentration of crocodiles in Jamaica is in the coastal wetlands in St. Catherine, Clarendon, Manchester, and St. Elizabeth Parishes. This core crocodile area extends from the Greater Portmore region near Kingston (Fig. 1) west to Luana Point/Fonthill, just west of Black River and includes Portland Bight, Canoe Valley, Black River and associated rivers and wetlands (Table 1). Locations within this area were described as containing a range of crocodile abundances, from small groups, to high density, to "crocodile country." One set of spotlight surveys in the Greater Portmore area in 2002 conducted by NRCA and University of Florida biologists resulted in sightings of 42 crocodiles (22 adults, 12 juvenile/sub-adults, 1 hatchling, and 7 unknown sizes) in 3 nights of surveys. This supports the impression that there were areas along the southern coast where crocodiles were common 10 years ago, although there is substantial evidence that the recent increase in crocodile consumption is potentially having catastrophic effects on population numbers.

Table 2. Location of known nesting areas of American Crocodiles in Jamaica. UWI= University of West Indies; SCS= South Coast Safaris; SCCF= South Coast Conservation Foundation.

Location	Parish	Notes (Sources)
Hellshire Hills	St. Catherine	3-4 nests were successful in 2008; Byron Wilson, UWI
Cockpit River	Clarendon	Nest adjacent to road; Charles Swaby, SCS
Portland Bight	Clarendon	2 nests with hatched shells were found in 1997; Brandon Hay, SCCF
Portland Point	Clarendon	Nesting Beach; Charles Swaby, SCS
Milk River	Clarendon	Nest on riverbank; Charles Swaby, SCS Les Garrick
Canoe Valley	Manchester	No specific Location; Les Garrick
Swift River/Three Rivers	Manchester	Possible nest in marsh; Charles Swaby, SCS
Calabash Bay	St. Elizabeth	Nesting Beach; Charles Swaby, SCS
Parotee Ponds	St. Elizabeth	Nesting Beach; Charles Swaby, SCS Les Garrick
Font Hill/Luana Point	St. Elizabeth	Nesting Beauch, "best nesting population"; Charles Swaby, SCS, Les Garrick

The south coast core crocodile area is also where nesting of crocodiles has been reported (Table 2). In addition to these confirmed records, there are anecdotal reports of crocodile nests in artificial habitats along the southern coast such as water and sewage treatment ponds, fish ponds, and canals. However, other than the fact that some nests occur in these manmade habitats, nothing is known about their location, number, or fate. In other locations such as southern Florida, nests on artificial substrates have to some extent compensated for nests lost to development of coastal habitat (Mazzotti *et al*. 2007).

Garrick (1982) described natural nest sites as either mounds on beaches or holes along elevated portions of riverbanks, similar to descriptions of nests in Florida (Mazzotti 1989). He commented that the maximum hatched clutch size (number of hatchlings caught adjacent to a hatched nest) was 30 but did not provide a sample size. He estimated size at first reproduction to be 2.2 m (7.3'), at 9 years of age.

Garrick (1982) also observed size-specific habitat use by crocodiles. Hatchling crocodiles near nest sites were found in shallow brackish ponds, or narrow creeks, similar to crocodiles in Florida (Mazzotti 1983). However, Garrick (1982) reported that, unlike in Florida, adult female crocodiles remained in the same ponds with hatchlings up to one month after hatching. After two months hatchling crocodiles dispersed to more open-water areas, remaining in the same general area for up to one year. Garrick described 2- and 3-year-old animals as dispersing farther away but also mentions that they are underrepresented in his samples, indicating that juveniles were in inaccessible habitats, had dispersed, or died. Of hatchling crocodiles tagged in Milk River in 1981-1982 Garrick found 46% alive after 2 months, 24% after 10 months, and 8.9% after 14 months. No sample sizes were given. He recognized that this was an underestimate of actual survival. This falls within the range of 12-month hatchling survival found in Florida (Mazzotti *et al*. 2007).

Garrick (1982, 1986) found that habitat loss (as a result of agriculture, aquaculture, peat mining, and pollution) and killing of crocodiles (mainly a result of uncontrolled fishing practices) were major threats to their conservation in Jamaica. More recently, habitat loss (exacerbated by development of nesting beaches and nursery habitat for touristic and residential projects) continues to diminish and fragment habitat, while killing of crocodiles as a result of persecution, and illegal harvesting for meat, both appear to be on the increase (Harrison 2010; Kelly 2006; Ritch 2006).

Conservation

Issues

Based on the above synthesis, it appears that in spite of decades of habitat loss and illegal removal and killing, crocodiles remain relatively widespread in Jamaica. However, while they are found in a variety of coastal habitats, populations appear small and there is little information on current status of crocodile populations.

Worldwide, the greatest problem facing crocodiles, and all wetland fauna, is loss of habitat, and Jamaica is no exception. Loss and fragmentation of coastal wetlands have been extensive in Jamaica, and are increasing as the country's population grows and people move farther into coastal areas. Major threats to these ecosystems identified by the NEPA include cutting, dredging and filling of mangrove habitats and other coastal wetlands as a result of urbanization and tourism-related development, agriculture, and fish farming. Other threats include pollution, and altered hydrology or soil salinity as a result of stream diversion or irrigation. Development of coastal beaches for resorts threatens critical nesting sites used by crocodiles (Ritch 2007). In many areas, natural habitat is being replaced by artificial wetlands such as canals or fish ponds, which when used by crocodiles can lead to conflicts with local people. This problem is compounded in Jamaica by the tendency of people to unwittingly provide food for crocodiles by throwing small fish or entrails into the water, by dumping dead dogs into mangrove ponds, or simply by creating garbage dumps along coastal wetlands. Under these conditions, adaptable crocodiles find food readily available in areas close to human habitation.

Conflicts between people and crocodiles in Jamaica arise as a result of destruction of the crocodile's natural habitat and the ability of American crocodiles to thrive in disturbed habitats close to people. American crocodiles are not considered to be a very aggressive species. Unlike Nile Crocodiles (*C. niloticus*) in Africa or Saltwater Crocodiles (*C. porosus*) in Asia and Australia, American Crocodiles do not regularly consider humans as food. They are potentially dangerous principally by virtue of their size in areas where people and crocodiles are not suitably buffered from one another. Over the last few decades, attacks on people have been rare but have occurred in Jamaica (eg Williams 2010).

Recently, a new threat to *C. acutus* in Jamaica has emerged - the increased killing of crocodiles for meat and the growing belief amongst Jamaicans that consuming crocodiles will lead to enhanced male sexual performance ('strong back'). This myth, perhaps precipitated by the influence of recent Asian immigrants (primarily Chinese), is creating a new market demand and by some accounts is driving up the price of crocodile meat (Harrison 2010; Wilson 2011). Unfortunately, this latest threat has not been effectively managed, and the illegal harvesting of crocodile meat continues to increase.

Recommendations

Efforts to protect the American Crocodile and other coastal wildlife in Jamaica would be most effective if done within the context of an overall coastal management plan that includes setting aside critical habitats as protected areas. Nevertheless, specific measures should be taken to address issues unique to crocodiles.

Management objectives for American Crocodiles in Jamaica should include ensuring survival of viable populations, reducing potential for conflict between crocodiles and people, public education, and developing programs whereby local communities can benefit from the presence of crocodiles. There are a number of actions that could be taken to attain these objectives; several are briefly discussed below.

Crocodile Surveys

The development of a crocodile management program in Jamaica requires an understanding of several key factors, including the present status and distribution of crocodiles, crocodile habitat, and the nature of crocodile-human conflicts. A survey of human-crocodile conflicts and attitudes of humans towards crocodiles would be helpful in developing educational campaigns and problem crocodile programs. A prerequisite for preparation of any management plan should be crocodile surveys to collect baseline data.

Crocodile surveys are typically based on indices of population size such as spotlight counts or nest counts. Both of these methods could be effectively employed in Jamaica as part of an initial effort to assess current status of crocodile populations. These surveys would provide a basis for long-term monitoring and could aid in obtaining future CITES approval to export crocodile products as part of an overall management plan. In addition to information on status and distribution of crocodile populations, it will be important to collect habitat data. Some of the most important factors that should be considered are the size and quality of habitat, land tenure and land use, and presence of other wildlife. An efficient way to merge all this information and make proper use of it for crocodile management purposes would be through the development of a geographic information system (GIS).

Provide Secure Habitat

Providing secure habitat for crocodiles would help meet two management objectives: securing crocodile populations and reducing conflicts with humans. Habitat loss and the resulting movement of crocodiles into areas of human occupation is one of the factors contributing to conflicts (Jamaica Information Service 2004). The long-term survival of crocodiles will require protection of critical habitats such as mangroves, freshwater wetlands, and nesting beaches. Many other species of wildlife, and many human activities, depend on the existence of healthy coastal wetlands. In this regard the crocodile could be used as a "flagship" species for the identification and protection of the most important of these natural wetland systems.

Public Education

Conflicts between people and crocodiles arise in part because of widespread misunderstandings about these large reptiles and the failure of people to take common-sense measures to reduce the potential for attacks. A public education campaign is needed to highlight the usually non-aggressive nature of the crocodile, and ways to avoid risky activity and inadvertent feeding of crocodiles (ie proper disposal of dead animals and fish). Similar efforts have had remarkable success in countries like Australia where crocodile attacks are a significant issue. Additionally, given the recent dramatic increase in consumption of crocodile meat, any public education campaign should expose the fallacy of the 'strong back' myth.

Problem Crocodile Program

No measures will completely eliminate conflicts between people and crocodiles. A transparent, government-managed (operated or regulated) program will need to be defined to deal with crocodiles that show up in inappropriate areas. A number of models for dealing with problem crocodiles could be used including examples from Australia, Florida (USA) and Mexico.

Crocodile Use Programs

Crocodiles can provide benefits for neighboring human communities. Worldwide, consumptive "ranching" programs based on the collection of eggs or hatchlings provide economic rewards through the sale of skins or meat. These types of programs must ensure that the harvest is sustainable and follow management guidelines that are enforceable. The non-consumptive use of crocodiles through ecotourism is also becoming a popular way for crocodiles to "pay their way" and benefit local communities. In fact, a booming business has developed in the Black River area of Jamaica based on river tours where crocodiles are the principal attraction.

Prognosis

Crocodiles occur in most locations in Jamaica where there is habitat to support them. Crocodiles are common enough to cause concern over an apparent increase in human-crocodile conflicts (Jamaica Information Service 2004) and support an ecotourism operation in the Black River Lower Morass. That crocodiles remain widespread in Jamaica, even with continued habitat loss and killing, is remarkable and evidence of their ability to survive in a human-dominated landscape in a mosaic of natural and artificial habitats. This is a demonstration of their basically secretive, non-aggressive, adaptable nature.

There is a new aspect to loss of crocodile habitat that may accelerate problems for crocodiles. Compared to the northern coast of Jamaica, the southern coast has remained relatively undeveloped in terms of resorts and residential development. However, the past decade has seen more rapid growth in both resorts and residential communities. Resorts tend to be built on nesting beaches and in adjacent nursery habitat. Development of residential communities and roads on beaches (eg Parotee Beach near Black River) can destroy nesting habitat and sever connections to interior nursery habitats (Fig. 2). Development of crocodile nesting beaches displaces crocodiles, causing an increase in interactions with humans in adjacent areas (Ritch 2007). Crocodile-human interactions further increase as a result of residential development in and adjacent to coastal wetlands that creates new crocodile habitats such as canals and ponds (Fig. 3). Such is the case in Greater Portmore, St. Catherine Parish, where crocodiles are now nesting near sewage ponds and causing concern in nearby neighborhoods (Mundle 2009).

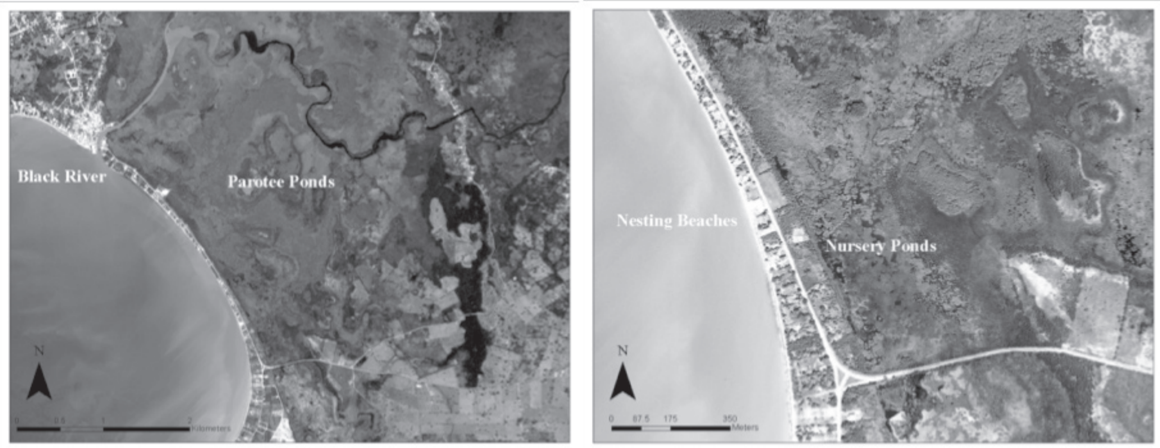

Figure 2. Development of crocodile nesting beach near the Parotee Ponds. Wider view (left) shows relation of nesting beach to ponds and Black River. Close up view (right) shows separation of nesting beach and nursery ponds by the road to Treasure Beach.

The solution for conservation of crocodiles in Jamaica, which will maximize options for dealing with problem crocodiles, earning foreign exchange, and increasing community participation in crocodile conservation, is the same as that recommended by Garrick (1982): protect core areas for crocodiles from further loss and degradation, and protect crocodiles from hunting by enforcing the *Wild Life Protection Act*. A very high priority should be placed on a countrywide survey of crocodiles and their habitat. A combination of habitat protection and increased knowledge of crocodiles could provide the basis for economically sustainable conservation of crocodiles in Jamaica.

Figure 3. Greater Portmore area showing juxtaposition of residential development and coastal wetlands southwest of Kingston, Jamaica. Canals and ponds create habitat for crocodiles in proximity to people.

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Spatial Ecology of the American Alligator (*Alligator mississippiensis*) and American Crocodile (*Crocodylus acutus*) in Estuarine Areas of Everglades National Park

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Abstract

Efforts are underway to improve Florida Bay and adjacent estuaries by improving water delivery. This may change salinities, water levels, and availability of nesting habitat. Alligators and crocodiles are among top predators within the Greater Everglades ecosystem; these species integrate biological impacts of hydrological operations, which affect them at all life stages through food webs, diversity and productivity, and freshwater flow. The purpose of our study was to determine home range and core-use areas for crocodilians in Everglades National Park. Kernel density estimation with site fidelity tests were used to quantify spatial habitat-use patterns over time. Through our analysis, we found habitat-use patterns of several individuals. Individuals traveled relatively low distances from capture sites, with 5.3 km mean displacement values. Core-use areas for 6 crocodiles with long-term data ranged from 51.6 to 155.2 km² (mean 86.8 ± 40.0 km² SD).

Development of a Conceptual Model Based on the Soft Systems Methodology for Evaluating Sustainability of *Caiman latirostris* and *Caiman yacare* Production in Argentina

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Abstract

Since the 1990s, five management programs on caimans (*Caiman latirostris* and *C. yacare*) have been developed in Argentina, all of them based on ranching. The aim of this work was to develop a conceptual model including economic, social and environmental variables, on caiman production in Argentina between 1997 and 2011. Four programs were evaluated (Formosa, Chaco, Santa Fe, Corrientes Provinces). The model was developed using the "Soft Systems Methodology" and data obtained through ethnographic techniques. We found cause-effect relationships between those variables that determined the activity: international demand for skins is influenced by the international regulatory framework, the global supply of crocodilian skins, the conservation status of the species under management, and customer's valorization of the product. These variables at a global scale impact on the amount of skins produced locally, and thus on the profit of ranches which could discourage business continuity, affecting its effectiveness as a strategy for conservation of species and their habitats.

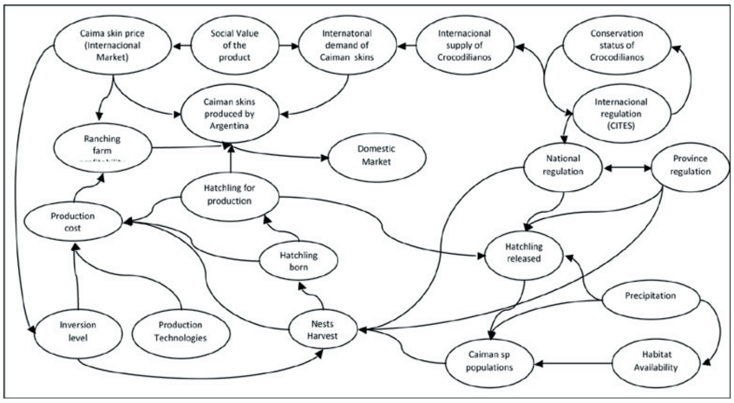

Figure 1. Argentine system of caiman skin production - conceptual model of cause-effect relationships.

Oxidative Stress and Antioxidant Defense Capacity Markers to be Applied in *Caiman latirostris* Blood

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Introduction

It has been indicated that one of the main mechanisms of pesticide toxicity is the production of reactive oxygen species (ROS).ROS include free radicals and other highlyreactive forms of oxygen (eg hydrogen peroxide, superoxide anionradical, hydroxyl radical). In excess, ROS can overwhelm the normal antioxidant buffering capacity of the cell, leading to significant damage to cellular components, including proteins, lipids and DNA.Cell damage caused by an excess of ROS,has been defined as oxidative stress (Azqueta *et al*. 2009). Organisms protect themselves from such damage with both enzymatic and non-enzymatic antioxidantdefenses. Three levels of protection have been considered: 1) prevention of ROS formation; 2) termination of the ROS using free radical scavengers or antioxidant enzymes; and, 3) repair of damaged cellular components (Storey 1996).

Measurements of lipid peroxidation products, such as malondialdehyde (MDA), as well as modifications in endogenous oxygen free radical (OFR) scavengers, including superoxide dismutase (SOD) and catalase (CAT), are used as effective biomarkers to study pollutant-mediated oxidative stress (Limón-Pacheco and Gonsebatt 2009). DNA damage induced by oxygen radicals occurs by oxidative modification of the bases in nucleicacids. Regarding this, the Comet assay modified by using repair endonucleases, such as formamidopyrimidine-DNA glycosylase (FPG), has demonstrated to be the most sensitive technique for measurement of oxidized bases (Collins 2009).

There was no previous data on the application of any oxidative stress technique in *C. latirostris* and only a few reports were found in crocodilians, including *C. yacare* (Furtado Filho *et al*. 2007) and *Alligator mississipiensis* (Gunderson *et al*. 2004; Lance *et al*. 2006). The aim of this study was to adapt oxidative stress biomarker techniques: 1) damage to lipids by Thiobarbituric Acid Reactive Substances (TBARS); 2) to DNA by Comet assay modified with the enzyme Formamidopyrimidine DNA glycosylase (FPG); and, 3) antioxidant defense: catalase and oxidized-reduced glutathione (GSH), for their application in blood of *C. latirostris* as early markers of pesticide effects in wild populations.

Materials and Methods

<u>Blood samples</u>: Blood samples were obtained from the spinal vein of 5 juvenile caimans from Proyecto Yacaré (Gob. Santa Fe/MUPCN), with heparinised syringes. Peripheral blood was used immediately for the modified comet assay. For the other techniques, blood was centrifuged,erythrocytes washed with saline solution and stored at -20^{o}C until analysis. Then erythrocytes were lysed with ice-cold distilled water and different dilutions were tested in order to determine the proper one for this species: 1:10, 1:20 and 1:40.

<u>FPG-Modified Comet Assay</u>: The alkaline Comet assay was performed with modifications required by *C. latirostris* erythrocytes, as described by Poletta *et al*. (2008). After lysis, slides were incubated with FPG or with enzyme buffer alone for 30 min at 37^{o}C, submerged in alkaline buffer for 10 min and then electrophoresed at 0.90 V/cm for 10 min. One hundred randomly selected comet images were analyzed, classified into 5 arbitrary classes, and a single DNA damage index (DI= n1+2 n2+3 n3+4 n4) calculated for each animal (Poletta *et al*. 2008). DNA breaks induced by oxidative damage are calculated by subtracting breaks with buffer from breaks with FPG as follows: FPG sites= Damage Index CA with FPG - Damage Index CA without FPG (Collins *et al*. 2008).

<u>Catalase(CAT) activity in erythrocytes</u>: CAT activity in hemolyzed erythrocytes was measured spectrophotometrically by monitoring the decrease in H_2O_2 concentration over time (Aebi 1984). The specific activity of each sample was calculated on the basis that one unit of enzyme activity was defined as the activity required to degrade 1 mole hydrogen peroxide during 60 s/g Hb.

<u>Lipid peroxidation in erythrocytes (TBARS)</u>: Malondialdehyde (MDA) as a marker of lipid peroxidation in red blood cells was determined by measuring the formation of the color produced during the reaction of thiobarbituric acid (TBA) with MDA (TBARS Assay) according to a modification of the method of Beuge and Aust (1978). The sample absorbance was determined at 535 nm and TBARS concentration was calculated using the extinction coefficient 1.56 x 105 M^{-1} cm^{-1}.

MDA concentration in erythrocytes was expressed as nmol/g Hb.

Reduced and oxidazed glutathione relation (GSH/GSSG): Lizederythrocytes were mixed with trichloroacetic acid. GSH was determined in the supernatant following the procedure described by Ellman (1961). For the determination of total glutathione (GSH + GSSG), GSSG was reduced to GSH with glutathione reductase and NADPH during appropriate time at room temperature. Reaction was stopped by acid precipitation with trichloroacetic acid. Dithionitrobenzoic acid (DTNB) was added to the supernatant and absorbance read at 412 nm. Total glutathione is expressed as μM of glutathione mg^{-1} Hb and then the relation GSH/GSSG is calculated.

Results and Discussion

Different modificationstested on the techniques protocols allowed the determination of suitable parameters for each biomarker to be applied in *C. latirostris* blood. We observed that the proper dilution for the determination of TBARS is 1:20, while for CAT and GSH it is 1:10.

All the studies previously made in crocodilians were applied in tissues from kidney, muscles, gonads or liver, so that animals have to be sacrificed or samples obtained from animals recently dead (Furtado Filho *et al*. 2007; Gunderson *et al*. 2004; Lance *et al*. 2006). In our study, different modifications were done to standard procedures in order to applythe techniques in *C. latirostris* blood.

There are still only a limited number of reports of the use of the CA in an ecotoxicological context, and very few of theseuse lesion-specific enzymes to detect specifically oxidised bases (Azqueta *et al*. 2009). Up to our knowledge no studies had been made evaluating DNA oxidative damage through the modified comet assay on reptile species, so that this is the first report on it. Considering our previous studies on the genotoxic effects of pesticides and pesticides mixtures on *C. latirostris* (Poletta *et al*. 2009, 2011), the possibility to add oxidative stress biomarkers represents an important advance for the evaluation of wild caiman populations, as we can obtain samples without causing any damage to the animals.

Acknowledgements

This work was supported by CONICET (PIP 112 to MDM), ProyectoYacaré and YacarésSantafesinos (Gob. Sta. Fe/MUPCN), and Universidad de Buenos Aires (UBACyT X154 to MDM).

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Red Fire Ant (*Solenopsis invicta*) Venom Effects on Physiological Responses and Survivorship in *Alligator mississippiensis* Hatchlings

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Abstract

In the United States, approximately of 20% of American Alligator (*Alligator mississippiensis*) nests contain *Solenopsis invicta* colonies, and at least 50% of surviving hatchlings shows signs of attacks, such as swelling of toes and eyes, as well as pustules. Studies have demonstrated that the venom delivered by bites of *S. invicta* affect bodyweight increases and survival of *A. mississippiensis* hatchlings. However, the physiological mechanisms and the implications for the immune system after *S. invicta* attacks are unknown. The aims of this study were to measure plasma corticosterone concentration, white blood cell counts and survivorship of *A. mississippiensis* hatchlings after exposure to a *S. invicta* colony. Exposure of hatchling alligators to *S. invicta* induced an increase in plasma corticosterone over time, a decrease in the total white blood cell counts, and survivorship that could be associated with physiological stress.

Introduction

The first experimental evidence of indirect effects of *Solenopsis invicta* (Hymenoptera: Formicidae) in herpetofauna was performed in *Alligator mississippiensis* (Crocodilia: Alligatoridae), demonstrating that the venom of bites reduced weight and survival (Allen *et al*. 1997). In the USA, 20% of alligator nests contain *S. invicta* colonies, and at least 50% of those hatchlings that survive show attacks evidence, such as swelling of the fingers and eyes, as well as visible pustules (Moloney and Vanderwoude 2002). However, physiological response mechanisms regulating these changes are still unknown, as well as the implications on the immune system that affect their survival. As in other vertebrates, reptiles have a well developed response to stress, which could affect the behavior, reproductive activity and intermediary metabolism (Guillette *et al*. 1995; Tyrrell and Cree 1998). Many of these changes are correlated with plasma concentrations of corticosterone, the major glucocorticoid produced by the adrenal gland in reptiles (Callard 1975). This work evaluated corticosterone production in *A. mississippiensis* hatchlings in response to *S. invicta* venom. Moreover, stress conditions and increased plasma concentrations of corticosterone have been repeatedly associated with immunosuppression in several species of crocodiles (Lance 1994; Morici *et al*. 1997; Rooney and Guillette 2001), so we analyzed if there are any alterations in white blood cells after contact with ants.

Methods

Solenopsis invicta colonies were collected in the wild and reared in plastic trays at the Louisiana Environmental Research Center (LERC), McNeese University, according to the technique described in Parachú Marcó (2011). For all experiments *A. mississippiensis* hatchlings from different nests were collected in the J.D. Murphree Wildlife Management Area, in Port Arthur, Texas, USA. Animals were kept isolated in plastic trays preventing any external factors that could generate them stress. During the preliminary test 12 hatchlings from different nests were used, being individualized through a non-invasive marking system that avoids excessive handling.

Animals from each clutch were divided into 4 treatments of 3 hatchlings each to be subjected to different blood sampling times after exposure to *S. invicta* bites. One group was used as control without exposure. All individuals were exposed at the same colony of *S. invicta*. The procedure consisted of placing all individuals in a tray with the same ant colony for 2 minutes, then washing them in water to remove ants from their bodies, and then placing each group in a different tray, and then bleeding a single time. Bleeding occurred at 0, 15, 30 and 60 minutes. Blood samplings were performed by extraction in the area of the vein cord at the cervical vertebrae (Zippel *et al*. 2003). After bleeding, animals were placed in trays, and raised and fed under routine techniques. An aliquot of whole blood was removed for white blood cell (WBC) determinations. The remain sample was centrifuged, and plasma stored at -20°C for determination of corticosterone with enzyme immunoassay (ARBOR ASSAY ®, Catalog N K014-H1). Total leukocytes were counted in Neubauer chamber. Leukocytes quantification was done by microscopic observation of whole blood smears stained with May Grunwald-Giemsa.

To assess whether the exposure to bites of *S. invicta* affects *A. mississippiensis* hatchling survival, in the second part of this study, 40 individuals from 4 nests were split into 5 different groups and exposed for different times to *S. invicta* bites (0, 0.5, 1, 2 and 4 minutes).

Results and Discussion

Preliminary results showed a gradual increase in blood corticosterone concentration in *A. mississippiensis* hatchlings after *S. invicta* attack (Fig. 1). Differences were found at 30 and 60 minutes compared to control (Tukey Test: $P \leq 0.05$). This suggests that red fire ants bites stress *A. mississippiensis* hatchlings. However, corticosterone concentration at 15 minutes after stings revealed no differences with control (Tukey test: $P \geq 0.05$).

Increase in glucocorticoid hormones cause characteristic changes in the leukocyte numbers that can be quantified and related to hormone levels (Barreno 2008). Leukocyte profiles are particularly useful in the field of conservation physiology because they are altered by stress and can be directly related to stress hormone levels (Davis *et al.* 2008). Indeed, we observed a decrease in total white blood cells (WBC) count in increased time blood samples from *A. mississippiensis* hatchlings. We found differences between 15, 30 and 60 minutes regarding control group ($P= 0.0154$, Fig. 2). As previously shown in response to glucocorticoids increase, circulating lymphocytes migrate to other tissues (lymph nodes, spleen, bone marrow or skin) where they are requested (Davis *et al.* 2008). On the other hand, neutrophils/heterophils are the primary phagocytic leukocyte, and proliferate in circulation in response to infections, inflammation and stress (Jain 1993; Campbell 1995; Thrall 2004). However, the heterophil-lymphocyte ratio in WBC differential count, showed no differences between treatments ($P= 0.4579$, Fig. 3).

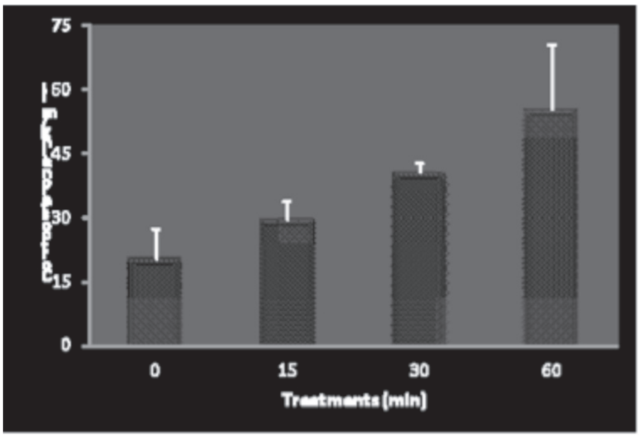

Figure 1. Mean plasma corticosterone (± SD) in *A. mississippiensis* hatchlings after exposure to *S. invicta*.

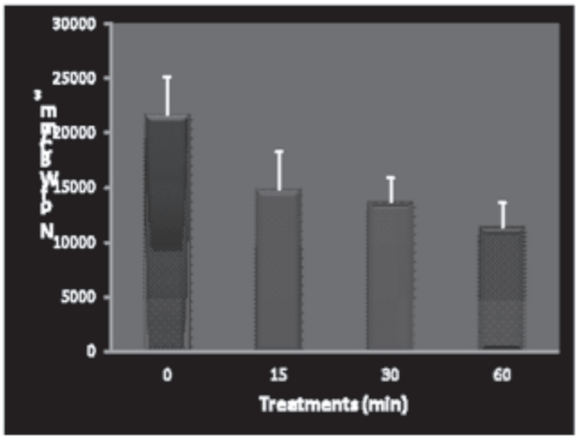

Figure 2. Mean total white blood cell (WBC) counts (± SD) in *A. mississippiensis* hatchlings after exposure to *S. invicta*. *= statistically significant differences with respect to the control group (ANOVA - Tukey).

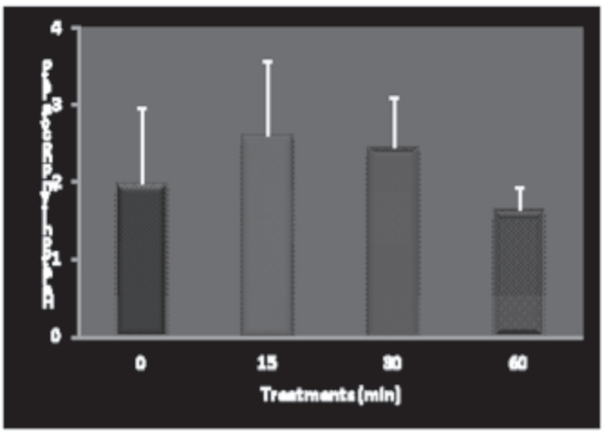

Figure 3. Mean heterophil-lymphocyte ratio (± SD) in *A. mississippiensis* hatchlings after exposure to *S. invicta*.

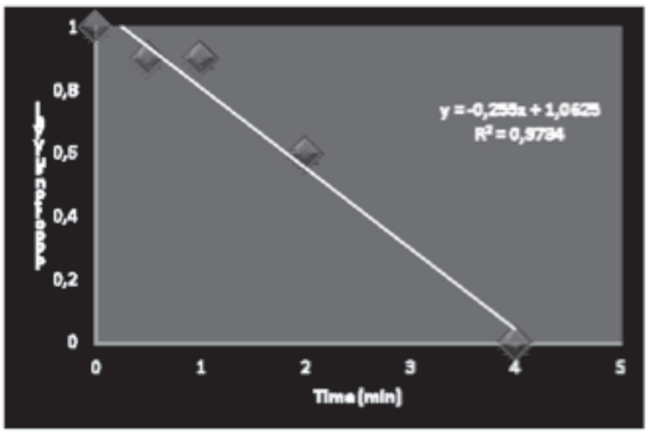

Figure 4. Survival of *A. mississippiensis* hatchlings after different times of exposure to *S. invicta* bites.

We found that survival of *A. mississippiensis* hatchlings decreased while time of exposure to *S. invicta* increased (Fig. 4). This relationship was reported in *Caiman latirostris* (Parachú Marcó 2011), although the survival of animals exposed to 4 minutes reached approximately 40%, while in *A. mississippiensis* there were no survivors after 4 minutes. This demonstrates that *S. invicta* bites have a greater effect on alligators than caimans.

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Detection and Characterization of Chitotriosidase Enzyme in *Caiman latirostris* Plasma

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Introduction

Crocodilians have demonstrated to have some immune components with an apparently higher activity than others animals, even humans. The ability to resist, with serious injuries in places with high concentrations of pathogen microorganisms without signs of illness, makes them interesting models to elucidate those mechanisms and components involved in the defense system. Among these components is chitotriosidase (CHT) enzyme, one of the main proteins secreted by activated macrophages. Chitotriosidase, also known as chitinase, is a glycosyl hydrolase secreted by activated macrophages (Hollack *et al*. 1994). For its specific expression, it is believed that CHT plays a role in mechanisms of immunity which hydrolyzes chitin and protection against chitin-containing pathogens (fungi, parasites, arthropods, etc.).There are two distinct isoforms of CT in humans, one that is 50 kDa and the other is 39 kDa (Renkema *et al*. 1997). The enzyme is located in specific granules of polimorphonuclears and secreted following stimulation with granulocyte macrophage colony-stimulating factor (GM-CSF). In addition, GM-CSF induces expression of CHT in macrophages that constitutively secrete the enzyme and partly accumulate it in their lysosomes. Our study was the first that revealed the presence of this enzyme in Caiman latirostris plasma, and based in its properties and functions, CHT activity was characterized under different laboratory conditions (pH, temperature, time, plasma concentration and salinity).

Material and Methods

Samples were collected from wild adult *C. latirostris* (7F, 6M; 1.51-2.31 m TL) in different areas of Santa Fe Province, Argentina. It is worth mentioning that, due to the influence of temperature on the physiology of these animals, the samples were collected during the summer. Animals were measured and returned to their environment within an hour of capture. The blood samples were collected relatively quickly after capture to avoid an increase in corticosterone concentration.

Blood samples were obtained from the spinal vein (Zippel *et al*. 2003) using heparin as an anticoagulant. Whole blood was centrifuged immediately at 4500 g for 20 min, at room temperature (approximately 24°C). The plasma was frozen at -20°C and CHT enzyme assays were conducted within 7 days of capture.

CHT plasma enzyme activity was determined as described by Hollack *et al*. (1994) using the artificial substrate 4-methylumbelliferyl-β-D-N,N´,N´´-triacetylchitotrioside (4 MU-chitotrioside; Sigma Chemical Co., St. Louis, MO). The enzymeassay mixture contained 15 µL of plasma and 100 µL (0.022 mM) of the substrate dissolved in citrate-phosphate buffer, pH 5.2, in a total volume of 115 µL. The reaction was stopped with 1 mL of glycine-sodium hydroxide buffer, pH 10.6. This mixture was incubated depending on determination dependence and fluorescence was read with spectrofluorometer (excitation and emission wavelength of 365 and 450 nm, respectively).

<u>Caiman CHT activity temperature dependence</u>: To evaluate the effect of temperature on enzyme activity, CHT assays were performed at different temperatures (from 5 to 40°C, at intervals of 5°C).

<u>Caiman CHT activity plasma concentration dependence</u>: To determine the effect of plasma concentration on CHT activity, different amounts of caiman plasma (0, 1, 2, 5, 10, 20, 50 and 100 µl) were added.

<u>Caiman CHT activity time dependence</u>: CHT assays were performed at different time intervals (0, 5, 10, 15, 20, 30, 60 and 90 min).

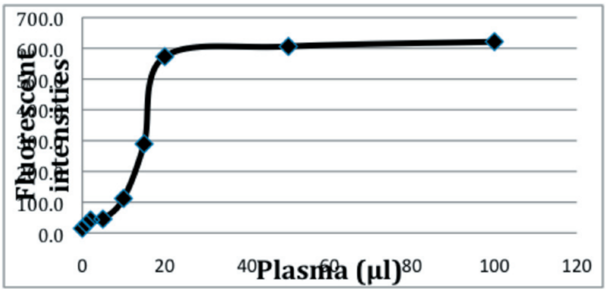
<u>Caiman CHT activity pH and salinitydependence</u>: To evaluate these activities, pH was changed by adding buffers ranging from pH 4 to 10, and salinity with different volumes of 1 M NaCl solution.

All assays were performed in quadruplicate and the results are expressed as fluorescence units (FU) ± standard error (SE).

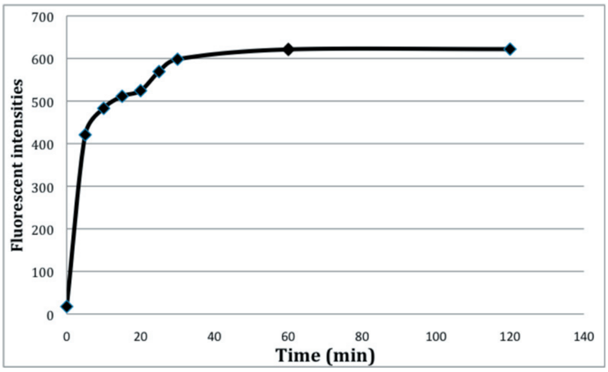
The effects of conditions variables on CHT activity were analyzed by linear regression for each species, and $p \leq 0.05$ was considered statistically significant.

Results and Discussion

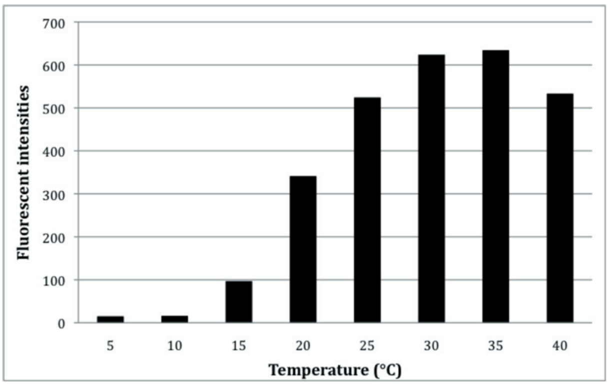
Caiman latirostris CHT plasma activity showed a positive relationship with increasing plasma concentration (Fig. 1). At the very beginning, small amounts of plasma produced a significant increase in CHT activity; 15 µL of plasma, CHT activity was 50% of maximum, approximately. This ability gives this technique an advantage, because with small volumes of plasma, reproducible results with very low variations can be obtained.



CHT activity in plasma of *C. latirostris* showed a positive relationship with time of incubation with substrate. Plasma caiman exhibited CHT activity immediately after 5 minutes of incubation with fluorescent substrate (Fig. 2). Within few minutes of the reaction time, high activity was demonstrated. These results coincide with those observed in similar studies made with dipeptidyl peptidase enzymes (DPPIV) (Siroski *et al*. 2011).

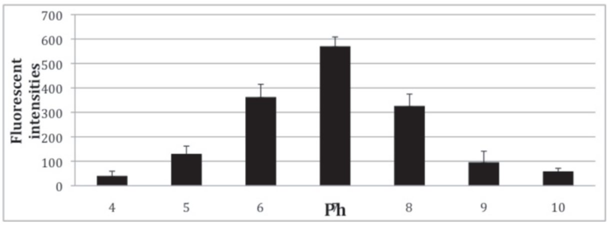


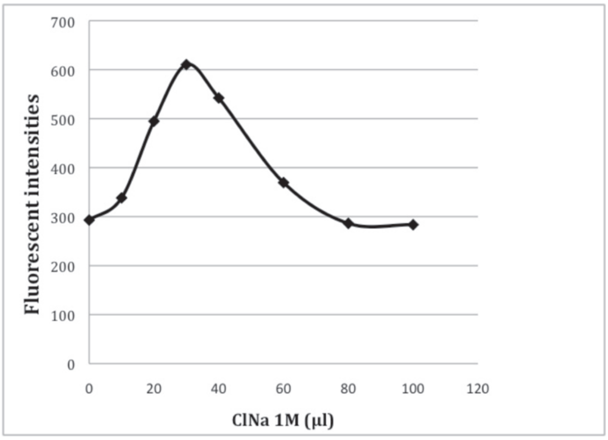
Ectothermic vertebrates are considered appropriate models to assess the influence of temperature on a variety of physiological functions (Pxytycz and Zkowicz 1994). Plasma CHT activity demonstrated a positive relationship with temperature (Fig. 3). At the lower temperatures (5, 10 and 15°C), CHT activity was low ($p<0.05$) until 15°C where it began to increase until 30-35°C. Enzymatic activity was dependent on the incubation temperature during the reaction assay. The activity at low temperature could be attributed to the greater climatic tolerance of *C. latirostris*, more than other species.



Crocodilians have preferences to maintain body temperature within a range of 28-33°C by using natural thermal gradients. This activity was detected at 35°C, approximately, close to caiman temperature preference selected to carry out normal physiological processes (Bassetti 2002).

The assays to evaluate the optimum functioning pH for CHT activity in plasma of *C. latirostris* are on Figure 4. The enzyme activity was highest at pH 7 and it was decreasing to both pH extremes, where practically no activity was detected.





The broad pH profile observed in our samples suggests the possible occurrence of distinct isoforms of macrophage (Renkema *et al*. 1997). It is expected that the pH optimum for each CHT isoform is close to the pH and osmolality of the environment in which it is active *in vivo* and may differ depending on tissue origin. In this case, we found values reasonable based on close pH optimum and salinity of the plasma tissue.

Polimorphonuclears, but not lymphocytes and monocytes, area major source of chitotriosidase in blood. Chitotriosidase hydrolyzes chitin substrates similarly to chitinases that are found in a variety of species (Boot *et al*. 1998), but this study is the first report about the presence and characterization of CHT in crocodilian, even in reptile plasma.

In conclusion, based on the parameters analyzed, it is presumed that the variation of these parameters may be useful to distinguish normal and abnormal organism. Considering the versatility of the results obtained in this study, CHT is a promising component of the caiman immune system and could be used for future applications in the veterinary area, in the study of immune phylogenetic mechanisms and as a biomarker of individual health status.

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Pentastomid Community Structure of *Sebekia mississippiensis* in the American Alligator, *Alligator mississippiensis*

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Abstract

Pentastomids parasitizing the lungs of *Alligator mississippiensis* (Crocodylia: Alligatoridae) from Florida and the East and West Zone of Louisiana were studied. Fifty-two of the 65 alligators analyzed (80%) were infected by pentastomids. Species richness was found to be depauperate as lung parasites were identified as juveniles or adults of one species, *Sebekia mississippiensis*. Males exhibited a higher parasitic prevalence than females (87% vs 71%) as well as parasite intensity (21.5 vs 10). Males from the West Zone of Louisiana had a higher prevalence than the East Zone of Louisiana and Florida (92%), but male hosts from Florida had a higher pentatsomid intensity than the other two locations (35.5). Female alligators from the East Zone exhibited a higher prevalence (80%) and intensity (14.1) than the other two locations. Host body size was found to be correlated with higher parasite intensity, which may be a result of the ontogenetic shift of alligators. In general, location, size and diet appear to be important factors in structuring lung parasite community of alligators (Overstreet *et al*. 1985; Hygynstrom *et al*. 1994; Delany and Abercrombie 1986; Boyce *et al*. 1987).

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WORKSHOP REPORTS

Crocodilian Capacity Building Manual Working Group

Participants: Charlie Manolis, Dietrich Jelden, Allan Woodward, Perran Ross, Geoff McClure, Matt Shirley, Paolo Martelli, Samuel Martin

At the 20th CSG Working Meeting, the CSG Executive agreed that a web-based CCBM was an important contribution that the CSG could make to Range States for crocodilians. The Steering Committee directed a working group chaired by Allan Woodward to assess the need for a comprehensive reference document, and suggest a structure for implementing the development of a CCBM (see SC Agenda Item 8.1).

At the 21st CSG Working Meeting, the CCBMWG was directed to assess the ways in which development of a CCBM could be implemented. The group's deliberations are summarised below:

1. The CCBMWG recognised that the successful development of a manual would rely on someone coordinating the project. Charlie Manolis agreed to take on this role temporarily, pending the identification of a permanent coordinator. The CSG Executive recognised that some funding may need to be provided for this "position".

2. The CCBMWG also proposed that:

    a. A revised version of the CCBM outline developed at the 20th Working Meeting (SC Agenda Item 8.1) be distributed by the end of June 2012, and input sought so that a final list of contents can be settled on.
    b. Volunteers be sought to contribute to specific sections of a CCBM. Some people have already indicated that they are able deal with specific sections (eg Dietrich Jelden, Matt Shirley, Allan Woodward, Charlie Manolis, Paolo Martelli, Perran Ross).
    c. The format of a CCBM be firmed up after some initial contributions have been received.
    d. The CCBM be developed in a simple form, with reference to published works, etc. - a "Wikipedia" approach. Matt Shirley indicated that many countries needed very basic information, and not necessarily at the more complex level at which much information is currently available. Although this is an important consideration, it was felt that the CCBM had to start somewhere, and specific "documents" may need to be developed over time to address it.
    e. Firm timelines be established.

Veterinary Science Group Report

The Veterinary Science Group met on 25 May 2012.

Participants: Paolo Martelli, Matt Plummer, Charlie Manolis, Samuel Martin, Charles Caraguel, Beatrice Langevin, Sam Seashole, Val Lance, Geoff McClure, Pablo Siroski, Robby McLeod, Marissa Tellez, Terry Cullen, Mark Merchant, Csaba Geczy, Adam Britton, Willem van de Ven

The Mission of the CSG Veterinary Science group is to:
- provide a platform for the exchange of and access to specific veterinary knowledge and advise the CSG on veterinary matters related to crocodilian conservation;
- contribute to advancing crocodile veterinary medicine and science; and,
- provide support to animals under human care: farms and zoological or educational institutions, biologists and researchers that require veterinary support in their work such as sampling, anesthesia, surgery, etc., conservation, research, NGO and Government organizations investigating *in-situ* mortalities and population health status.

Agenda and report

1. Overview of past 2 year's activities, next 2 years

    Overall the group has fulfilled its mission (see above). The group is mostly active *ad hoc*.

    1.1. There is a feeling that more could be done and the group tried to identify how its level of activity could increase.

An increase in requests for assistance would achieve that because we are a consultative/group. There is insufficient awareness of the existence and mission of the group so we need to increase awareness. Matt Plummer will work on increasing awareness amongst farmers, Marissa Tellez will contact herpetological societies in North America and Africa, Pablo Siroski will do the same in Central and South America. Paolo Martelli will do the same with the AZA, EAZA and ARAZPA.

1.2. Charlie Manolis suggested the inclusion of Cathy Shilton (Australia). Paolo Martelli will contact her.

2. Review website information and reassign tasks

Review of documents to be put on the CSG website. The following are ready to go and will be loaded by the first week of June 2012.

a. Necropsy procedures, with English, French and Spanish versions
b. Checklist of parasites
c. Anesthesia literature
d. Link to histopathology site

The following are pending and have been assigned:

Topic	Champions	Target
veterinary procedures (general exam, sampling, medication, etc.)	Samuel Martin, Terry Cullen	May 2013
Literature resources	Kent Vliet, Val Lance, Paolo Martelli, Charlie Manolis	pending political and legal issues
Imaging database and techniques	Charlie Manolis, Cathy Shilton	December 2012
Introduction techniques for new animals in captivity	Samuel Martin, Terry Cullen, Geoff McClure	May 2013
Parasite database Excel format	Marisa Tellez	December 2012
Manual for parasite collection and preservation	Marisa Tellez	July 2012
Share information on histopath database technicalities to facilitate adding material	Paolo Martelli	August 2012
Facilitate movement of histopathology slides in and out of USA	Terry Cullen	in place
Scientifically sound study on effect of various electrical parameters on crocodiles subjected to EI	Mark Merchant	Update May 2014

3. Review the list of relevant research topics encouraged by CSG

These were areas of veterinary science and medicine that will benefit crocodile medicine, conservation and biology. This is not a comprehensive or exclusive list. It is intended to stimulate and guide scientists.

- anatomy
- immunology acquired and innate
- Stress: stress indicators, response to stress, stress monitoring, patho-physiological effects of stress
- epidemiology emerging diseases and biosecurity, including at international levels
- nutrition
- health assessment and screening in general and in the context of reintroduction following IUCN Reintroduction Specialist Group
- crocodile-specific veterinary training of managers and veterinarians in various areas
- Behavior, medical and husbandry training of the animals
- husbandry and welfare, electric immobilization
- intellect, cognition
- endocrinology/reproductive physiology
- genetics
- physiology

4. Review of stunning

   The Veterinary Science group is not competent to pronounce on political or legal matters on this topic. The views of the members of the group with direct experience of this technique were shared. There was unanimous agreement that:

   4.1. Stunning is a term dedicated to delivering electrical current prior to culling. Electrical immobilization is the term dedicated to delivering electrical current for the purpose of temporary immobilization is "electrical immobilization".
   4.2. The group unanimously agreed on the following: "like every tool electrical immobilization must be used by trained staff using well maintained equipment. To the best of our observations there are no reasons to consider that EI is more detrimental to the individual or the group it is in than manual capture. There is evidence that it less stressful to the animal (Franklin *et al*.). Studies are underway and more studies are encouraged."

5. Present and assign tasks for the Crocodilian Capacity Building Manual group

   See also above Point 2 above. Also:

   5.1. Euthanasia/killing methods. Paolo Martelli will draft a document and circulate it to the group to describe humane methods for killing crocodiles. The documents will then be shared with the Executive and posted on the website.
   5.2. All members are to share with the CCBM Working Group web resources or other resources the members use. Links can be added to the CSG website.

6. Other matters

   Geoff McClure suggested that we need more husbandry specialist participation in the Veterinary Science group and CSG. To this effect he proposed that:

   6.1. With Chris Banks, to request people maintaining adult or breeder *C. mindorensis* to submit a 'floor plan' and photo/s of their breeder pen design with comments on the behavior/compatibility of their animals. Submissions will be collated to ascertain any features of pen design that will effect breeding. All submissions will be acknowledged and presented at the next CSG working meeting in 2013. This initiative will be included in a database - it should be noted that there is no current 'studbook'.

   6.2. The croc husbandry challenge - to design self-cleaning accommodation for 50 2-year-old crocodiles. Interested people should list the attributes or criteria that should be considered when designing self-cleaning accommodation. The design should consider among other attributes species-specific issues if any, shape, area, land and pond dimensions, and plumbing. Heating, cooling and insulation may be omitted. All submissions should be e-mailed to Geoff McClure. They will be acknowledged and presented at the 2013 CSG working meeting.

Human-Crocodile Conflict Working Group

Participants: Charlie Manolis (Chair), Allan Woodward, Terry Cullen, Colin Stevenson, Rambli Ahmad, Oswald Bracken Tisen, Ahmad Abdul Hamid, John Breuggen, Jennifer Breuggen, Robert Pahl, Tarun Nair, Adam Britton, Joe Wasilewski, Shaun Heflick, Alvaro Velasco, Vic Mercado, Chris Kri Ubang, Robby McLeod

The objectives of the Human-Crocodile Conflict Working Group (HCCWG), established in 2002 (Gainesville), are to provide technical advice and assistance for the avoidance and mitigation of HCC globally. In 2004 (Darwin), it was agreed that the HCCWG would: a. Compile information on crocodile attacks in the form of a database; b. Produce a fact sheet for media use - pre-emptively and after crocodile attack incidents; and, c. Provide guidelines for the avoidance and mitigation of HCC at a national level.

The HCCWG met twice at the 21st CSG Working Meeting, and the results are summarised as:

1. Despite the limited progress on the proposed activities (a-c above) over the last 8-10 years, it was agreed that the HCCWG would continue to operate until the 23rd CSG Working Meeting (May 2014).

2. Database

   Information on crocodilian attacks continues to be collected at a National level in many countries, and this information has often been sent to Rich Fergusson for inclusion in a database. However, as indicated in the HCCWG report to the Steering Committee (Agenda Item 6.2), some information has yet to be placed in the database. The database has not been available to other HCCWG members, and nor has there been discussion on how the database could be utilised effectively in the long-term.

   The importance of a database on HCC was recognised. However, caution is also warranted, as gaps in historical HCC information make lead to misinterpretation of trends. At times data on indirect HCC (ie on livestock) is collected, but at other times it is not. Same situation with "near misses".

   Adam Britton indicated that the HCC database being developed by Brandon Sideleau would most likely be available to the public, but some work remained to be done to integrate search functions, etc. They hope that the final design of the database will allow new information to be entered independently by others. Adam undertook to discuss with Brandon the possibility of CSG linking to their website.

   A HCC reporting form was developed by the HCCWG previously, to allow cases of HCC to be collated for submission to the database. This form will be integrated into the HCC page on the CSG website.

3. Fact Sheet

   No progress has been made. The HCCWG agreed that it is difficult to produce generic document that could be applied at a global level.

4. Guidelines for avoidance and mitigation of HCC

   No progress has been made. The HCCWG agreed that it is difficult to produce a generic document that could be applied at a global level. There is information available on how countries have approached this issue, and case studies may be an avenue through which this type of information could be made available through the website. Specific examples of other mitigation measures (eg aversion learning) could also be included as they are developed.

5. The concept of a mini-symposium at the 23rd CSG Working Meeting (May 2014) was proposed by Allan Woodward. This would allow the HCCWG to produce a tangible "product" that could assist wildlife managers, etc. The feasibility of incorporating a HCC session will be discussed with Mark Merchant (2014 meeting organiser), and if possible a draft agenda will be circulated for input. Invited speakers on Human-Wildlife Conflict, including crocodiles, would be need to be identified. The participation of people from developing countries would also need to be considered.

6. The HCCWG agreed that regular communication was essential. A google group has been established, and this could continue to be used. More regular meetings of the HCCWG was considered desirable, although problematic given the distribution of members and costs that would be involved.

7. Colin Stevenson reported that the Madras Crocodile Bank is organising a mini-symposium on HCC in August 2012, in Bangalore, India, under the auspices of the SCB meeting.

8. As Rich Fergusson has stepped down as Chairman of the HCCWG, Allan Woodward agreed to take on the position until 2014.

Industry Group Report

Participants: Charlie Manolis (Chairman), Matt Plummer, Simone Comparini, Paolo Martelli, Terry Cullen, Charles Caraguel, Yoichi Takehara, Geoff McClure, Greg Mitchell, William Belo, Buddy Chan, Heintje Ong Limketkai, John Caldwell, Chieko Abe, Ben Solco, Daniel Barlis, Careen Belo-Solco, Erik Wiradinata, Marcos Coutinho, Michael Cruz

In the absence of the Industry Vice Chair Don Ashley, Charlie Manolis chaired a meeting of the industry group to review the issues raised in the detailed report to the Steering Committee (see Agenda Item 4.2).

One of the key issues with which Don Ashley has been involved in during 2011-12 relates to welfare, which was given prominence following a recent documentary dealing with the harvest of snakes in Asia for the skin trade. The industry group recognised that opposition to the use of wildlife by some NGOs was often philosophically driven, and that welfare was used as a means to raise "public concern" unduly.

It is difficult for the CSG, which is not legal entity, to come out openly in defence of specific cases (eg current situation in South Africa). The group agreed that efforts should continue through involvement in various forums (eg CITES, IUCN, UNCTAD), but also through individual business contacts and consumers generally, to reinforce the positive benefits of trade on crocodilian conservation. Meeting the "problem" head-on with scientifically based information was considered important.

The issue of whether the group should consider the development of "generic" Best Management Practices to guide industry in countries where no guidelines or codes currently exist, was discussed. BMPs could be addressed within the context of the Crocodilian Capacity Building Manual (see CCBM Working Group Report), but no decision was reached by the group on whether it should be pursued as a specific project.

Tomistoma Task Force Report

The TTF group met on 25 May 2012.

Participants: Colin Stevenson (meeting chair), John Brueggen, Jen Brueggen, Scott Pfaff, Joe Wasilewski, K. Robert Pahl, Steve Conners, Anthony Pine, Rambli Ahmad, Bekky Muscher, Matt Shirley, Mark Bezuijen

Since the 20th CSG Working Meeting in 2010, the TTF has achieved some good progress. An update on this progress was given at the CSG Steering Committee meeting (SC Agenda Item 6.1).

Red List Assessment

The CSG is currently reassessing many crocodilian species under the IUCN Red List. The Tomistoma account is being worked on, with a first draft being circulated to species assessors and reviewers. Once this draft is finalised, it will be distributed to other TTF members for comments. After this, it will be submitted to the CSG for final review and forwarding to the IUCN. At this stage, it seems that Tomistoma will remain as "Endangered".

Mesangat

Lake Mesangat in Kalimantan remains critical to Tomistoma, and is recognised also for its Siamese crocodile population - along with other endangered taxa. Whilst there are talks about attaining RAMSAR site status for Mesangat through the TTF, members familiar with the area are cautious, and recommend moving slowly forward with this. It seems that political sensitivity requires such caution.

There is a further study by Agata Staniewicz planned for Lake Mesangat this year, as well as TTF member Rob Stuebing's continuing work in this region. TTF will continue to seek Rob's advice on the Mesangat situation and return to the CSG with an update so that a way forward can be made.

Bruce Shwedick will contact Fernando and update the group on his project in West Kalimantan.

Sumatra

Sumatra was identified in the 2010 Action Plan for Tomistoma for clarification of the species' status. Mark Bezuijen informed us that there is an NGO working in the area, with whom TTF could perhaps link up. These people know the area, the politics, and the people, and are open to helping us with Tomistoma. This is under the Merang REDD Pilot Project, and involves Peat swamp forest conservation. The meeting agreed that this sounded promising. Mark Bezuijen agreed to follow up with this NGO and report back to TTF.

Sarawak

Another key area in the 2010 Action Plan is Sarawak. Rambli Ahmad told the group that although *C. porosus* was the main focus of crocodile action in Sarawak, there are clearly some Tomistoma populations there. He suggested that training is required of forest officials, and also funding support. TTF members agreed that this is something TTF should follow up on.

Fundraising

The TTF was informed of a new non-profit organisation in the USA formed by Dr. Sam Seashole: the Crocodilian Conservation Institute, South Carolina. This organisation is keen to support Tomistoma and offers an effective way for us to raise funds within the USA. Dr. Seashole will be contacted to ensure that he is supportive of this.

The idea of having further fundraising events such as that held in Miami Zoo in 2006 (with a follow-up planned for 2013) was discussed. Colin indicated that whilst he and Bekky Muscher are in Singapore Zoo following this meeting, they will raise the issue with the curator, who has supported TTF in the past. Similarly, there is the opportunity to hold such an event at Madras Crocodile Bank in India. Colin will keep members updated on both issues.

Tomistoma Fund

The newly formed non-profit in the USA was discussed at a separate meeting with its founder Anthony Pine. The outcome was that this is an opportunity for TTF to collaborate with Anthony on fundraising for Tomistoma, as well as offering Anthony a scientific assessment group that he could run his projects past for review. Anthony will be added to the TTF Google group, and we look forward to positive collaborations with him.

Other

There was a call for us to reassess how we want Tomistoma to be perceived - both in our fundraising and awareness work. Tomistoma is not a publicly well-known name. Species such as the Saltwater Crocodile and American Alligator are well-known and readily identifiable to the public, and other crocodilians within the region are Critically Endangered, and hence will have the main focus of study proposals and fund-raising.

Suggestions were that we approach Tomistoma conservation from the habitat perspective: if we drive for peat swamp forest conservation, and use Tomistoma as a representative species within that habitat. This is something that will be discussed within the TTF group itself and an approach can be decided on.

The Tomistoma Husbandry Manual is still in preparation. Given the difficulty in breeding Tomistoma outside of range states, this manual will provide some important information on maintaining and breeding the species in zoos around the world. However, perhaps there is a need for TTF to research/compile some detailed breeding-specific information in order to really improve this facet of keeping Tomistoma and establishing proper breeding groups in zoos.

Summary

The CSGs Tomistoma Task Force has had some good progress toward several of the targets detailed in the 2010 Action Plan. This progress is set to continue in the following period, with several projects already lined up and good leads on other work as well as collaborations that will help us achieve our goals.

Siamese Crocodile Working Group

Participants: Heng Sovannara, Luon Nam, Sam Han, Oudomxoy, Kristian Robert Pahl, Steven Platt, Kumthon Suaroon, Bundit Kullavanijaya, Chanthone Phothitay, Charlie Manolis, Smith Thummachua, Terry Cullen, Yosapong Temsiripong

A meeting was held on 23 May 2012. Participants included government representatives from Cambodia, Lao PDR and Thailand, and researchers working in the Range States (see above).

Yosapong reported that Dr. Parntep Ratanakorn had asked him to discuss the establishment of a Siamensis Crocodile Task Force. The main goal of this Task Force would be to improve networking and communication about *C. siamensis* conservation activities within and between Range States. Proposed positions are: Chairman: Dr. Parntep Ratanakorn (Chairman), country contacts: Cambodia, Heng Sovannara; Vietnam, TBC; Indonesia, TBC; Lao PDR, Chanthone Phothitay; Thailand: Bundit Kullavanijaya, Yosapong Temsiripong.

Specific Terms of Reference will be developed as soon as the Task Force is approved by the CSG Executive. The Task Force would operate under the umbrella of the CSG. During the short period of the meeting, several issues were discussed, some of which could be addressed by the Task Force:

1. Capacity Building: regional training and workshop, community based conservation. Dr. Ratanakorn hopes to have a Sub-regional Workshop for the Siamensis Crocodile Task Force at Mahidol University, Bangkok. Training material will be developed. Community-based conservation has been developed in Lao PDR and Cambodia, which can be shared among Range States. Cross border co-operation has been discussed to stop international illegal trade in live *C. siamensis*.

2. Captive Management: standard marking system, hybridization, trade obstacles. marking system, Cambodia offer to mark wild crocodiles and released crocodiles with both microchip and scute-clipping. Other range states may have more systems. There are still other issues not yet discussed in the meeting for example, hybridization. There is a need to develop a better understanding of the degree to which hybrids can be discerned from external morphology (morphometrics, scale and colour). Farmer Association is another issue not yet discussed. However, Thailand and Cambodia have already established such associations. that work closely with Government.

3. Restoring wild population: re-introduction program. Terry Cullen offered to test DNA upon request. Some re-introductions have already occurred (eg Vietnam in 2000, Cambodia in 2004, and Thailand in 2005). The Thai Government plans to release in Kaeng Krachan National Park and is preparing an awareness program. There is no demand for collecting wild crocodiles into farms. Moreover, each country already has internal control of the trade and movement of the animals. Problem in Vietnam around Cat Tien NP is that poor people may occasionally poach wild crocodiles for food, not for commercial purposes. Robert Pahl proposed that each country set up core area for crocodiles, Cambodia consider stopping fishing in known crocodile habitat around Tonle Sap, and an incentive program for accidentally caught wild crocodiles in exchange for rice. Steve Platt suggested a population viability analysis to prioritize potential release sites.

In summary, Government policy in range states is to sustainably use this species by following the country master plan to continue re-introducing purebred *C. siamensis* into protected areas as well as facilitate trade of the species by helping legal trade in terms of issuing practical regulation and reducing trade obstacles. The transfer of the species from CITES Appendix I to Appendix II was discussed and proposal will be developed in parallel to the increasing wild population and better protection, which will need advice from CSG.

Jamaican Crocodile Conservation Working Group

During discussion of SC Agenda Item 2.8.1 (Jamaican Crocodile Conservation; prepared by P. Ross, B. Wilson, F. Mazzotti, M. Cherkiss, L. Henriques), the Chair asked Perran Ross, Allan Woodward and Joe Wasilewski to convene a working group and develop a recommended plan of action.

The working group met on 21 May, and following consultation with people who were not present at the meeting (Byron Wilson, Frank Mazzotti, Mike Cherkiss, Jeff Beauchamp), but who have been involved with the issue, the following "6-step" action plan was submitted.

The problem is not unique to crocodiles in Jamaica, but is one component of limited internal capacity and complex social and economic issues affecting the conservation of many Jamaican endemic and endangered species, including but not limited to: Jamaican iguana, Jamaican hutia, Yellow- and Black-billed parrots, "many species of endemic birds", skink species, Jamaican skink, ~80% of island's 21 endemic frog species, sea turtle species, Jamaican boa, American Crocodile.

In response, the CSG should do the following:

1. Identify external international NGO and government partners who have interest in any of these species [eg San Diego Zoo, International Iguana Foundation, Disney Company (Iguanas), AZA SSP (Dino Ferri and Jamaican boa), Amphibian groups (to be named), Audubon Society, WCS or WWF, Iguana Specialist Group, Sea Turtle Specialist Group, Bat Conservation International]. Mike Fouraker, Director of the Fort Worth Zoo, has recently established an NGO called the Caribbean Conservation Alliance.

2. Convene a meeting somewhere in the USA to form a consortium and strategize an approach. This meeting should take place in the next few months.

3. Reach out to identify and recruit internal (Jamaican) partners with interest and capacity/leverage to provide support for conservation efforts (any company that has a crocodile, turtle, bird or any other wildlife species in their logo).
   a) Sandals
   b) International Bank of Jamaica

c) Appleton rum
d) Red Stripe beer
e) Dole Company (?)
f) Air Jamaica
g) Royal Caribbean Cruise Line (or other cruise lines that visit Jamaica)
h) Ecotourism Lodges
i) Hope Zoo/Tour boat facility
j) Partnering within the entertainment industry (eg The Bob Marley Foundation)?

4. Initiate an outreach and public education program with Jamaican partners. Here, the primary link would be JET (Jamaican Environment Trust), run by Diana McCauley, arguably the island's most competent and effective environmental advocate. JET has an environmental lawyer on board.

5. Establish, fund and monitor effective protected areas at crucial locations (ie Hellshire Hills/Manatee Bay, Font Hill, Black River. Establishing the Hellshire Hills area as a "World Heritage Site").

6. Acquire funding to conduct a systematic countrywide survey to identify the current status of crocodiles and their habitat, particularly the extent, distribution and success of nesting.

Steps 1 and 2 would initiate this process. The CSG Steering Committee is requested to empower Perran Ross, Joe Wasilewski, Byron Wilson and Frank Mazzotti to start Step 1.