respectively. Hatchlings handled had the following abnormalities: missing parts of tail (0.26%), damaged eyes (0.07%), and missing feet (0.03%).

PIT tags used on alligators ≥ 1.52 m total length had a mean yearly retention rate of 0.836 (95% CI: 0.683, 0.946) (Table 1). Gun shots to the head of the alligators during harvest caused 45% of the losses. The remaining missing tags had apparently backed out of the injection hole soon after application. Monel web and PIT tags were placed simultaneously on 100 alligators of which 37 were recaptured within 60 days. No alligators were missing web tags. Using a binomial distribution and a 0.05 probability level in association with 0 cases of missing tags and 37 cases of retained tags, the retention rate up to 60 days was estimated to be no less than 0.922. Three of these same alligators were missing PIT tags. PIT tag retention averaged 0.919 (95% CI: 0.831, 1.000). Forty-nine of 77 adults were relocated under natural conditions and identified using the PIT tag only. Of these adults, 15 alligators were identified without handling using the water resistant probe and 3 of these were identified while still underground within the den.

A total of 204 previously marked hatchlings were reexamined over the 3-year study and yearly PIT tag retention was estimated at 0.972 (95% CI: 0.781, 1.00) (Table 1). All of these losses were for hatchlings handled within the first year of the study. Hatchlings marked and recaptured during 1999 (n = 23) also retained 100% of PIT tags, however, there were only a few weeks between recaptures.

One alligator with a cranial reflector was observed and re-identified using the reflective tag; this occurred 3 days after the original capture. Of the 18 alligators fitted with this tag type, 6 were rehandled at least 90 days after application and no scar or evidence of the tag or its placement remained. Using a binomial distribution and a 0.05 probability level in association with the 6 cases of missing tags and no cases of retained tags, the estimated retention rate after 90 days was no more than 39.3%.

The detection distance for harmonic radar tags used to relocate alligators was under 15 m when the tag was less than 2.54 cm from or below water level, and tag orientation effected signal strength. Overall, the tags detection range (m) was equal to 0.2717 times the height (cm) of the tag above water level (n = 60) but was reduced by 8.6 m when parallel to the receiver ($F_{2,57} = 239.42$, $P < 0.001$, $R^2 = 0.89$).

Table 1. Probability of an alligator retaining a passive integrated transponder (PIT) tag between recaptures from 1997 through 1999, Sabine National Wildlife Refuge, Louisiana. Size is the total length of alligators; Year is the year in which the alligator was originally tagged; year 1999_a includes alligators tagged with both web and PIT tags and were excluded from the 1999 sample; r_{ni} is the number of alligators reexamined within interval ∞; l_{ni} is the number of alligators missing a PIT tag at interval ∞; K_i is the probability of tag retention during the recapture interval ∞.

		∞ ≤ 1 Yr			**1 Yr < ∞ ≤ 2 Yrs**			**2 Yrs < ∞ ≤ 3 Yrs**		
Size	**Year**	$\mathbf{r_{ni}}$	$\mathbf{l_{ni}}$	$\mathbf{K_i}$	$\mathbf{r_{ni}}$	$\mathbf{l_{ni}}$	$\mathbf{K_i}$	$\mathbf{r_{ni}}$	$\mathbf{l_{ni}}$	$\mathbf{K_i}$
≥1.52 m	1997	21	3	0.857	22	5	0.773	22	6	0.727
	1998	12	1	0.917	13	2	0.846			
	1999	23	3	0.870						
	1999_a	21	4	0.810						
	All	77	11	0.857	35	7	0.800	22	6	0.727
< 1 m	1997	97	10	0.897	32	1	0.969			
	1998	52	0	1.000						
	All	149	10	0.933	32	1	0.969			

DISCUSSION

This manuscript describing the capture, handling and marking of alligators from southwestern Louisiana was written as a reference during the initial stages of starting a research project. Little literature exists that describes safe and effective methods to handling alligators.

We successfully captured a large number of hatchling and adult alligators using knowledge learned primarily through the process of trying. Our estimates of tag retention rates are preliminary and may not reflect those rates realized after many years, but to our knowledge these rates are some of the only ones available.

We found that safe and effective capture and handling of adult alligators was possible with an experienced 2 person crew from a boat. We learned several important lessons when handling alligators. Alligator should be allowed to spin freely prior to being restrained. Alligators mouths can be closed easily while pulling forward on the noose rope and stomping downward on the snout while the alligator is pulling backwards. And, heavy downward pressure over the chest and abdomen or lifting the alligator so it is air born only increases the amount of struggling.

Harassment of alligators out of dens, although time consuming, proved to be highly successful and allowed the capture of animals that would otherwise have been excluded. Care should be taken to agitate the alligator only to induce increased activity through the den roof and not to harass through the den entrance. Alligators appear to become more obstinate as they become aware of the consequence of leaving the den. Baited lines allowed capture of alligators in deepwater habitats that would have otherwise escaped capture. Alligators in deepwater habitat easily out maneuver the noose. The baited line allowed some control prior to noosing. Care should be taken to use a weak enough twine to prevent drowning, due to the line becoming entangled underwater, but strong enough to provide a noosing opportunity while the alligator is still underwater.

When marking alligators for later identification we found no perfect tag available. Within this study only web and hatchling PIT tags had retention rates approaching 100%. Our web tag retention rates, however, were based on only 60 days. Monel tags used on sea turtles also have demonstrated excellent short-term retention, and 100% of tags are suspected lost after a 10-year period (Limpus 1992). Alligators may possibly live well over 50 years (Hutton 1991), and farm-raised alligators are routinely harvested without web tags (Sabine National Wildlife Refuge, unpublished data). Additionally, monel tags that are appropriate for adults are not suitable for use on newly hatched alligators that are only 5% of adult size. Conversely, if an animal retains a PIT tag after the injection wound is healed, the probability it retains the PIT tag is expected to be relatively high and stable over time (Schooley et al. 1993).

Adult alligators have been marked routinely using what are considered permanent body marks consisting of toe clipping and notched tail scutes. Alligators, however, naturally lose various body parts including parts of feet and segments of tails. Our cranial reflectors, even when mechanically attached to the skulls of alligators, demonstrated very low retention rates; these tags were apparently sheared off leaving no scars or other indication of their presence. We found cranial mounted reflectors and radar tags were ineffective for alligators in this habitat.

PIT tags are a viable alternative to other tags for identifying individuals of all size classes. PIT tags allow for unlimited numbers of individuals to be marked and a potential for remote identification of individuals. Retention of 100% may be possible if care is taken where the PIT is applied. The placement in the earflap of adults made the tags susceptible to loss by migration and gunshot damage. The style of tag (i.e., true anti-migration characteristics) may also protect against tag loss. Alligators have a less sophisticated inflammatory response to foreign bodies than mammals and little swelling occurs following injection; the earflap and its restricted size allowed movement toward the injection site. Most preventable losses of PIT tags appear to occur prior to the healing of the injection wound, after this time minimal natural loss should occur. Sealing the injection wound prior to release may additionally help reduce PIT tag loss. We recommend that a different application site with more soft tissue, possibly the neck or cheek, be used in the future. The site should remain close to the head to augment remote reading unrestrained alligators.

Planning a large scale nesting ecology study was difficult due to a lack of information referencing alligator capture and handling techniques and tag retention rates. The goal of monitoring large numbers of individual female alligators and their young over successive years meant dependency on untried methodologies. The unexpected amount of time alligators spent in a subterranean environment (dens) had additional effects on the

ease of identifying individuals. Web tags proved to be an excellent tag for adults, however, required alligators to be handled to read tags and identify individuals. Every handling involves risks of injury to researchers and alligators. PIT tags were the only tags tested that provided for the individual identification of all size classes of alligators under various field conditions. PIT tags allowed the identification of individuals without handling or even seeing the animal, even with the limited range (< 7.5 cm) of the water resistant probe.

ACKNOWLEDGMENTS

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Stress in Farmed and Captive Crocodiles: Stressors and Effects

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ABSTRACT: The stressors of farmed and captive crocodiles consist of any limitations imposed on the expression of the natural behaviour of crocodiles: fear and the inability to escape a frightening event (capture, restraint, transport), the inability to thermoregulate (lack of environmental thermogradient) and the inability to establish or leave a territory (overcrowding). The effects depend on the severity and duration of the action of the stressor, on the age of the animal and on environmental conditions (immunosuppression by cold, malnutrition). Consequently, they may be expressed in different degrees of severity and they can be behavioural or somatic. The behavioural changes caused by stress are anorexia and hydrophobia or in less severe cases reduced appetite and growth rate. The somatic changes are immune suppression and septicaemia, gastric ulcers and chronic dermatitis, as well as metabolic disorders, particularly lowered calcium levels with resulting poor dental mineralization and osteoporosis.

INTRODUCTION

Stress is one of the natural defense mechanisms common to all vertebrates and it is designed to function in a natural environment. However, captive and farmed crocodiles are frequently subjected to conditions that may be severely stressful. Consequently stress, its mechanisms and its impact have been discussed repeatedly at previous meetings (Lance, 1994; Smith and Marais, 1994; Huchzermeyer, 2000a; Lance *et al*., 2000). The purpose of this paper is to identify important stressors commonly challenging captive and farmed crocodiles and to describe the clinical manifestations of stress in these animals.

STRESSORS

In the context of this paper stressors can broadly be defined as any limitations imposed by captive or farming conditions on the expression of the natural behaviour of crocodiles. These limitations can be seen as inabilities. The stressors can act acutely or over extended periods, chronically.

Fear

The inability to escape a frightening event whether expected or actually happening is commonly present in the following situations:

Hatchlings are afraid of being left in the open. Any disturbance induces them to hide under a cover or to escape into deep water. Even when kept indoors they do not perceive the ceiling or roof as protection. They need cover close to the ground (Fig. 1). The water usually is kept shallow because of the necessity of daily water changes and therefore cannot play a role as refuge either.

Figure 1. Nile crocodile hatchlings seeking refuge under a hide board

All crocodiles are frightened of being caught, restrained and handled, as this also leads them into an inescapable situation. Also affected are the crocodiles

witnessing the capture of their pen mate, being unable themselves to escape. The transport of a caught and restrained crocodile is just another extension and prolongation of the inescapable situation.

Thermoregulation

Thermoregulatory behaviour is essential to the well-being of crocodiles. In the natural environment there always is a thermogradient along which the crocodile can choose and adjust its internal temperature. In farming and captive situations such a thermogradient usually is greatly reduced or entirely absent. Suboptimal as well as excessive temperatures occur quite commonly. The inability to thermoregulate under such circumstances is a very common and severe chronic stressor.

Territoriality

In hatchlings and juveniles territoriality is more a function of competition for food while in sexually mature animals the establishment of a breeding territory becomes important, probably with large species-specific differences. In older subadult juveniles there may also be the inborn need to disperse and migrate (Hutton, 1989). In captive and farming situations there is an inability to escape from the other pen mates, to disperse or to establish an own territory when chased away by a stronger or more dominant individual. Consequently overcrowding has long been identified as a major source of stress (Elsey *et al*., 1990; Morpurgo *et al*., 1992).

CLINICAL EFFECTS

The combined effects of several stressors are additive and their effects depend not only on their individual or combined severity and duration but also on the physiological and nutritional state of the animal subjected to the stressor(s). In addition environmental factors can also play a role, viz. the immune suppression by cold. The effects can be behavioural and/or somatic.

Behavioural

In crocodiles, the range of behavioural responses to stress is relatively restricted. Most commonly seen are hydrophobia, the refusal to go into the water, and anorexia, the refusal to feed. Usually the two are combined. In a less severe form the anorexia leads to a lowered feed intake and thereby to a reduced growth rate. It is suspected that the swallowing of large quantities of stones and other foreign bodies could also be a form of stress behaviour.

Somatic

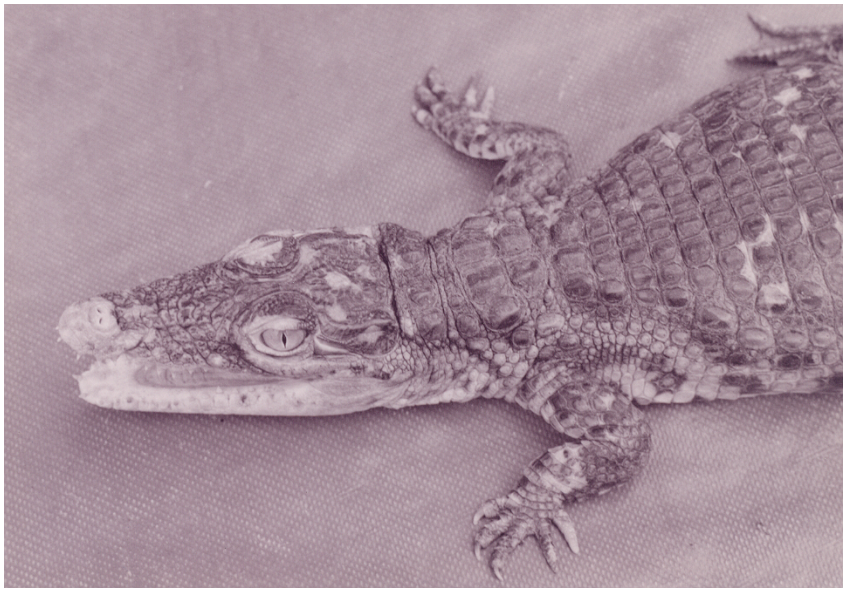

Figure 2. Chronic stress dermatitis characterized by white patches of desquamation, particularly around nostrils and eyes

There is clinical evidence at least in ostriches and crocodiles that under severe stress intestinal bacteria can become translocated through the mucosal barrier into the blood circulation and cause septicaemia, similar to the shock septicaemia in human trauma and burn patients (Deitch *et al*., 1996). This septicaemia is aggravated by the immune suppresion caused by high corticosteroid levels in the circulation, particularly under prolonged or chronic stress or under conditions of cold and frequently it develops into a polyarthritis (Huchzermeyer, 2000b). Gastric ulcers found in severely stressed crocodiles may partially be a sequel of anorexia. They are commonly associated with a chronic dermatitis characterized by the formation of white patches of desquamation (Fig. 2) and sometimes also by an extremely severe polyarteriitis (Huchzermeyer and Penrith, 1992). The "chronic stress dermatitis" mentioned above may occur also on its own without stomach ulcers.

A stress-induced metabolic disorder is the reduction of circulating calcium levels (Morpurgo *et al*., 1992). Clinically this becomes visible as poorly calcified teeth (Fig. 3) and pathologically as osteoporosis. Initially this osteoporosis had been mistakenly diagnosed by myself as exostoses possibly caused by an overdose of vitamin D (Huchzermeyer, 1999). It real nature became clear when I stared to see many more cases. Decalcification of teeth was an outstanding sign in adult breeding Nile crocodiles which had been translocated during winter and were subsequently dying from stress septicaemia (own unpublished case). However, lowered calcium levels with similar effects could also be due to the difficulty of digesting small particles of bone (bone meal or minced whole chicken) because of their rapid passage through the stomach, before the bone is dissolved by the gastric acid. Glassy teeth are also seen in cases of osteomalacia caused by lack of nutritional calcium (Huchzermeyer, 1986).

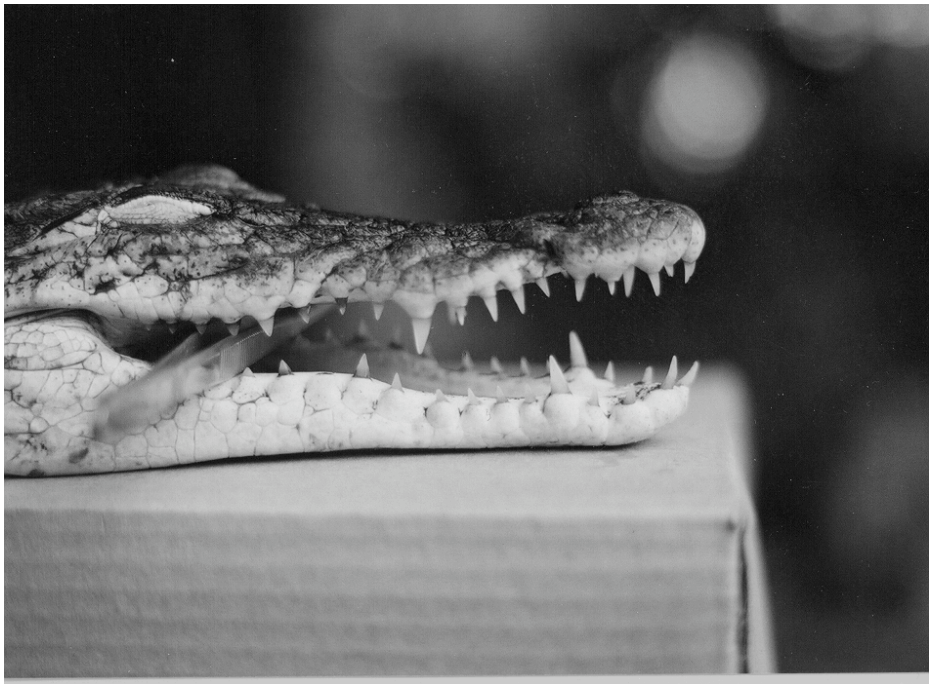

Figure 3. Decalified teeth in a chronically stressed slaughtered Nile crocodile

CONCLUSION

Very few contagious diseases cause high mortality rates on crocodile farms. Instead, most outbreaks are either caused or aggravated by severe and chronic stress. Being able to diagnose the effects of stress and to identify the stressors is the first step towards the prevention of stress-induced disease and mortality.

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Tissue Lead Levels in Captive-Reared Alligators

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ABSTRACT: Alligators from artificially incubated eggs were reared in captivity since hatch in 1972 and 1973 at the Rockefeller Wildlife Refuge. This group of alligators has been the subject of intense study on captive reproduction: they were first observed to reproduce at 6 yr, but egg quality, hatchability and fertility declined over the years. By 1998 successful reproduction in this captive group had essentially ceased, but many of the females continued attempts at nesting. In 1999 they were euthanized and complete necropsies were performed. All females had extensive reproductive tract pathology. The males appeared normal. However, upon examination of stomach contents lead pellets were discovered in both males and females. It was determined that the lead pellets came from the ground nutria (*Myocastor coypus*) meat that the alligators had been fed over the years. Liver, kidney, muscle, bone, and yolk from large preovulatory follicles were collected for analysis. For comparison, tissue samples from wild alligators were used. The tissue samples were digested in concentrated nitric acid and analyzed on a graphite oven atomic absorption spectrophotometer for lead, chromium and selenium. Tissue chromium levels were very low in both wild and captive samples and were not significantly different from one another. Liver lead concentrations in the captive group, however, were extraordinarily high, higher than any previously recorded values in reptiles. Lead in the liver of the wild alligators was very low. Lead was also very high in the yolking follicles of the captive alligators

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Antimicrobial Activity in the Blood of the Saltwater Crocodile, *Crocodylus porosus*

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ABSTRACT: The saltwater crocodile shows a low incidence of infection from serious injuries sustained during intraspecific aggression, in spite of the microbe-laden environment in which it lives. This suggests a well-developed innate immune system, which provides a rapid, non-specific first line of host defense. In other aquatic species such as amphibians and fish, this defense is found in the mucous skin secretions as antimicrobial peptides. Due to the anatomy of the crocodile, we reasoned that a homologous defense would be found in the circulatory system, either as soluble factors or as agents expressed in phagocytic cells. To address the first hypothesis, we extracted serum from wild saltwater crocodiles to isolate naturally occurring antibiotics. The serum was maintained at -80C until fractionation. Serum was fractionated by Reverse-phase HPLC on a C-18 column with a 0-60% acetonitrile gradient, and fractions were assayed for antibiotic activity against *E. coli* in a modified radial diffusion assay. Preliminary results indicated strong antibiotic activity in several fractions. We have taken a single fraction, eluting at 13% acetonitrile, for further characterization. Based on our initial observations, we predict that the crocodile exhibits both peptide and non-peptide based antimicrobial activity in its blood.

Antibacterial Properties and Complement Activity of Serum in the American Alligator (*Alligator mississippiensis*)

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ABSTRACT: Treatment of alligator or human serum samples with E. coli resulted in a 10-fold lower bacterial survival rates in the alligator serum after 1 hour. When inoculated with E. coli, alligator serum exhibited a time- and concentration-dependent inhibition of bacterial proliferation. In addition, the antibacterial spectrum of alligator serum was shown to be much broader than that of human serum, with growth inhibition occurring in 100% of bacterial strains tested (compared to only 35% for human serum). Additional results showed that the antimicrobial activities of alligator serum could be completely inhibited by preincubation with proteases, indicating the proteinaceous nature of the antimicrobial activities. Furthermore, incubation of serum at 56^{o}C for 30 min. (classical human complement inactivation conditions) obliterated all antimicrobial properties of the alligator serum. Immunofixation of electrophoretically-resolved alligator serum proteins with antihuman C3 polyclonal antibodies resulted in recognition of alligator C3 protein. Incubation of alligator serum with sheep red blood cells in the presence of antihuman C3 antibodies reduced complement activity by 60%. These data suggest that the antibiotic properties of alligator serum are likely due to the presence of a complement-facilitated humoral immune response analogous to that described in mammalian systems. The complement activity in alligator serum is shown to be much more potent and has a broader spectrum of activity than human serum. This study represents the first detailed characterization of reptilian complement protein system and may provide a partial explanation for disease resistance in the American alligator.

INTRODUCTION

Eukaryotic organisms must continuously defend themselves against infiltration and colonization by microorgamisms. The humoral immune response comprises a significant portion of the immune system and acts as an initial defense mechanism against microbial growth shortly after infection occurs. The serum complement system, an important component of the humoral immune response, is composed of 25-30 proteins that can be activated to initiate the inflammatory response, recruit leukocytes to the site of infection, mediate opsinization of particulate foreign materials and to kill microorganisms directly by the assembly of a multiprotein membrane attack complex in the outer membrane of microbes (Muller-Eberhard, 1986, Dalmasso et al., 1989). Because of the immunological importance of the serum complement system, a deficiency or mutation in any complement protein is almost always associated with multiple recurring infections (Morgan and Walport, 1991, Pascual and French, 1995).

Complement proteins are expressed and circulated as inactive precursor proteins that can be activated in a very precise and highly coordinated fashion (Campbell et al., 1988). The complement cascade can be initiated by two distinct mechanisms, an antibody-dependent classical pathway and an antibody-independent alternative pathway, that result in the modulation of immune function. The serum complement system has been well-characterized in humans as all of the proteins have been purified to homogeneity, their functions in each pathway identified, and their genes isolated (Campbell et al., 1988). Although several studies have reported the

presence of complement components in a variety of reptiles (Koppenheffer, 1987), the serum complement system is not well-characterized in reptilian systems. The results from this study provide evidence for a potent and broad-acting complement system in the serum of the American alligator (*Alligator mississippiensis*).

MATERIALS AND METHODS

Chemicals and biochemicals. Nutrient broth and nutrient agar were purchased from ISC Bioexpress (Kaysville, Utah). Lyophilized ATCC bacterial strains were purchased from Chrisope Technologies (Lake Charles, LA). The following ATCC-registered strains were used: Klebsiella oxytoca (49131), Providencia stuartii (33672), Escherichia coliform (25922), Proteus mirabilis (43607), Enterobacter aerogenes (49469), Salmonella typhimurium (14028), Pseudomonas aeruginosa (27853), Citrobacter freundii (C109820), Shigella sonnei (25931), Shigella dysenterae (13313), Salmonella poona (4840), Yersenia enterocolitica (9610), Staphylococcus pyrogenes (19615), Streptococcus epidermitis (19615), Staphylococcus aureus (6538), and Enterococcus faecalis (29212). Protease derived from Streptomyces griseus was purchased from Sigma Chemical Company (St. Louis, MS). Goat antihuman C3 polyclonal antibodies were obtained from Rockland Immunologicals (Gilbertsville, PA).

Treatment of animals. Alligators were captured and housed at the Rockefeller State Wildlife Refuge in Grand Chenier, Louisiana. Numerous juvenile alligators, which were hatched in captivity from eggs collected in the wild, were maintained at Rockefeller Refuge in fiberglass-lined concrete tanks approximately 12' long x 6' wide. Several small alligators (up to 3' in length) are housed in a single tank. Adult alligators were typically captured at night with use of a wire snare.

The environment in the tanks consisted of 50% dry bottom and 50% water of approximate 6" depth. The temperature is maintained at a constant 87-88ºF. The alligators are fed formulated dry pellets four times per week and the cages are cleaned five times per week. Blood samples were drawn from the supravertebral branch of the internal jugular vein using a heparinized 1_" 18 gauge needle and a 10 ml syringe (Olsen et al., 1977) and transferred to either serum or plasma (50 mM EDTA) vacutainer tubes.

Bacterial cultures. Bacteria were maintained on nutrient agar slants at 4^{o}C. The day before an experiment, a 4 ml nutrient broth liquid culture was inoculated from the slant with a sterile cotton swab. The bacteria were allowed to incubate at 37^{o}C overnight to obtain log-phase culture. Serial dilutions of the log-phase cultures were plated on nutrient broth agar in 100 mm Petri dishes to determine the colony-forming units (CFUs) in each culture.

Determination of CFU. Fifty µL of a dilution of each sample was spread onto the surface of nutrient broth agar plates to determine the CFUs for each sample. Samples were typically plated at three different dilutions to obtain plates with a quantity of colonies such to provide a reasonable estimate of bacterial density (50-400 CFUs/plate).

Concentration-dependent antibacterial properties of human and alligator sera. Two ml samples containing various concentrations of human or alligator sera in 12 x 75 mm culture tubes were inoculated with approximately 1 x 10^5 E. coli bacteria from a log phase culture and incubated for 12 hr at 37^{o}C. The optical density of each sample was measured at 0, 3, 6, and 12 hrs using the Varian Cary 50 spectrophotometer at 430 nm.

Antibacterial capacity determination. E. coli cultures in log growth phase were used to make 10-fold serial dilutions in sterile saline. Fresh alligator or human sera samples (450 µl) were treated with 50 µl of bacterial culture or a dilution containing different amounts of bacteria. The samples were incubated at 37^{o}C for 1 hr and the CFUs for each culture was determined by the solid medium bacterial growth assay as described above.

Effects of serum on the growth of different strains of bacteria. Nutrient agar was dissolved in boiling water and 30 ml aliquots were transferred to 70 ml culture tubes, autoclaved, and held in liquid phase in a 45^{o}C water bath. The aliquots of sterile nutrient broth agar were inoculated with 100 µL of a log phase culture of one of various bacterial strains and then dispensed into 145 x 20 mm Petri dishes. After the agarose set, wells were cut with the large end of sterile, cotton-plugged Pasteur pipettes attached to a vacuum line. Twenty-five µL of

serum was pipetted into each well and allowed to incubate at room temperature for 3 hrs. Another 30 ml aliquot of sterile nutrient broth agar (45^{o}C) was poured onto the top of the original agar layer and allowed to set. The plates were incubated in an inverted position overnight at 37^{o}C and the zones of bacterial growth inhibition were measured. For slower growing species of bacteria, plates were incubated for 48 hrs to observe the results.

Immunofixation Assay. Detection of C3 complement protein in human and alligator serum was achieved by immunofixation using a SPIFE Combo protein analyzer (Helena Laboratories, Beaumont, Texas). The analysis was performed according to the manufacturer's instructions, with the exception that goat polyclonal antihuman C3 antibodies were used for immunofixation. The human serum was analyzed at a 1:3 dilution while the alligator serum was analyzed undiluted.

Sheep Red Blood Cell (SRBC) Hemolytic Assay. The functionality of the alligator serum complement system of proteins was investigated by a SRBC lysis assay modified from the method of Mayer (1967). Six hundred µL of 1% SRBCs were mixed with 700 µL of undiluted alligator serum. The incubate was increased to a total volume of 1500 µL using saline, or a C3 antibody solution. The optical density of the samples was measured at 525 nm using a Varian Cary 50 UV/Vis spectrophotometer.

Statistics and controls. All experiments were performed in quadruplicate to obtain valid statistical evaluation of the results. CFUs/ml for each sample were calculated by multiplying the number of colonies counted by the dilution factor and then by ten (due to the fact that only 50 µL were plated on each dish). Zones of growth inhibition were measured and the width of the well subtracted prior to statistical calculations. All results represent the means ± standard errors.

RESULTS

Table 1 exhibits the effects of heat-treatment and preincubation of alligator and human sera with proteases on the antibacterial effects. Treatment of human or alligator sera for 30 min. at 56^{o}C completely obliterated antimicrobial activities, as determined by a solid phase growth inhibition assay as described in the Materials and Methods. In addition, pretreatment of a 100 µL sample of human or alligator serum with 20 uL of a mixed protease cocktail eliminated antimicrobial activities.

Table 1 Effects of Protease- and Heat-treatment on the Antibacterial Activity of Human and Alligator Serum. Twenty µL of each serum sample were transferred to 6 mm wells cut into nutrient agar that had been inoculated with E coli. The samples were allowed to incubate in the wells for 3 hr, nutrient top agar was poured over the surface, and the plates were incubated overnight at 37^{o}C. The results are expressed as the means ± standard errors for four determinations.

	ZONE OF GROWTH INHIBITION (mm)
Human Serum	
Untreated	***2.3 ± 0.4***
Heat-treated	***ND***
Protease-treated	ND
Alligator Serum	
Untreated	6.5 ± 1.0
Heat-treated	***ND***
Protease-treated	***ND***

Inoculation of nutrient broth with approximately 10^6 CFUs of E. coli resulted in a time-dependent increase in bacterial proliferation as measured by spectrophotometry at 430 nm (Fig. 1). Inclusion of different concentrations of human or alligator serum in the broth produced a concentration-dependent decrease in bacterial growth. 10% alligator serum reduced bacterial growth by 84% at 3 hours, compared to only a 47% reduction by human serum (Figures 1A and 1B). The addition of 10%, 25%, 50%, 75%, and 100% alligator serum resulted in statistically significant ($p<0.001$) decreases in bacterial growth at all time points after zero. Inoculation of 100% alligator serum that had been thermally inactivated (56^{o}C, 30 min) produced only a 16% decrease in growth as compared to 97% growth inhibition by serum which had not been heat-treated (data not

shown). Likewise, the growth of bacteria in 100% human serum that had been heat-treated showed only a 18% reduction in growth, relative to nutrient broth cultures (data not shown). The ability of thermally-inactivated alligator serum to inhibit bacterial proliferation demonstrates that the antibacterial effect is due to the presence of a heat-labile factor(s) and not to the dilution of nutrient in the growth medium.

Figure 1A

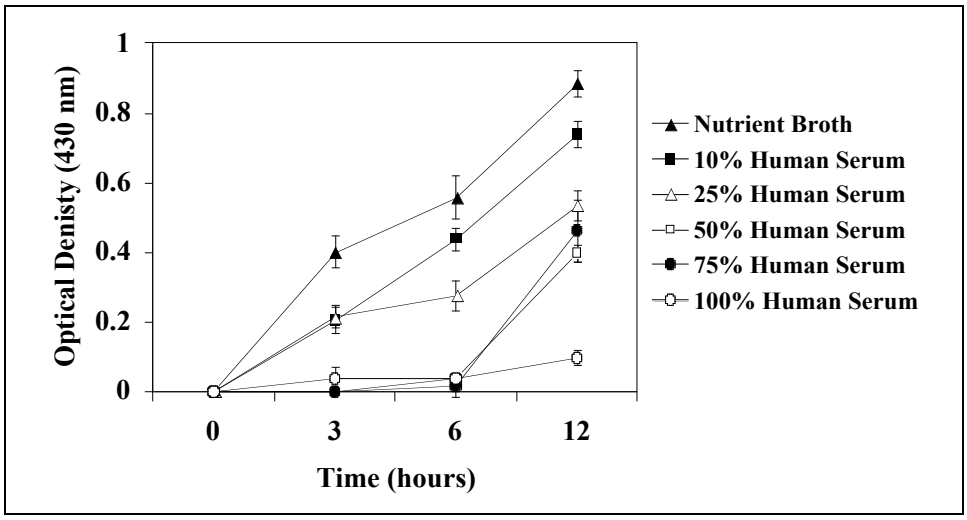

Figure 1B

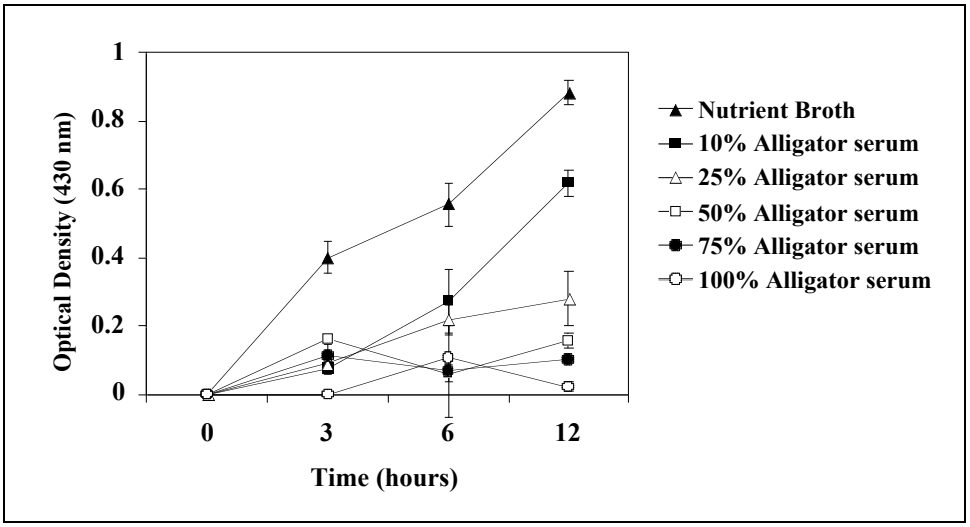

Figure 1. Concentration-Dependent Inhibition of Bacterial Growth by Human and Alligator Serum. Culture tubes containing 2 ml of 0, 10, 25, 50, 75, or 100% human (1A) or alligator serum (1B) in nutrient broth were inoculated with 10^5 E. coli. The cultures were incubated at 37^{o}C and their optical densities were measured (430 nm) at 0, 3, 6, and 12 hr post-inoculation.

Measurement of the antibacterial capacity of human and alligator serum (Fig. 2) revealed that alligator serum killed approximately 11- or 9-fold more bacteria when serum samples were challenged with 104 or 103 CFUs/ml, respectively. Inoculation of alligator serum with 105, 104, or 103 CFUs/ml of E. coli resulted in a stepwise decrease in bacterial survival of 54, 3, and 5%, respectively. In contrast, treatment of human serum resulted in 66, 44, and 44% survival for the same inoculations. Inoculation of cultures with 106-108 CFUs of E. coli overloaded the capacity of the serum complement to kill the bacteria, resulting in 100% bacterial survival in all three sample groups for both human and alligator serum. However, inoculation of serum with 105 CFUs/ml resulted in a 46% and 34% decrease in bacterial survival for alligator and human serum, respectively.

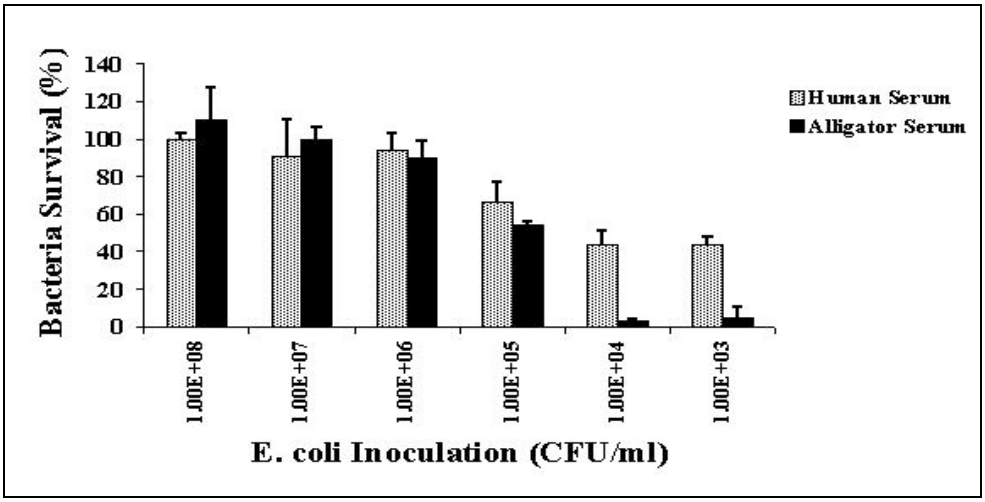

Figure 2. Bactericidal Properties of Human and Alligator Serum. Serial dilutions of a log phase E. coli culture were added to 0.5 ml serum samples and incubated for 20 min. Dilutions of each sample were plated to determine CFUs. Results are expressed as bacterial survival ([CFUs after incubation/CFU added to sample] x 100.

Treatment of 17 different strains of bacteria (representing 13 different genera) with alligator serum resulted in 100% effectiveness of growth inhibition (Fig. 3). In comparison, human serum was only effective on 35% of bacterial strains. Human serum exhibited antibacterial activities toward only 6 of 13 gram negative strains and was completely ineffective against gram positive strains. Furthermore, the degree of antimicrobial activity was significantly lower for all bacterial strains tested in human serum than that of the alligator.

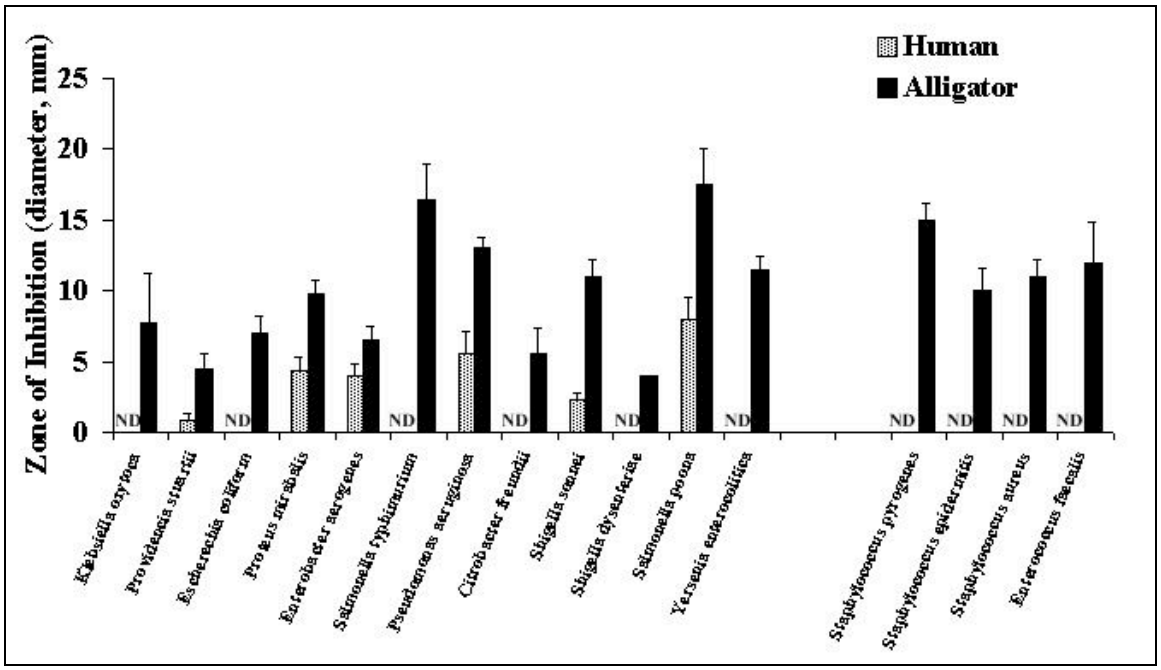

Figure 3. **Antibiotic Spectrum of Human and Alligator Serum**. Aliquots of nutrient agar in liquid phase (45oC) were inoculated with one of various strains of bacteria and poured into 125 mm Petri dishes. Samples of alligator or human sera were transferred into aseptically formed wells. After a 3 hr incubation at room temperature, top agar was poured over the plates and the samples were incubated overnight at 37oC. ND = not detected.

The native agarose gel electrophoretic analysis of human and alligator serum revealed remarkably similar protein patterns (Fig. 4). The albumin band in the alligator serum constituted only 25% of the total serum protein, as determined by densitometry, compared to approximately 50-60% for human serum. The albumin

band migrated slightly more anodal than that of the human serum. Immunofixation of the human C3 protein showed a single band just cathodal to the application point on the gel (Fig. 4, lane 2). The same antibodies (goat antihuman polyclonal C3) detected a single band in alligator serum (Fig. 4, lane 4) that migrated slightly more anodal than the human C3. The appearance of only one band indicates the specificity of human C3 antibodies for the alligator C3 protein. The interaction of the alligator protein with the human C3 antibodies and the similar migration of the protein band lead us to believe that this protein represents a crocodilian complement component analogous to the mammalian C3 protein.

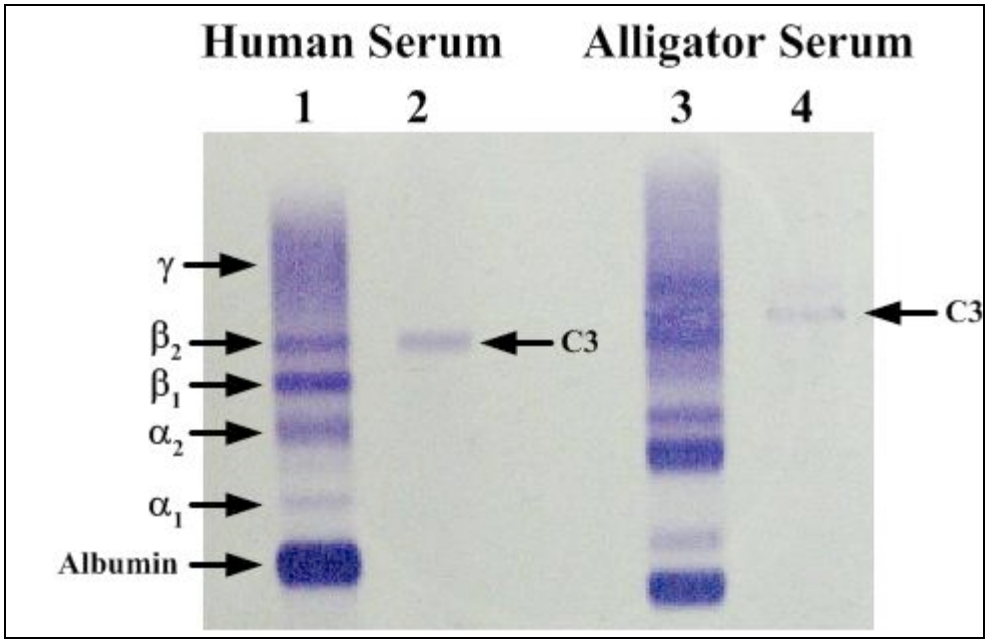

Figure 4. Detection of Serum Complement C3 Protein in Human and Alligator Serum. Serum samples were resolved on a 1.2% native agarose gel and the total protein was precipitated using 5% acetic acid (lanes 1 and 3). Protein was visualized using Acid Violet Coomassie-type stain. The cathodic C3 protein band was detected by immunofixation (lanes 2 and 4) using goat antihuman C3 antibodies.

The data in listed in Table 2 illustrate the ability of alligator serum to disrupt SRBCs in vitro. Incubation of 50% alligator serum with 1% SRBCs (v/v) resulted in a 60% increase in the optical density at 525 nm. The serum-mediated hemolysis of SRBCs was decrease 40% by the addition of antihuman C3 polyclonal antibodies.

Table 2. Effects of C3 Antibodies on Alligator Serum Complement . Alligator serum was incubated with sheep red blood cells in the presence or absence of antihuman C3 antibodies. The resulting hemolytic activity was measured spectrophotometrically at 525 nm.

Sample	Abs (525 nm)
Alligator Serum	0.347 ± 0.010
Heat-treated Serum	0.092 ± 0.003
Serum + antiC3 antibodies	0.140 ± 0.009

DISCUSSION

The immune systems of crocodilians have not been well-characterized. However, several descriptive reports have described the presence of cellular components of the immune system in the American alligator. Cuchens and Clem (1979) have reported the presence of B-like and T-like lymphocytes in the alligator. In addition, Mateo and coworkers (1984) described the morphological and cytochemical characteristics, and relative abundance of various types of alligator peripheral blood cells. However, no description of a humoral immune response has been reported in alligators.

Several investigators have reported the presence of an active serum complement system in a variety of reptilian species (Koppenheffer 1987, Sunyer and Lambris 1998, Sunyer et al. 1998). For instance, Kuo and coworkers (2000) described the complement-mediated killing of the Lyme disease spirochete (Borrelia burgdorferi) in the western fence lizard. Koppenheffer (1986) has demonstrated the presence of both classical and alternative pathways in turtle serum. Other studies have focused on the complement activities in snake serum (Kawaguchi et al., 1978, Dias et al., 1984). The most extensive characterizations of reptilian complement have been in the cobra (Vogel and Muller-Eberhand, 1985a, Vogel and Muller-Eberhand, 1985b, Fritzinger et al., 1992). Sharbanay et al. (1999) described the antibacterial activity of crocodile serum. However, to our knowledge, a detailed functional description of crocodilian complement has not been published to date.

Alligators seldom exhibit detrimental health conditions due to infection. Alligators often sustain serious injuries and, despite the septic conditions under which they live, heal very rapidly and almost always without infection. Crocodilians have been known to live with opportunistic pathogenic bacterial infections but exhibit no physiological effects (Manolis et al., 1991, Madsen, 1993, Madsen et al., 1998). While crocodilians are not completely immune to microbial infections (Gorden et al, 1979, Novak and Siegel, 1986, Brown et al., 2001), these species do exhibit remarkable resistance to microbial colonization. The results from this study provide the first evidence that the American alligator has an active serum complement system.

Human complement contains heat-labile proteins that are thermally inactivated when incubated at 56^{o}C for 30 minutes. The fact that the antibacterial activity of alligator serum can be obliterated under these conditions (Table 1) provides circumstantial evidence that these activities are due to the presence of a crocodilian complement immune system component. The concentration-dependent increase in antibacterial activity by alligator serum (Figure 1) also suggests the presence of complement activity. The inclusion of a thermally-inactivated 100% alligator and human serum controls show that the bacterial growth inhibition is due to the effect of a heat-labile serum factor(s) and not dilution of the nutritive value of the liquid growth medium by the addition of increasing amounts of serum.

The data listed in Table 1 provides evidence that the antimicrobial activities observed in alligator blood are due to the serum complement system of protein present in higher eukaryotes but not yet characterized in reptilian systems. Incubation of alligator serum at 56^{o}C for 30 min. (classical conditions for the inactivation of human complement) completely depletes the serum of antibacterial activities. In addition, pretreatment of serum with proteases also diminishes the antimicrobial character indicating that these activities are dependent on the action of protein(s).

Results from the investigation of the range of antibacterial activity demonstrated that alligator serum has a much broader spectrum of antibacterial activity than that of human serum (Fig. 3). Human serum complement proteins are typically ineffective against gram positive bacterial strains. However, the alligator complement was highly effective as an antibacterial agent against all four gram positive strains tested (Fig. 3).

Complement protein C3 plays a pivotal role in the activation of the serum complement activation by both the classical and alternative pathways. C3 is a 195 kDa protein present at 1.3 mg/ml in human serum, making this protein the most abundant of the complement factors. The data depicted in Fig. 4 reveal the presence of C3 complement protein in alligator serum. The densitometric analysis of the C3 bands (data not shown) show a 2.3-fold decrease in intensity of the C3 band in alligator serum despite a three-fold increase in sample load on the gel. Although the interactions are relatively weak, the fact that antibodies directed toward human C3 protein bind to alligator C3 complement shows the similar surface antigenic character of human and alligator proteins. These findings contrast those reported by Eggersten et al. that showed no cross-reactivity of human C3 antisera to cobra C3 complement protein. In addition, the same approximate cathodal migration on the native agarose gel reveals a similar charge/mass ratio for the alligator and human C3 proteins. The presence of the C3 protein in both humans and alligators exhibits the immunological importance of this protein in host defense.

The use of sheep red blood cell (SRBC) hemolytic assays has been used for years to assess the functionality of serum complement in the clinical laboratory. The data shown in Table 2 exhibits the ability of human and alligator serum to disrupt SRBCs. The ability of alligator serum to hemolyse SRBCs is another similarity of the complement-like activities of alligator and human sera. The incubation of sera at classical complement inactivation conditions (56oC, 30 min.) disabled the hemolytic capacity of the samples. The addition of C3 antibodies neutralized the ability of the serum complement system from both species to hemolyze the cells.

The study describes potent and broad-spectrum antibacterial activities in the serum of the American alligator. The results also represent the first report of serum complement activity in a crocodilian species. The complement system is shown to exhibit similar functional and molecular properties of mammalian complement. We believe that the complement system may be responsible for the antibacterial properties of alligator serum.

ACKNOWLEDGEMENTS

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Pix Skin Disease in the American Alligator (*Alligator mississippiensis*)

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ABSTRACT: "PIX" is the trivial name for a type of scar found on alligator hides. PIX scars are small pits about 1mm in diameter, located primarily in the ventral neck, abdomen and tail region, and designated as PIX by the tannery because they appear to be small pits in the hide made with an ice pick. The incidence of PIX in alligator hides from farms has dramatically increased since November of 1999. The potential seriousness of an increased incidence of PIX becomes apparent when the hides are presented for grading, where damaged hides may be downgraded by as much as 50% and more recently, some hides were branded as total rejects (no value). Hides from alligators greater than four feet in length have the highest incidence of PIX, which indicated that an increase in the incidence of PIX was associated with increases in age of the alligator and suggests prolonged exposure of the animals to an infectious agent. Histological studies showed that the PIX pit results from eruption of an epidermal granuloma. Therefore, epidermal granulomas are considered to be pre-PIX lesions. *Hortaea werneckii*, a primary pathogenic fungus found in the skin of animals has been isolated from a granuloma found in a severe case of PIX. Studies on the occurrence of PIX scars on hides from farms with a high incidence of PIX showed that more than 40% of the hides from a single lot may be severely affected and that 100 or more scars can be present on a single hide. This finding suggested that an increased incidence of PIX may follow a systemic mycosis caused by a primary pathogenic fungus such as *Hortaea werneckii*. Milder PIX infections may be caused by a systemic mycosis of opportunistic fungi that are of lower pathogenicity but can infect a host with a weakened immune system. Our studies indicate that good sanitation and the treatment of pen water with an antifungal agent such as chlorine should reduce the severity of PIX infections. Preliminary results indicate that these preventative measures are effective.

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Environmental Contaminants, Gonadal Development and Alligators: From Genes to Populations

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ABSTRACT: Many chemicals introduced into the environment by humans adversely affect embryonic development and the functioning of the vertebrate reproductive system. It has been hypothesized that many developmental alterations are due to the endocrine disruptive effects of various ubiquitous environmental contaminants. The endocrine system exhibits an organizational effect on the developing embryo, altering gene expression and dosing. Thus, a disruption of the normal hormonal signals can permanently modify the organization and future functioning of the reproductive system. We have worked extensively with contaminant-exposed and reference populations of the American alligator (*Alligator mississippiensi*s) as well as performed a number of experimental studies exposing developing embryos to various persistent and non-persistent pesticides. Using this specie*s*, we have described altered steroidogenesis and follicular morphology in the ovary of females living in polluted environments. A number of these alterations mimic those reported in DES-exposed rodents. Egg dosing studies, with total egg pesticide exposures of 100 picomolar or greater have produced alterations in gonadal steroidogenesis, secondary sex characteristics and gonadal anatomy that are similar to those reported in wild populations. Further, studies of males have documented differences in testicular release of inhibin B and gene expression of $P450_{arom}$ and SF-1, using homologous probes and quantitative rtPCR. Following our the recent cloning of alligator estrogen receptor alpha and beta ($ER_{\alpha,\beta}$), as well as the progesterone (PR), thyroid (TR_{β}) and androgen (AR) receptors, studies have begun to examine ontogenic and environmental influences on ovarianand testicular expression and function[1]. In summary, these descriptive and experimental studies have begun to provide an understanding of the causal relationships between embryonic pesticide exposure and reproductive abnormalities that have been lacking in previous field studies of wildlife populations. An understanding of the developmental consequences of endocrine disruption in wildlife can lead to new indicators of exposure and a better understanding of the most sensitive life stages and the consequences of exposure during these periods for other crocodilians. Research funded in part by grants from the US EPA (#R824760-01-0; CR826357-01-1) and the NIEHS (#PR471470).

[1] Work performed in collaboration with the laboratories of Prof. Taisen Iguchi, National Institutes of Basic Biology, Okazaki Japan, Dr. Caren Helbing, Dept. Biochemistry and Molecular Biology, Victoria University, Canada and Dr. Teresa Woodruff, Biochemistry and Molecular Biology, Northwestern University

Developmental Alterations as a Result of Embryonic Exposure to the Pesticide Metabolite p,p'-DDE in *Alligator mississippiensis*

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ABSTRACT: Altered circulating hormone concentrations and *in vitro* steroidogenesis, and reduced phallus size have been reported in several juvenile alligator populations Florida. It has been hypothesized that these abnormalities are the result of contaminant exposure during embryonic development. Organochlorine pesticides such as DDT and its metabolites DDE and DDD have been implicated as being hormonally active—possibly acting as environmental estrogens or anti-androgens in developing embryos. The purpose of this study was to determine the effects of *in ovo* exposure to DDE on neonatal alligator development and endocrine function. Eggs were obtained from Lake Woodruff National Wildlife Refuge and treated topically with either p,p'-DDE or estradiol-17β (E_2) in 50 μl of ethanol at doses of 100, 0.1, or 0.0001 μg/kg just prior to the temperature sensitive period of sex determination. Following incubation at 32° C, untreated and ethanol treated (vehicle control) eggs resulted in 60 % females, whereas 33.5° C produced 97 % males. DDE altered sex determination at 32° C only at the highest concentration used (87.5 % females) and did not affect sex determination at 33.5° C, whereas E_2 produced a greater percentage of females at both temperatures. There were no detectable differences in plasma testosterone (T) or *in vitro* E_2 production among any of the groups. Thyroid to body mass and spleen to body mass ratios were greater in DDE treated animals than controls at 32° and 33.5° C respectively, whereas E_2 increased spleen and liver mass respective of body mass at 33.5° C. These results indicate that DDE has weak estrogenic or anti-androgenic effects on sexual differentiation and development, but does not explain some of the alterations in endocrine function reported in contaminant exposed populations.

Thiamine Status and Mortality of Adult American Alligators (*Alligator mississippiensis)* in Lakes Griffin and Woodruff in Central Florida during 2000 and 2001.

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ABSTRACT: Abnormal behavior and mortality of adult American alligators (*Alligator mississippiensis)* and reduced alligator hatch rates have been observed in Lake Griffin in central Florida. Schoeb et al., 2002 and our own continuing work has found no association between mortality and environmental contaminants, toxins, viruses, or cyano-bacteria. However, results of brain tissue histology and lethargic behavior in these animals suggested a similarity with early mortality syndrome (EMS) in salmonids (Schoeb et al. 2002). The present study was conducted to evaluate thiamin status in eggs, liver and muscle of Florida alligators collected in 2000 and 2001. Total egg thiamine in 2000 was lower in eggs from Lake Griffin (0.4 nmol/g) and Lake Apoka, (0.6 nmol/g) than from reference lake (Orange Lake 3.1 nmol/g). Muscle total thiamine values were lower than in liver which is similar to data from salmonids. Samples from farmed alligators were found to have the highest total thiamine (liver, 3.58 nmol/g, muscle 0.49 nmol/g). Thiamine content of alligator muscle is more similar to walleye than salmonid muscle. Significant differences in total thiamine were noted in adult alligator muscle and liver in 2000 between sick alligatorsand alligators classified as healthy but absolute thiamine values may

have been affected by freeze-thaw before analysis. In 2001, correctly handled liver and muscle again showed differences in thiamine. We propose that large populations of Gizzard Shad (*Dorosoma cepedianum*)in Lake Griffin may be a source of thiaminase. These results provide supportive evidence that thiamine deficiency is involved in alligator mortality of adults and developing embryos. Alligator mortality has declined markedly in spring 2002. One possible explanation is that over one million pounds of gizzard shad were removed from the lake by commercial fishing thus reducing the source of thiaminase. Preliminary data indicate that alligators ate fewer shad in this period than in earlier years.

LITERATURE CITED

Schoeb et al. 2002, J. Wildlife Disease 38(2): 320-337

Ecotoxicology of Morelet's Crocodile in Belize

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ABSTRACT: Reproductive impairment and population declines have been reported for American alligators (*Alligator mississippiensis*) inhabiting contaminated lakes in Florida, USA. The principle objective of this study is to examine exposure and response of another crocodilian, Morelet's crocodile (*Crocodylus moreletii*), to environmental contaminants in Belize and assess the effect of these chemicals at the individual and population levels. In 1995, we found contaminants including mercury and multiple organochlorine (OC) pesticides in Morelet's crocodile eggs from three lagoons in northern Belize. We now hypothesize that crocodiles inhabiting contaminated lagoons contain higher contaminant concentrations in their tissues than individuals in non-contaminated areas, and that differences in crocodile morphology, blood hormone levels, serum chemistry, reproductive success, population density, and survival exist between contaminated and non-contaminated sites. From 1997-2000, approximately 650 crocodiles were captured, sampled, and released from areas in north-central Belize for examination of exposure and response to environmental contaminants. In addition, various endpoints of reproductive success, population density, and survival were measured. In this paper, we present and discuss results obtained to date.

Crocs in the Pews: Interfacing Crocodilian Conservation with Church Mission Activities

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ABSTRACT: Although the major religious denominations of the world have always been at the forefront of concerns for moral and ethical issues, environmental concerns, particularly those related to the conservation of wildlife resources, have only recently been addressed by the church. Church mission work, in many under-developed parts of the world, takes place in many regions that are important for the survival of endangered wildlife. In such regions, church infrastructures offer a vast but as yet untapped potential resource for the development and support of programs for the sustainable use and conservation of wildlife. As an example, we describe the potential for the inclusion of crocodilian conservation concerns in the overseas mission work of the Presbyterian Church (USA) in Sudan, Niger and Nigeria. Specifically, we suggest ways in which church support could prove helpful in providing: (1) travel and logistical support for researchers and conservation workers in remote regions, (2) information/data on the status, distribution and potential for the utilization of crocodilian populations, (3) expanded opportunities for contacts to gain the support of local government officials, and (4) promotion of the local acceptance of the principles of sustained-use, particularly as they apply to the management of wild crocodilian populations. In return, development of crocodilian resources on a sustained-use basis can provide local economic development and support for church social programs in the region.

INTRODUCTION

Throughout the course of human history, a number of important causes have come on the scene only to sputter, stumble and stall until they became the focus of national or global organized religious denominations. Although such causes often enjoyed significant grass-roots support in their early stages, it really wasn't until the organized religious establishment of the time became active in support of the cause that national thinking began to transform generational thinking and genuine change and progress began to take place. A good example of this process has been the issue of racial relations and civil rights in areas such as the southern United States. Here, churches eventually became a seat of activism and coordination in pursuit of what had previously been a more disorganized and certainly less effective effort to transform national attitudes. The result has been the replacement of a widespread attitude of racial hatred and suspicion with more rational thinking, sympathy and support at both the national and individual level.

The so-called "Green Revolution" of environmental concern began to sweep the more developed countries of the world with the initial Earth Day in the early 1970's. However, in many of the

underdeveloped parts of the world where critical natural resources may still remain available for management or exploitation, environmental thinking and concerns have only recently begun to become parts of national programs and priorities. The church itself, with the exception of certain eastern religions such as Hinduism and Buddhism, has similarly only recently begun to accept the fact that it has a responsibility to not only support but become active in the conservation and wise/sustainable use of environmental resources, including wildlife populations. Generally considered as "creation restoration", this thinking is based on the premise that God is the creator of the earth and all of its environmental resources. It is therefore man's responsibility to provide wise stewardship for this creation upon which all life on this planet depends (Stivers, 1989; Andrews, 1990).

We present here as an example of this process, a brief history of the development of environmental awareness, leading to activism and finally institutionalized programs in the area of environmental concerns and conservation within a major North American Church denomination, the Presbyterian Church (USA) (PCUSA). We will then show how some of these institutionalized programs have now begun to interface with the issue of the conservation of wild crocodilian populations through the concept of sustainable use based on sound scientifically-based management programs. We will show how this interface has particularly developed within the Africa Office of the Worldwide Ministries Division of the PCUSA's General Assembly Office in Louisville, Kentucky, USA, with support from the church's Office of Social/Environmental Concerns. Finally we will suggest specific ways in which this newly developing interface may soon bear fruit in terms of concerns for the assessment of the status and conservation/sustainable use of wild populations of crocodilians in Nigeria, Niger and Sudan.

ENVIRONMENTAL CONCERNS AND THE PRESBYTERIAN CHURCH: A BRIEF HISTORY

The first concrete recognition of the importance of environmental stewardship by the PCUSA came in the form of a 1990 report (Andrews, 1990) that identified these concerns as a legitimate and scripturally-justified part of the church's mission in the world today. This position statement was written as a response to an earlier grass-roots report (Stivers, 1989) which had been submitted to the national administrative body of the PCUSA a year earlier. The resulting church position statement was then distributed throughout the church body with a recommendation that it be used within congregations as a study document. The aim was to encourage further dialogue, thinking and commitment concerning the restoration and conservation of all forms of environmental resources - particularly those that had been in the past or are now being degraded, threatened or endangered by man's activities. One of the most important and tangible results of the dissemination of this report was to commission and train a nationwide network of "Restoration Creation Enablers" (RCE's). The RCE program was administered by the PCUSA Office of Environmental Justice which had been established at the church's national General Assembly headquarters. RCE's serve as grass roots volunteers, with one being appointed within each of the church's 173 geographic administrative units (presbyteries) within the United States. It then became the responsibility of each commissioned RCE to stimulate and coordinate environmental initiatives within their local area, although broader thinking on national and even global scales was also strongly encouraged.

An often-unappreciated aspect of the church's efforts to establish appropriate environmental programs has been the importance of including within such programs, a sound foundation of basic principles of environmental science (ecology). These scientific principles have been developed from programs of research and are based on a familiarity with the published scientific literature. They are needed (although the need is often not appreciated within many church circles) to determine which positions are appropriate for the church to support and/or which actions are proper for it to take. Appropriately, there is now, within the PCUSA, a movement to better understand and reconcile the interface between science and religion. This movement is being undertaken by the Presbyterian Association for Science, Technology and Christian Faith (PASTCF). However, most of the membership in this organization and much of the focus of its activities has been in the areas of the philosophy of science, cosmology and physics; until recently there has been relatively little interaction between this group and the environmental activities of the church. In the summer of 1998 however, an opportunity arose to combine the environmental concerns of the PCUSA with the interests of the PASTCF in

bringing environmental science into an active role in the life of the church. Furthermore, as will be explained below, the focus of both of these areas was then brought to bear on an issue of particular importance to the work of the IUCN Crocodile Specialist Group (CSG). This issue is the way in which science has now demonstrated that economic benefits can be associated with sound conservation policy through the application of sustainable-use (Balmford et al., 2002; Leshner, 2002).

THE CHURCH MEETS SCIENCE: SUSTAINABLE - USE

As indicated above, many elements of the major religious denominations have adopted a strongly pro-environmental stance whether or not they have actively put it into practice. In the case of some denominations such as the PCUSA, this stance has been embodied in strong statements supporting the maintenance of world biodiversity and the protection of endangered species. The biblical story tells of the first endangered species project - Noah and his ark. The teaching is clear, that God wills for each species on earth to continue (Andrews, 1990).

Supporting this position, many well-meaning elements of the church joined the call from the "Green Revolution" in deploring the utilization of products from endangered species. Andrews (1990) for example, in establishing a template for environmental action of the Presbyterian Church (USA), called for the implementation of a policy to, "Prohibit trade in endangered wild animals and endangered plants, or products derived from them".

Unfortunately, this well-intentioned policy became yet one more element of a general societal attitude of prejudice against the use of legitimately marketed wildlife products such as crocodilian leather goods, which may be similar in appearance to those of closely related endangered forms. As described by Ross (1998), misunderstanding by the public in this regard has been exacerbated by misguided information promoted by some conservation groups discouraging the use of all such wildlife products. Information of this kind, when disseminated to the public, invariably fails to make a distinction between those products produced by legitimate sustained-use programs of harvest or ranching vs. those obtained illegally from endangered species. Moreover, as also pointed-out by Ross (1998) and Webb (2000), any resulting decrease in the worldwide demand for luxury crocodilian leather goods depresses market prices for raw crocodilian hides. This in turn reduces the profits that may accrue to local people who own or at least have harvest/management rights to legitimately usable non-endangered crocodilian resources. This in turn then reduces the incentives of such people for managing these resources on a sustained-use basis. Thus, in an ironic way, well-meaning programs initially intended to save and protect endangered crocodilian species may, in some instances, actually now be working to hinder their recovery. This hindrance is now coming in the form of threats to those very programs of sustained-use harvest which have repeatedly proved their importance in the recovery of crocodilian populations in many parts of the world (Webb, 2000). All of this stems from a basic lack of understanding on the part of the church and other elements of today's society of the basic principles of sustained-use science, particularly as they pertain to crocodilians.

As explained in detail by Webb (2000) and Messel (1991), the science of sustained-use management of crocodilian populations is based on the fact that wild nests of these species invariably produce many more young than ever reach maturity and enter the breeding population of subsequent generations. Management programs, generally in a form known as "ranching", begin with removing all or nearly all of the eggs from wild nests. These eggs are hatched in captivity and the young are raised to a size at which they can be considered to be safe from the myriad of predators and other sources of mortality that frequently befall small hatchlings in the wild. A percentage of these partly-grown "head started" immature animals are then be returned to the wild population while the remainder are marketed as meat and leather to produce (often substantial) income for local peoples. The keys to the success of this approach are important advances in nutrition and other aspects of the husbandry of captive crocodilians (e.g. Brisbin et al., 1990). These advances can now, under appropriate conditions, allow enough hatchlings to be marketed to realize financial profits while still returning more animals to the wild than would ever have resulted from the wild nests in the first place. Careful monitoring programs are required however, to ensure that levels of egg removal are not having detrimental effects on the numbers or population dynamics of the wild populations from which they are being taken.

As illustrated by Temsiripong et al. (2000), such monitoring programs must be based on sound scientific principles of population biology and must consider a number of factors such as hatch rates of wild vs. harvested clutches of eggs, growth and survival rates of repatriated vs. wild immatures, etc. Any of these factors moreover can interact in compensatory or contradictory ways, again requiring a thorough understanding of the basic population biology of the species under consideration in the particular region where harvest/ranching programs have been proposed.

Because of the long-term experience of many of its members in studies of these very scientific principles, the CSG has become an important player in the monitoring and evaluation of the effects that any proposed program of sustained-use harvest and/or ranching may be having on wild crocodilian populations. In this capacity, the CSG can bring together the interests of governments who want to establish harvest programs within their national boundaries, with those interests of conservation groups, (and/or churches), which want to ensure that such programs do not harm the very populations upon which they are based.

CHURCH MISSION AND CROCODILE CONSERVATION

The initial interfacing of the interests of the PCUSA in the areas of science and environmental concern with the work of the CSG came about during the meeting of the latter's Steering Committee in Singapore in July, 1998. At this meeting there was discussion of a request from the Wild Life Conservation Administration of the Ministry of Interior of the Republic of Sudan seeking assistance in initiating a program to collect data on the status and distribution of crocodile (*Crocodylus niloticus)* populations in that country. The eventual aim was to use this information to justify a program of sustained-use harvest and/or ranching of crocodiles, resulting in the production of products for export into the world trade. There was great interest within the Steering Committee in seeing crocodile surveys initiated in that nation. The Sudan has variously been claimed to have the largest crocodile population of any nation in Africa with total numbers estimated as high as one million animals (Elobeid, 1990). However logistic constraints quickly came to light, related to a long on-going state of civil war which has prevented safe access to many areas of important crocodile habitat, particularly in the southern part of that country.

The PCUSA however has had a long-standing program of humanitarian missionary concern in parts of the southern Sudan (Welch and Ryan, 2000). It was therefore hoped that the considerable infrastructure and familiarity of those involved with "on the ground" PCUSA missionary activities in that region could somehow help to facilitate plans for the collection of the needed crocodile survey data. Then, when military conflicts are finally resolved, the required information database could be more expeditiously collected and sustained-use programs of crocodile harvest/ranching could begin to provide badly needed economic benefits to all of the people of that nation. Unfortunately, the continuation of military conflicts in Sudan has so far prevented any such survey activities from taking place. However, the thinking outlined above has at least had the benefit of now bringing-together the program interests of the Africa Office of the Worldwide Ministries Division of the PCUSA and the work of the CSG. It was this contact that eventually resulted in the extension of thinking similar to that outlined above, for the nations of Niger and Nigeria where there have been more opportunities to develop cooperative program interests between the PCUSA and the CSG.

THE PRESBYTERIAN CHURCH (USA) IN AFRICA AND CROCODILES: WHERE CAN WE GO FROM HERE?

As indicated above, on-the ground Sudan crocodile surveys, even those taking advantage of church missionary infrastructure in that region, can not begin until a stable peace has been established allowing open contact and cooperation in this effort between the government in the north and rebel forces in the south. However humanitarian relief efforts in the south of Sudan, such as the United Nations' Operation Lifeline Sudan, have operated relief flights into the south from a base camp in Lokichokio, Kenya (Spinney, 1996). These flights have provided valuable information in the past on the status and distribution of other wildlife resources, particularly large grazing herbivores, in this region. It may therefore be possible for church/humanitarian groups to work through such programs to begin to obtain

at least some of the preliminary information needed by the Sudan government, concerning crocodile status and distribution in this region.

In the meantime however, the interests of the church (PCUSA) in developing the concept of sustained-use crocodile harvest/ranching in Sudan has attracted the interests of Presbyterian church elements in at least two other African nations, Niger and Nigeria. Of the two, by far the greater potential for the development of crocodile resources is in Nigeria where the extensive mangroves and marsh habitats of the Niger River Delta in the southeastern part of that country, offer the possibility of extensive suitable crocodile habitat (Luiselli and Akani, 2002). However as pointed-out by those authors and by references cited therein, Nile crocodiles are generally rare in this region which is under heavy pressure of habitat disturbance and destruction from oil-exploration activities. Thorbjarnarson (1992) reports declines of crocodiles in Nigeria attributed to hide hunting, and Luiselli et al. (2000) report that juvenile *C. niloticus* occasionally appear in the bush-meat trade. Crocodiles can still be found however in the less accessible areas of mangrove creeks and marshes that also seem to serve as refuge habitat for African rock pythons (*Python sebae*; Luiselli and Akani, 2002). These pythons are also hunted intensively by local peoples for meat and leather, so there does seem to be a potential for a formalized commercialization of trade in reptilian meat and leather goods in this area. However, exploited populations have apparently yet to be harvested on a sustained-use basis since Luiselli and Akani (2002) report that like crocodiles, pythons are widespread but also in general decline throughout this region. Competing oil interests almost guarantee that any efforts to establish crocodile harvest/ranching programs in this area will have to present an economically competitive alternative use of habitat resources - particularly with regard to economic returns to local people. It is precisely in this latter respect however, that CSG-fostered programs of sustained use (sensu Webb, 2000) can make an important contribution, particularly if they can be introduced and promoted by a church-mediated program establishing grass-roots support and education through local church congregations.

In the case of Niger, crocodile populations are almost certainly less abundant than in either Sudan or Nigeria, due mostly to lesser amounts of suitable habitat. Moreover there is relatively little known about crocodile populations in that country and no recent information exists for numbers or distribution. On the ground inquiries however, conducted in the spring of 2002 in the course of other activities of the Africa Office of the Worldwide Ministries Division of the PCUSA (DMW), suggest that crocodiles are rarely hunted in Niger. Thus their population numbers are probably relatively stable in that country. Nevertheless, crocodiles are officially protected by law 98-07 which was enacted in April 1998 to regulate hunting and the protection of wildlife. Crocodile meat is consumed to some extent by local peoples in Niger and local church contacts suggest that there is interest in pursuing the idea of crocodile farming/ranching. However technical guidance would need to be provided by outside groups (e.g. the CSG) which are experienced in this area of in situ local economic development.

As in the case of Niger described above, our initial inquiries suggest that the potential is high for church connections to assist in the development of viable and economically profitable crocodilian farming/ranching programs in a number of African countries. The only exceptions are those cases where current conditions would make on-the-scene visits either dangerous or impractical, as is currently the case with Sudan. Even there however, experience gained from the use of church connections to initiate such programs in other countries (e.g. Niger and Nigeria) could help convince governmental officials and local peoples alike of the value of such an approach to combining conservation concerns with local economic development once national peace has been achieved. If nothing else, our initial experiences as outlined above, suggest that the time is now right to begin to "spread the word" to other church denominations in Africa as well as other parts of the world, that sound conservation of crocodilian resources can go hand-in-hand with badly needed local economic development in these same regions.

A FINAL THOUGHT: THE CHURCH, CROCS AND BUSH-MEAT

Like so many other topics of environmental concern, the conservation and management of world populations of wild crocodilians through sustained-use is not an isolated issue. Rather it is part of the larger issue of the science of sustained-use for a wide variety of environmental resources. This issue has recently come to the fore of global environmental thinking and action (Balmford et al., 2002; Leshner,

2002). Both of these citations however show that there must be a firm understanding of the basic science involved in order to have reasonable expectations of a successful outcome for such programs.

Nowhere is thinking on conservation through sustainable-use more ripe for application than in the developing countries of the African continent. Yet nowhere is there now a clearer need for the input and assistance of those experienced in all aspects of the science of sustainable-use. In the case of crocodilians, this role can and should, we maintain, be played by the CSG. Furthermore, we believe that the church may be just the vehicle needed in some instances to introduce CSG thinking in such a way as to maximize the probability of its acceptance by local peoples. When so "preached from the pulpits" of local congregations, the sustained-use of crocodile products such as meat and leather would almost certainly have a greater probability of acceptance. In addition, a church-CSG collaboration may also prove more effective than either would be alone in avoiding some of the common pitfalls that often plague small-scale business/production start-up activities in under-developed parts of the world. In return, the substantial economic benefits that could be provided to local peoples by such programs would help fulfill many aspects of the church's mission of humanitarian outreach in such regions.

When introduced in this way, the sustained-use of crocodilian leather and particularly meat could also offer an alternative to the meat of great apes and other wildlife species that currently form part of the bush-meat trade, but not on a sustained-use basis (Pearce, 1996; Ammann, 1999). Juvenile crocodiles and other reptilian species are already traded and are apparently accepted as part of the bush-meat trade (Akani et al., 1998; Luiselli et al., 2000). We believe that meat from crocodiles that have been carefully ranched under conditions guided by the best principles of the science of sustained use (Webb, 2000); (Messel, 1991), have the potential to provide an even cheaper source of higher-quality protein than bush-meat from other wild species. Moreover, its use in this way could also serve the purposes of the conservation value system that should be espoused by church mission activities in these areas.

Realizing such a win-win situation will almost certainly demand considerable mutual cross-education and a redirection of thinking on the part of both the church and the world conservation community. We believe that the CSG is in a position to now begin to make just such cross-education and redirected thinking a reality for model programs for the conservation and sustainable-use of African crocodiles in countries such as those discussed above. Furthermore, we feel that such efforts may yield dividends of a broader conservation significance than to just crocodilians alone - if for no other reason than for the simple importance of initiating a dialogue between church mission activities and conservation interests in this and other parts of the developing world.

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Human-Crocodile Conflict in Belize: A Summary

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Two species of crocodiles are indigenous to Belize, Morelet's crocodile (*Crocodylus moreletii*) and the American crocodile (*Crocodylus acutus*) (Groombridge, 1987). While Morelet's crocodile is typically found in interior freshwater habitats and the American crocodile in offshore saltwater habitats, the two species are also known to live sympatrically in areas of brackish water along the coast (Platt and Thorbjarnarson, 1997; Rainwater et al. 1998). While anecdotal testimony suggests crocodile attacks on humans in Belize have historically involved American crocodiles, the majority of the documented cases appear to involve only Morelet's crocodiles.

Previous accounts of crocodile aggression and attacks on humans in Belize persist in the literature (Morelet, 1871; Sanderson, 1941; Abercrombie et al. 1982; Marlin et al. 1995; and Rainwater et al. 2000). Attacks have also been documented in Guatemala and Mexico (Neill, 1971 and Alvarez del Toro, 1974). Additional anecdotal accounts exist in Belize as oral testimony, but the accuracy of these accounts is questionable. In each of the cases listed, causal factors responsible for attacks on humans appeared to vary.

On the afternoon of 12 August 2001, while swimming in a canal in the Belama area of Belize City, a 13 year-old male named Jamaal Swift drowned as a result of attack by a large crocodile. Following the attack, Mr. Swift's body was recovered and inspected by the Belize City coroner who confirmed the cause of death to be drowning. Only limited puncture wounds were observed about the legs (M. Windsor pers. comm.).

On 14 August 2001, at approximately 2200 hours, a group comprised of personnel from Belize Forestry, Texas Tech University, and Lamanai Outpost Lodge captured an adult male Morelet's crocodile in a canal adjacent to (and approximately 500 meters away from) the location of the attack. The specimen captured measured 284.5 cm (total length), weighed 97.5 kg, and upon necropsy stomach content analysis revealed chicken remains, a nail, and multiple rocks (M. Windsor pers. comm.).

Following the death of Mr. Swift, media and concerned citizens stressed the need for a nationwide crocodile management strategy in Belize. As a result, the Belize government contacted the Florida Association of Volunteer Agencies for Caribbean Action who in turn contacted Dr. Frank Mazzotti to make assessments and recommendations for the mitigation of human-crocodile conflict.

Morelet's crocodile is currently recognized as endangered under the United States Endangered Species Act (ESA), listed on Appendix I of the Convention on International Trade in Endangered Species of Flora and Fauna (CITES) and is protected domestically under the Belize Wildlife Protection Act of 1981. Following near-extirpation in the late 1970's, Morelet's crocodile populations experienced

substantial recovery as a result of its protection status. In addition to an increasing crocodile population, Belize has also experienced rapid growth in its human population over the last few decades (Figure 1).

Increases in both human and crocodile populations potentially increase contact between the two groups and thus increase the possibility of conflict. Exacerbating this problem is the development of Morelet's crocodile habitat around Belize City. A similar problem exists offshore with the construction of resorts and the development of crucial nesting habitat for the American crocodile.

Development of a crocodile management plan is needed in Belize to effectively address nuisance crocodiles, development and encroachment of crocodile habitat, and most importantly public education regarding crocodiles.

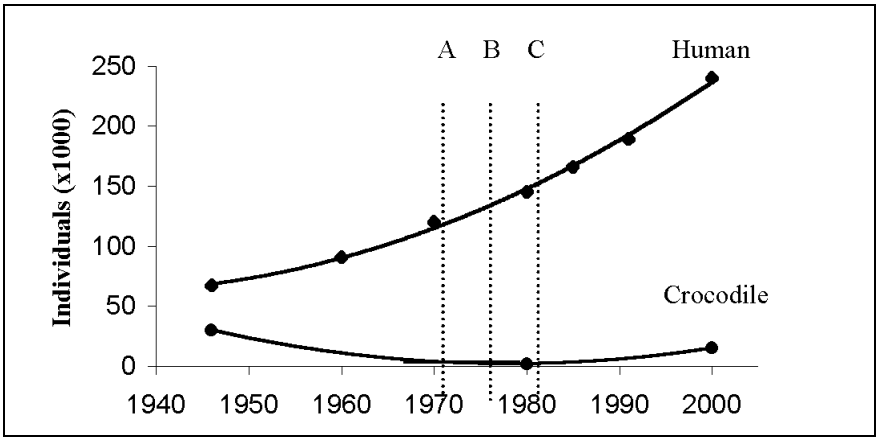

Figure 1. Estimated Human-Morelet's crocodile population size changes in relation to conservation legislation (A. ESA listed in 1970, B. CITES listed in 1975, and C. Belize Wildlife Protection Act of 1981). Human population data provided by the Belize Central Statistical Office.

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Interacción Hombre-Cocodrilo en la Costa de Jalisco, México.

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ABSTRACT: The coast of Jalisco has 52 water bodies and 35 have crocodile populations. The principal problem of the conservation of the species in this State is because the accidents happened with crocodiles. That is why it is important to study the problem to determine solutions for the extinction of these animals. To carry out this project we visited most of the water bodies where we made interviews to the local people trying to find people who had been attacked by a crocodile. We are registered 16 accidents since 1958, most of them with local people, adult crocodiles and related with fishing activities. Some of this accidents occurred during reproductive season. We found since 1993, the accidents are increasing because the raise the human activity near the estuaries as well as the growth of crocodile population. Therefore it is necessary to begin environmental education with focus in the crocodile and the importance of its habitat in addition to inform them about the risks and how to avoid them.

Keywords: Accidents, *Crocodylus acutus*, Coast of Jalisco, Mexico.

RESUMEN

La costa de Jalisco cuenta con 52 cuerpos de agua costeros, de los cuales, en 35 existen cocodrilos. El principal problema para la conservación de la especie en el Estado se debe a los accidentes ocurridos con cocodrilos; por esta razón es importante estudiar este problema para determinar y dar solución para evitar la extinción de estos animales. Para llevar a cabo este proyecto se visitaron la mayoría de los cuerpos de agua donde se hicieron entrevistas a la gente local, para encontrar gente que hubiera sufrido un accidente con cocodrilos. Se registraron 16 accidentes desde 1958, la mayoría ocurridos durante la temporada reproductiva, además, desde 1993, los accidentes se han incrementado debido al crecimiento de las actividades humanas cerca de los esteros así como el crecimiento de las poblaciones de cocodrilos. Sin embargo, es necesario comenzar un programa de educación ambiental con foco en los cocodrilos y la importancia de su hábitat, además de informar a la gente sobre los riesgos que existen y como evitarlos.

Palabras Clave: Accidentes, Crocodylus acutus, Costa de Jalisco, México.

INTRODUCCIÓN

Cuando el hombre apareció en la Tierra, los cocodrilos ya se encontraban establecidos en ella y nuestros ancestros vivían en armonía con los cocodrilos. Sin embargo, como sucede en el mundo entero donde habita el cocodrilo, el incremento de la población humana cambia el ambiente provocando día a día su fragmentación y la invasión de su territorio. Ya que se trata de un depredador, que además representa un valor económico en su piel, ha sido cazado durante décadas produciendo la declinación de las poblaciones. Además, éstos son destruidos indirectamente con la destrucción de su hábitat (Butler, 1987).

En México, durante los años 40 y posteriormente en los 60´s se presentó la explotación irracional de la piel de cocodrilo, junto con la de tortuga marina. Con esto se vino una disminución de la especie que la llevó casi a la extinción. A partir de 1970 se decretó la protección de las tres especies de cocodrilianos

mexicanos. A partir de ahí y a pesar de existir la cacería furtiva, las poblaciones se han ido incrementando, gracias también a la creación de reservas en donde el cocodrilo es protegido. Por otro lado, el crecimiento de la población humana ha ido invadiendo terreno que una vez perteneció a los cocodrilos, por esta razón los habitantes de la costa creen que el cocodrilo es el que ha invadido las lagunas costeras.

Desde principios de los 90 se ha llevado a cabo un monitoreo sobre los accidentes y sus causas para analizar los problemas y buscar soluciones adecuadas a estos, que se incrementa día a día. Este proyecto forma parte del Plan de Conservación del "caimán" (*Crocodylus acutus)* que se realizó para el estado de Jalisco (Ponce y Huerta, 1997).

Durante las diferentes investigaciones que se han realizado en torno al cocodrilo en Jalisco para su conservación, al igual que otros lugares como Costa Rica (Jiménez, 1998), se encontró que uno de los principales problemas se debe a los accidentes ocurridos generalmente con la gente local.

Debido a esto es importante estudiar la problemática hombre-cocodrilos (accidentes) ya que cada vez que ocurren accidentes se pretende eliminar a la especie, de tal forma que es necesario encontrar nuevas perspectivas para que la gente comprenda su importancia. Esto se puede lograr con programas productivos en donde los cocodrilianos aporten recursos para las comunidades.

METODOLOGÍA

Para llevar a cabo las investigaciones sobre los accidentes se aprovecharon las visitas hechas a la mayoría de los cuerpos de agua costeros monitoreados, y se entrevistaron pescadores en las principales cooperativas de la costa, gente local en poblaciones cercanas a bocas de ríos, esteros, lagunas y áreas inundables. Los datos principales que se analizaron fueron, fuente o informante, si la información de este fue por terceras personas, por testigos de los accidentes o los mismos accidentados. Además se tomaron datos del lugar donde ocurrió el accidente, datos sobre el animal involucrado, información general del accidentado como edad, sexo, actividad que realizaba, si tenía conocimientos de la presencia de cocodrilos en el lugar, nivel de agua en la persona, si nadaba o buceaba durante el accidente, entre otros. Algunos de los casos se les dio seguimiento durante varios años, en diferentes visitas y con diferentes entrevistados. En ocasiones se habló con el accidentado o con testigos, para verificar la validez de la información y de ésta forma evitar reportar la información no confiable.

RESULTADOS

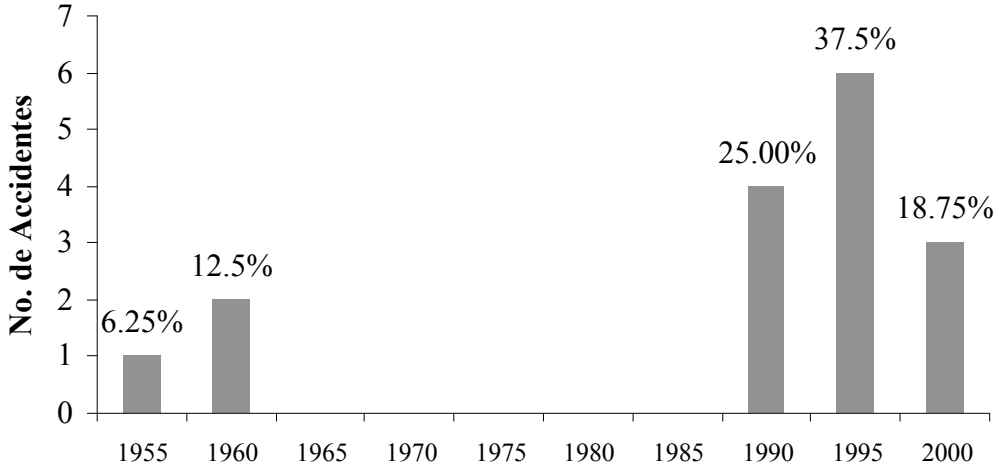

Figura 1. Gráfica donde se muestra el número de accidentes por cada 5 años

El accidente más antiguo registrado data de 1958, y para los años 60 se reportan 2 más. Sin embargo a partir de ahí no se reporta otro accidente hasta el año de 1991. Esto se debe a que a finales de los 60 habían exterminado casi por completo las poblaciones de cocodrilos en la costa, y para los 90 hubo una

recuperación de estas, las cuales se fueron extendiendo por todos los cuerpos de agua costeros. Sin embargo, para finales de los 90 y principios del 2000, se reportan más de la mitad (56.15%) de los accidentes registrados.

El 37.5% de las personas accidentadas dijeron haber tenido el agua arriba de la cintura, mientras que el 31.25% debajo de la misma. El 18.75 % se encontraba nadando o buceando y el 12.5% no se determinó. De acuerdo a las edades de estas personas, el 62% eran adultos mayores a los 25 años, el 19% jóvenes entre los 15 y 20 años de edad y el otro 19% niños entre los 3 y los 10 años. Solo en 2 de los accidentes resultó con pérdidas humanas, un niño de 3 años aproximadamente en los años 60 y un buzo de Tecomán que se metió a pescar en el Río Marabasco. En estos dos casos no se vio al animal que los atacó, solamente desaparecieron.

El 62.5% de las personas accidentadas fueron gente local, el 12% turistas y el 25% de ellos eran residentes de la costa que fueron a pescar a otra localidad. En total fueron 15 hombres y una mujer. El 87.5% de los encuentros fueron con animales adultos de más de 2 m, de los cuales, el 28.6% fueron animales arriba de los 3 m de longitud total y el 14.3% animales de más de 2m.

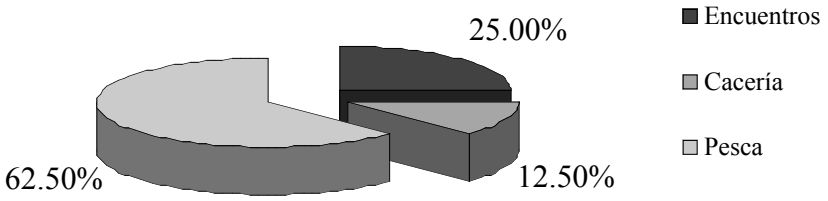

Figura 2. Porcentajes de accidentes debida a encuentros accidentales con cocodrilos, cacería de cocodrilos o pesca en los esteros.

DISCUSIÓN Y CONCLUSIONES

Dos de los accidentes registrados coinciden con la temporada reproductiva, cuando la especie es más activa y agresiva (Lazcano-Barrero, 1996). Nueve accidentes coinciden con la temporada de nacimientos. Seis accidentes se relacionan con actividades de pesca con el agua a la cintura o más arriba, 3 de estos por traer pescado colgando, al igual que 4 de los 7 accidentes reportados por Lazcano-Barrero (1996) en Quintana Roo.

A diferencia de otras partes del mundo, en Jalisco solo se ha reportado dos muertes causada por cocodrilos. En Madagascar, Behra (1996) reporta 140 ataques de los cuales, 57 causaron la muerte de la víctima y Jiménez (1998) reporta 3 muertes desde 1995 en Costa Rica. Según la información obtenida en todos los casos donde se han reportado accidentes, las personas sabían de la presencia de cocodrilos al igual que los accidentes ocurridos en Jalisco con excepción del niño turista y de la persona que falleció a principios de los años sesenta.

A partir de 1993 se han incrementado los accidentes, pero la frecuencia no ha sido tan alta como la reportada por Lazcano-Barrero (1996) quien reporta siete accidentes en un periodo de seis meses. Este incremento se asocia con el aumento de la actividad humana en los esteros, principalmente la pesca. De 1995 a la fecha han ocurrido nueve accidentes. El accidente más grave ocurrido después de la pérdida humana, es el del río Cuitzmala.

Por medio de las investigaciones que se han llevado a cabo a través del plan de conservación del cocodrilo en Jalisco, se están determinando categorías para los diferentes cuerpos de agua costeros y

áreas inundables con el fin de establecer prioridades y estrategias de conservación con base en la problemática.

Algunas de las acciones urgentes que se pretenden realizar en la costa de Jalisco, es la creación de centros de investigación y conservación del "caimán". En primer plano, estos lugares servirán para introducir animales problema, además de exhibirlos, hacer investigación, implementar programas de educación ambiental, evitar la captura y el tráfico ilegal de la especie, entre otros. Esto se puede apoyar con lo ocurrido en Quintana Roo, Jalisco y otros estados, donde los animales problema son llevados a un zoológico o a otros lugares que generalmente no tiene la capacidad suficiente para todos estos animales.

Otra acción importante es informar a la gente local, pescadores y turistas para que conozcan los riesgos y como evitarlos por medio de trípticos, letreros y pláticas a diferentes niveles. Esto se está comenzando en el estero de La Manzanilla con la participación de profesores locales, profesores de la Universidad Pedagógica Nacional y David Collins.

AGRADECIMIENTOS

Agradecemos las atenciones y la información que proporcionaron los accidentados y la gente local que nos apoyó como Gilberto Hernández y Juan Cervantes, Sr. Andrés Aceves Franco, Don José en la Cruz de Loreto. También queremos agradecer al Biól. Marciano Valtierra de Cuxmala, A. C. y al Biól. Julian Gamboa voluntario de Bosque Tropical, A. C

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Crocodilians: Fact vs. Fiction

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ABSTRACT: This is a discussion of crocodilian behaviors witnessed at the St. Augustine Alligator Farm. There are four specific topics discussed:

Crocodilians have the ability to swallow prey under water: We have witnessed *Crocodylus johnsoni, Crocodylus porosus,* and *Tomistoma schlegelii* swallowing their food without coming to the surface.

Crocodilians eating their vegetables: Is it possible that biologists have been assuming too much when doing stomach content surveys on crocodilians? We have compelling evidence that alligators in captivity, at least, may seek out vegetation in their diet.

Siamese Crocodiles as parents: A two-year account of a pair of *Crocodylus siamensis* raising young on exhibit at the St. Augustine Alligator Farm.

Crocodilians feeding their young?: Observations of a female *Crocodylus siamensis* allowing her young to feed from a piece of meat in her mouth.

INTRODUCTION

The St. Augustine Alligator Farm has been in existence for almost 110 years. Its name is a little misleading. It is not a true alligator farm. There is no production of skins or meat at the facility, and there never has been. It is a zoological park, accredited with the American Zoo and Aquarium Association (AZA). The facility started in 1893 by displaying only American Alligators, *Alligator mississippiensis*. It has now grown to include other reptiles, birds, small primates, and the world's only complete collection of all 23 species of crocodilians. The following are four accounts of observations of crocodilian behavior that tend to go against the current literature or common thought.

Crocodilians have the ability to swallow prey under water: The palatal valve, in the back of a crocodilian's mouth, is a unique adaptation that seals the throat off from both air and water. With the palatal valve shut a crocodilian can grasp food underwater and not have the water flood past into the esophagus or glottis. Essentially the inside of a crocodilian's mouth is outside its body. Crocodilians obviously prefer to keep this palatal valve closed while submerged, and come to the surface to swallow their food, thus avoiding water rushing past the palatal valve. It is often assumed that crocodilians are unable to swallow food underwater. However, we have witnessed three crocodilian species swallowing their food underwater. The first is a female Freshwater crocodile, *Crocodylus johnsoni,* housed alone. On several occasions she has picked up pieces of meat from the bottom of the pool and proceeded to eat them without surfacing. Once this behavior was noticed, it became a regular observation.

The second observation is of a female saltwater crocodile, *Crocodylus porosus*, housed with its mate. This female swallows both above the water and below, seeming to not have any preference for one over the other. When she surfaces, after swallowing underwater, she has been observed purging excess water at the surface. This appears to be done by contracting muscles in the throat. Water can be seen spraying out of the mouth from the palatal valve.

The third species we have observed swallowing underwater is a female false gavial, *Tomistoma schlegelii*. She is currently housed in a large exhibit with another female and a male. This exhibit affords visitors a complete underwater view of the entire pool though four large glass panels. Soon after moving the female to this exhibit, I witnessed her taking a piece of meat to the bottom of the pool and holding it. After about five minutes, she very deliberately partially opened her mouth, then opened her

palatal valve, and quickly moved her head forward and swallowed the meat. She remained in a resting position on the bottom of the pool for another ten minutes. Because she had recently come to us from Audubon zoo, I called the reptile staff there and asked if they had witnessed this behavior. They said that they had. Apparently the male *Tomistoma* at this facility was in the habit of stealing the female's food if she surfaced with it. I have witnessed her swallowing underwater on only one other occasion. I believe the behavior is being extinguished by our training efforts, as our male does not have an opportunity to steal meat from the females.

Figure 1: Male Siamese Crocodile, *Crocodylus siamensis* showing palatal valve.

Crocodilians eating their vegetables: Scientific literature is filled with research regarding the stomach contents of crocodilians. Almost all of them refer to the plant material found in the animals' stomachs as either an accident (i.e., the crocodilian got leaves in its mouth while trying to swallow a prey item), or secondary (i.e., the crocodilian swallowed a prey item that had grass or leaves in its stomach). One such article lists plants as a "nonfood item", but notes that the plants were found in ninety percent of the animals sampled. In the summer of 2000, some of our keepers said that they had been seeing American alligators eating fruit from the elderberry plants in the Swamp exhibit. Of course I wanted to blame this on the fact that the alligator must have seen an anole or some other animal in the plants and lunged for it. The keepers were fairly persistent, saying that the alligator had gotten a mouth full of elderberry, swallowed, and then gone back for more. Reports of the alligators eating elderberry, as well as wild grape, from plants in the Swamp exhibit happened several more times that year and have continued over the following years.

In May of 2001, we began a mixed species exhibit which includes: American alligators, Chinese alligators (*Alligator sinensis*), brown caiman (*Caiman crocodilus*), dwarf caiman (*Paleosuchus palpebrosus*), dwarf crocodiles (*Osteolaemus tetraspis*), mugger crocodiles (*Crocodylus palustris*)*, and* red-foot tortoises, (*Geochelone carbonaria*). The keepers reported observing American and Chinese alligators eating out of the tortoises' dishes. Again, I was inclined to explain this away by saying that the alligators must have been attracted to the food dishes by the movement of the tortoises, and just accidentally eaten some lettuce. However, it is now a common sight to see the alligators at the tortoise bowls eating romaine lettuce and yellow squash. Sometimes the alligators even beat the tortoises to the dishes. We have had plenty of opportunity to record this behavior on videotape.

There are several citrus trees in this mixed species exhibit. Occasionally, we have witnessed alligators running around with an orange or lemon in their mouth, trying to keep it away from the other animals. It eventually gets chewed up or torn by other animals and swallowed. In March of this year, we watched as an American alligator raised itself into the lower parts of a small kumquat tree and grabbed fruit directly from the tree. In the course of a few minutes, we observed this same individual swallowing the fruit and going back for several more kumquats.

In August 2002, some of our park visitors reported seeing an American alligator "sit-up" and remove a lime from one of the trees and proceed to chew it up and swallow it.

We are not exactly sure why alligators in our park are eating their vegetables. It is possible that our animals, in captivity, are lacking something in their diet that makes them seek out vegetation. Or, is it possible that crocodilians deliberately consume vegetation as part of their normal diet?

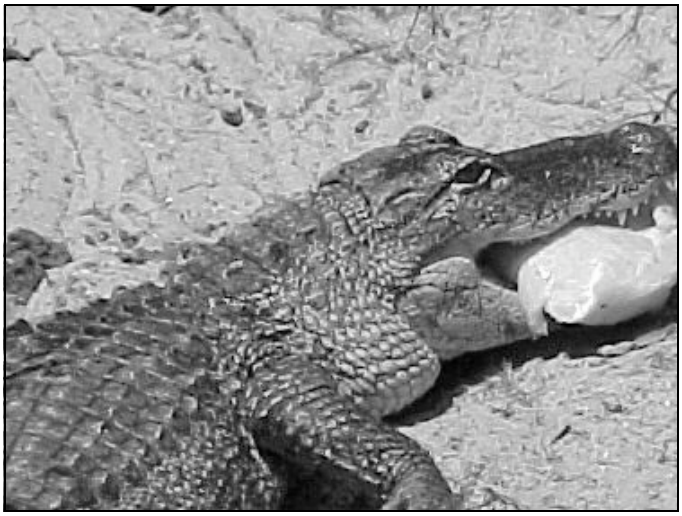

Figure 2: American Alligator, *Alligator mississippiensis* eating a lemon from a tree in its exhibit.

Siamese Crocodiles as parents: In May of 2000 the St. Augustine Alligator Farm decided to do things a little differently. Typically, when the alligators or crocodiles lay eggs the keepers collect the eggs and put them in an incubator. There are several reasons for this. The success rate of artificially incubated eggs is usually higher, the sex of the animals can be controlled by the temperature at which they are incubated, and it keeps the larger animals in the enclosure from eating the hatchlings. Though mother alligators are usually very good parents, some literature implies that male American Alligators tend to be unconcerned with their offspring, or worse yet, have been known to eat the hatchlings. Because of multiple paternity, it is possible the males don't even know which hatchlings are theirs. It is also possible, that observations have been misinterpreted. Neill points out, in his 1971 book, that except in very controlled circumstances, it is difficult to say with any accuracy whether an alligator is male or female just by looking at it, much less know if it was, in fact, the parent of the alligator being eaten.

While Neill is not willing to authenticate observations of adult alligators cannibalizing their own young, he is quick to disregard any possibility of crocodilians playing a significant role as attentive parents. However, the following two accounts might have changed even Neill's mind.

On May 21, 2000 our female Siamese crocodile built a beautiful nest right in the front of her exhibit. We decided to leave the nest alone for all of the visitors to see. We had no idea what would happen. There were any number of things that could have gone wrong. The nest could have been too dry, fire ants could have claimed the eggs, or the male could have eaten all of the young as they came out of the nest. We just had to wait patiently. It turns out the nest was built over a sprinkler head which kept the inside of the nest quite moist. Ants were seen several times at the nest, but were controlled. And when it came time to prove the male's intentions, he performed admirably.

We are unsure exactly when the female actually laid the eggs in the nest mound. One week after the mound was built, we gently opened the top of the nest and removed the top three eggs. They were nicely banded and we put them in our incubator, just to make sure some of the clutch would survive.

At 7:30 am, on August 13, 2000, about seven weeks later, the female was seen lying on the nest mound with her head cocked sideways appearing as though she were listening to the nest. By 8:30 am eight hatchlings were out of the nest and eggshells were noticed floating, or laying on the bottom of the pool. As we watched, the female slowly used her front legs to pull dirt away from the top of the nest. When she uncovered a hatchling, she gently picked it up in her mouth and carried it to the water. If she uncovered an egg that was not yet hatched, she gently broke the egg with her mouth, scooped up the baby and again carried it to the water. Sometimes the hatchlings were still attached to the egg by their yolk and both baby and egg would be carried to the water. One little guy had quite a struggle as the egg he was attached to started to fill up with water and began to drag him under. He was pulled partially under water before he managed to wiggle his way free.

By 12:30pm the female got out of the pool and started to bask. There were seventeen hatchlings all huddled together at the edge of the pool, and it was assumed that this would be all that would hatch. But, at 2:45 the female returned to the nest and removed four more hatchlings.

The adult male stayed in the water during this entire process, and was quite curious as the female brought the babies to the pool. He swam over to almost every new release and watched as things went on. He never made an aggressive move toward any of the hatchlings.

On several occasions the female brought whole, unopened eggs to the water and released them. The eggs floated, and she left them, with no apparent interest in their future. The adult male bumped into one of these eggs as he was patrolling the pool. He gently picked it up in his mouth. At first, it looked as though he swallowed it, but he rolled it around on his tongue for almost a minute, and then gently broke it open. He then rinsed the shell out of his mouth, but the egg was empty. There is no way of knowing for sure, but it appeared that he was trying to open the egg and release a baby, just as the female had done. After opening the first egg, he seemed to patrol the pool more diligently, even diving to the bottom of the pool and gently breaking open what was left of hatched eggs. The male never went to the nest to retrieve eggs or young, but did open several of these infertile eggs. His basking site is near the nest mound, and twice during the day he crawled out and basked near the nest.

One week after hatching, the baby crocodiles started chasing and eating crickets and mealworms that were tossed into the exhibit. They are fed worms, crickets, and gator chow every two or three days. On August 25, 2000, twelve days after hatching, many of the babies were seen basking on the back of the parent crocodiles. This has been a common sight on warm afternoons ever since. When in the water, babies tend to congregate around the parents' heads, some even resting on the adults' heads as if they were a floating island.

The goal of leaving the nest alone was to allow our visitors be able to see the nest, the hatching, and now a family unit of crocodilians on display. It has been a great success. Visitors who take the time to look carefully can see many of the baby crocodiles usually, lined up at the edge of the pool. This is not the first time that the St. Augustine Alligator Farm has hatched Siamese Crocodiles, but it is the first time that we have allowed the parents to do all of the work. The three eggs that we put in the incubator from the nest hatched three days after the eggs in the exhibit hatched. We have since introduced these three babies back to the exhibit and they have been accepted into the family unit.

As long as we had this unique setup, we decided to try a couple of experiments. First, three yearling crocodiles were added to the exhibit to see how the parents reacted. These yearling crocodiles were offspring from the adults, but had never seen their parents, as they were artificially incubated and raised separate from the adults. The adults accepted these juveniles in the exhibit as well, and all are living comfortably together.

Secondly, we introduced a couple of hatchling American alligators. This introduction was very interesting as well. The alligators did not seem to mind being with the crocodiles their own size, but were intimidated by the adults. While the juvenile Siamese crocodiles would congregate around the adults (even the yearlings), the juvenile alligators would swim away from them. Early on, there were

several occasions that the alligators were seen around the adult crocodiles, but the alligators seemed shocked when the adults moved, and they swam away quickly. This test was performed to see if the parent crocodiles could distinguish between hatchling species. It is generally accepted that some crocodilian species will guard their offspring in nurseries. In other words, one female may guard offspring from several females in the area. One of the hatchling alligators did not survive in the exhibit. It appeared to have been accidentally crushed by a basking adult. However, one American alligator now seems to be just as comfortable as the young crocodiles and can still be seen, more than two years later, swimming, feeding, and basking with its surrogate family.

Figure 3: Male Siamese Crocodile, *Crocodylus siamensis* allowing the young to bask with him.

Crocodilians feeding their young?: More and more we are realizing how closely birds and crocodilians are related. They have many similar adaptations and behaviors. However, one distinct difference is that crocodilians are not known to feed their young. Unlike most birds, hatchling crocodiles are ready to feed on their own soon after hatching. In spite of this, there have been occasional observations that may suggest that some parent crocodilians provide a little extra help to their offspring.

McIlhenny, in his 1935 book, claimed to have seen American Alligators feeding their young on eight different occasions. A private individual in Florida claims to have seen his broad-snouted caiman, *Caiman latirostris* tearing pieces of meat from a large feeder rat and feeding the smaller pieces to their hatchlings. Blohm, in 1982, said that he witnessed an adult Orinoco crocodile, *Crocodylus intermedius* offering food to hatchling animals.

On two occasions, we have witnessed our adult female Siamese crocodile allowing her hatchlings to eat meat from her mouth. This has only happened twice in almost two years, and did not occur until the hatchlings were more than a year old. The adult Siamese crocodiles are shifted off exhibit when they are fed. This gives the keepers a chance to count the hatchlings, clean the exhibit, and trim the plants. Both adults usually swallow their food almost immediately. However, on these two occasions the female still had a large piece of nutria in her mouth when she was released from the shift cage. The female sat for more than an hour with the meat protruding out of her mouth, allowing the hatchlings to tear off small pieces of meat. The adult male attempted to take the meat away from the female, but she just got up and walked away. Once, she even got in the pool to avoid the male's attempts, but she crawled right back on the bank and held the meat in her jaws until the hatchlings began feeding again.

Many people have read my account of this event, and there are many skeptics that think I might be exaggerating what I saw. However, once they see the event captured on video, there is no doubt that the female allowed the hatchlings to feed from the meat in her mouth. I do not pretend to know what this means. I am not assuming that all crocodilians feed their young, or even that this particular female intended to feed her young. I can only say that she did not mind the hatchlings eating her meal. It is entirely possible that she was just not hungry, and decided to hold the meat for later.

The adult male in this exhibit has been a great father to the hatchlings; he is protective and cautious around them. He is also very food motivated and has never been seen attempting to share his meals with the hatchlings. In addition to attempting to steal meals from the female, I have even seen him try to take the occasional large piece of meat from the hatchlings. He is very gentle about it; trying to only get a hold of the meat without touching a hatchling, even to the point of quickly pulling his head back if a hatchling was accidentally in the way. To prove his good intentions, I have seen him basking with his mouth open just after eating. Hatchlings have crawled in his mouth looking for the meat that they can still smell. One even tried to bite his tongue. The male is very patient, but really does not like the young crocodiles in his mouth, and he gently turns his head and shakes them out.

Figure 4: Female Siamese Crocodile, *Crocodylus siamensis* allowing her young and one adopted American Alligator, *Alligator mississippiensis* (closest to the adult) to eat some of her meal.

CONCLUSION

Crocodilian behavior is often overlooked because we tend to think of them as prehistoric, and therefore too primitive to have complicated behavior. We have also been quick to dismiss all of a particular author's writings, because they were not completely accurate in everything they wrote. I am not the first to suggest that crocodilians swallow under water or that they may feed their young, but other authors have often been ignored because of errors elsewhere in their observations or because so few others have witnessed these behaviors for themselves. It is my assertion that crocodilian behavior deserves a more in depth look, as I am confident they have much more to teach us.

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DNA Tools and Resources for Crocodilian Research

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ABSTRACT: Many new tools for the analysis of DNA from crocodilians have been developed. These tools can be used for the study and identification of individuals, family groups, populations, and species. The biological and genetic basis for 2 types of markers now available for most crocodilians – mitochondrial DNA and microsatellite DNA loci – will be explained. Examples from our previous research will be used to illustrate the potential utility of these DNA tools. Specific information about the DNA tools now available, PowerPoint slides used for this presentation, abstracts of research presented at the 2nd International Crocodilian DNA Workshop, as well as a variety of additional resources are available from the Crocodilian DNA Information Repository (follow links from http://BadDNA.srel.edu).

Nuclear RAG-1 and Mitochondrial Control Region Sequences of the Order Crocodylia: Phylogenetics Implications with Emphasis on the Family Crocodylidae

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ABSTRACT: Crocodilians comprise an ancient and ecologically diverse group and the phylogeny of the Order Crocodylia has been the subject of numerous molecular and morphological studies. While several recent studies have focused on the so-called "gharial problem" – a conflict between morphological and molecular data sets on the relationships between the true and false gharials and their position relative to the rest of the order -, the intrafamilial relationships of the Crocodylidae have been largely ignored. Here, we address both aspects of Crocodylian phylogenetics using the nuclear RAG-1 gene for higher-level relationships and the more variable mitochondrial control region sequences for relationships within *Crocodylus*. Results of nuclear sequence analyses conform to other molecular studies by placing the alligators and caiman as ancient sister lineages, uniting the two gharials as sister taxa of a possibly recent origin, and confirming the recent radiation within family Crocodylidae. Mitochondrial control region sequence analyses agree with morphological studies by uniting the New World forms with the Nile crocodile and by placing *Crocodylus cataphractus* and *Osteolaemus tetraspis* as basal within the family.

Genetics on *Caiman latirostris*

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ABSTRACT: *Caiman latirostris* is an endangered mid-sized South America crocodilian. In the state of Sao Paulo there is an increasing interest on its economic use and also on the conservation of its remaining wild populations. In order to provide genetic management for the captive breeding program of the species by farmers and also in order to understand the use of space by the species in the wild we have developed molecular markers from $(ACC\text{-}TGG)_n$ and $(AC\text{-}TG)_n$ enriched microsatellite DNA library. Since 1988 the captive colony of the species managed by the University of Sao Paulo has already furnished more than 500 captive-born animals from F1, F2, and F3 generations to nine farmers. The animals are individually marked and annually reported by the Regional Studbook of the species in Brazil, managed by the University of Sao Paulo in SPARKS software by ISIS. Parentage tests have been applied to the captive colony when necessary in order to establish individual pedigrees by the use of molecular markers. Combined use of morphometric and genetic methods to assess site of origin of wild individuals on a microgeographic scale has been relatively inefficient, but effectiveness might be improved by an increase in sample size and subject variables (i.e., morphological measurements and DNA markers). Preliminary genetic results suggest, however, that the wild groups sampled are composed predominantly by related individuals. A possible combination between high mortality and low natality rates results in a low number of successfully dispersed individuals per generation. Future studies might help us to understand metapopulacional processes that are possibly occurring with the species in São Paulo, Brazil.

Population Genetic Structure of Amazonian Crocodilians: Preliminary Results

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ABSTRACT: We used the mitochondrial cytochrome *b* gene to study the population genetic structure of *Melanosuchus niger* (from Negro, Purus and Amazonas rivers, and French Guiana), and *Caiman crocodilus* (from Purus and Amazonas rivers). We found 11 haplotypes in both species. In both species one common haplotype predominated, while the other haplotypes were rare. Tajima´s D statistic test was not statistically significant in *M. niger*; it showed that the population is at a genetic equilibrium. However, this test was significantly negative in *C. crocodilus* (D=-2.239, $p<0.01$), i.e. there is a significant excess of the number of segregating sites compared to the average pair-wise sequence divergence. This pattern is frequently observed in populations undergoing a rapid demographic expansion. Gene flow in *C. crocodilus* was also high (Nm = ~16). *Melanosuchus niger* showed significant population structuring and differentiation among all population (F_{ST}=0.31; $P<0.05$) with lower gene flow (Nm = ~3). These results are compatible with the life-style of the two crocodilians; *C. crocodilus* is a habitat generalist and appears to disperse rapidly to newly available habitats, while *M. niger* is a more sedentary habitat specialist.

INTRODUCTION

The smaller spectacled caiman (*Caiman crocodilus*) and the black caiman (*Melanosuchus niger*), have a long history of overexploitation in the Amazon. Intense commercial skin hunting that started in the 1930's continued into the 1980's (Medem, 1983). The commercial importance of the Amazon crocodiles can be appreciated in the descriptions of Fittkau (1970), Medem (1971; 1983), Magnusson (1979), Smith (1980), and Rebêlo and Magnusson (1983) documenting that millions of Amazonian crocodilians were slaughtered for their skins. The commercial hunting drastically decreased the numbers of these two crocodilians, particularly *M. niger*, throughout the Amazon basin. Despite the Brazilian Federal Laws that prohibit all commercial hunting, and IUCN conservation dependent status of *M. niger*, illegal hunting continues throughout the year, threatening the survival of these species (Da Silveira and Thorbjarnarson, 1999). While historical hunting was primarily for the skin trade, current hunting of crocodilians is primarily for their meat fueled by local demand (Da Silveira and

Thorbjarnarson, 1999). Therefore it is necessary to formulate and implement a realistic conservation policy for both species that takes local socioeconomic needs into account.

Preliminary ecological studies suggest that these two crocodilians from the Amazon are very different in terms of life history and reproductive patterns (Da Silveira et al., 1997; Da Silveira and Magnusson, 1999; Thorbjarnarson and Da Silveira, 2000). However, nothing is known about the genetic structure, or about the population genetic indicators of effective population sizes and gene flow among populations of these species. These data are vital for the conservation management of wild populations, as well as for the management of captive breeding efforts. It is with this motivation that we proceed to investigate the population genetic structure of the Amazon crocodiles.

The analysis of mitochondrial DNA (mtDNA) polymorphisms within and between populations is useful for defining Evolutionary Significant Units (ESUs) and Management Units (MUs) (Moritz, 1994) since the distribution of genetic variability within and among populations is affected by historical events and by recurrent factors (Templeton et al., 1995). The high substitution rate of the mitochondrial DNA has facilitated reconstruction of intraspecific phylogenetic relationships and resolved population structure in a variety of taxa. The mitochondrial cytochrome *b* gene is widely used in systematic studies to resolve divergences at many taxonomic levels from "deep" phylogenies to the population and recent divergence levels (Johns and Avise, 1998), including crocodilians (Glenn et al., 2002). We therefore sequence the cytochrome *b* gene, and use the sequence data to conduct a preliminary study the biogeography and the population genetic structure of *Melanosuchus niger* and *Caiman crocodilus* in Amazônia.

MATERIAL AND METHODS

Tissue samples were collected from the tail scales obtained during the marking of adults and hatchlings. Scales were preserved in 95% ethanol, and once in the laboratory stored in a freezer. Samples of *Melanosuchus niger* were collected from Anavilhanas archipelago (N=17), Lake Janauacá (Solimões-Amazonas River, N=8), and Purus River (N=8), and from Kew Swamps (N=13). Samples of *Caiman crocodilus* were collected from Lake Janauacá (N=11) and Purus river (N=13). Tissue samples were dissolved and digested with a Proteinase K/SDS solution, followed by phenol-chloroform extraction, the addition of 5M NaCl, and a final 70% ethanol precipitation of DNA product (Sambrook et al., 1989).

The complete mitochondrial cytochrome *b* gene was amplified by Polymerase Chain Reaction (PCR) using the primers listed in Table 1. Protocol is as follows: denaturation at 94°C for 35 seconds, annealing at 50°C for 35 seconds, and extension at 72°C for 1:30 minute repeated for 35 cycles. Sequencing reactions were performed according to the manufacturers recommendation using the Terminator Cycle Sequencing Kit (Amersham Pharmacia), and resolved a MegaBACE automated sequencer (Amersham Pharmacia).

Table 1. Sequencing primers used in the present study.

Primer Sequence	Reference
L14254 (5'-ATGACCCACCAACTACGAAAAT-3')	(Glenn et al., 2002)
L14731 (5'-TGTCGTGCCATGAATTTGAG-3')	(Glenn et al., 2002)
H14779 (5'-CGAATGGAAGGAGGAAGTG-3')	(Glenn et al., 2002)
H15454 (5'-GGTTCCGTCCACTTCTGTCTTACAA-3')	(Glenn et al., 2002)
H15982 (5'-TCCTRGCTTTGGTAGCCAGG-3')	This study

DATA ANALYSIS

Homologous protein-coding regions were aligned manually and confirmed by translating the DNA data into putative amino acid sequences in the program BioEdit (Hall, 1999). The present data set is

composed of nearly complete cytochrome *b* gene sequences, which did not show any insertions or deletions (indels). A total of 46 individuals of *M. niger* from four locations, and 24 individuals of *C. caiman* from two locations were scored; the cytochrome *b* segment represented of 11 haplotypes defined by 12 segregating sites in *M. niger*, and 11 haplotypes defined by 15 segregating sites in *C. caiman*.

A number of statistical methods have been developed to infer historical processes shaping observed patterns of genetic distribution and diversity. The genetic equilibrium of mtDNA alleles was tested using Tajima's D test (Tajima, 1989). Although this test have been formally designed to test for selection, a significant deviation from genetic equilibrium in mtDNA alleles is most likely a result of recent population expansions or bottlenecks in situations where no selective advantage among haplotypes exists (Hartl and Clark, 1997). The hypothesis of a recent genetic bottleneck was tested for by looking for significant excess of common and of medium frequency alleles at the expense of rare alleles (Watterson, 1978). Population subdivision and structure was examined using an analysis of molecular variance (AMOVA) (Excoffier et al., 1992), and pair-wise population F_{ST} significance test (Cockerham and Weir, 1993) as implemented in the program ARLEQUIN (Schneider et al., 2000).

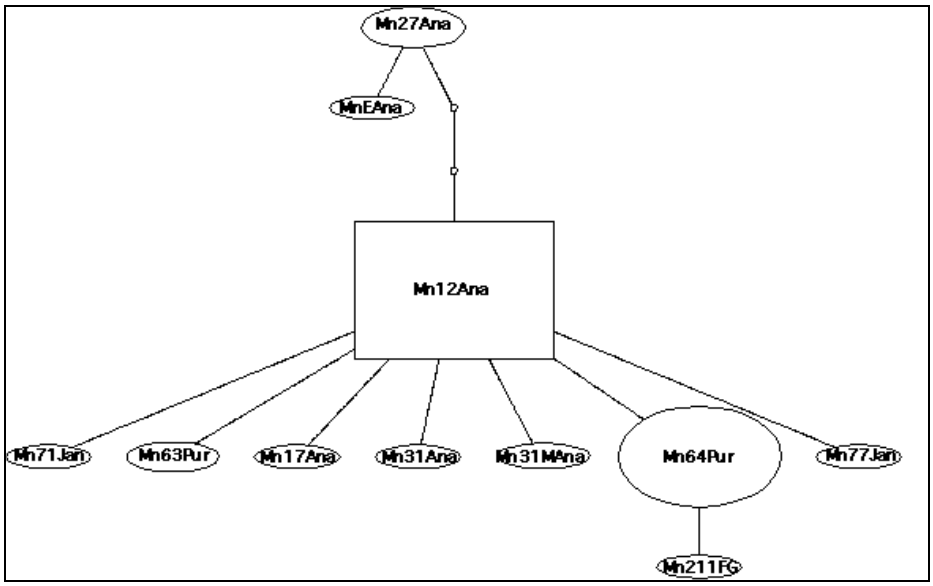

Figure 1. A minimum spanning network of *Melanosuchus niger* haplotypes. Mn12Ana is the most likely ancestral haplotype from which most other haplotypes are derived. Each oval represents a haplotype and the size of the oval is proportional to the number of individuals possessing that particular haplotype.

RESULTS AND DISCUSSIONS

We used the mitochondrial cytochrome *b* gene to study the biogeography and population genetics structure of the two Amazon crocodilians. Here we describe the first population genetic results for *Melanosuchus niger* (black caiman) and for *Caiman crocodilus* (spectacled caiman) from the Amazon basin.

Melanosuchus niger

A total of 46 individuals of *Melanosuchus niger* from four localities were scored. The final alignment comprised 871 positions of partial sequence of cytochrome *b* defining 11 haplotypes separated by 12 segregating sites (Fig. 1). The mean frequency base composition was 28% A, 24% T, 35% C and 13% G confirming a slight under representation of Guanine, as is normally observed in the mitochondrial genome (Zhang and Hewitt, 1996). One common haplotype predominates, while the other haplotypes are composed of rare haplotypes or singletons. Tajima´s D statistic test is not significant (D=-1.44, P>0.05), i.e., it shows that the population is at a genetic equilibrium with respect to mtDNA alleles. The negative result of Tajima´s D is suggestive of a population expansion, however,

this result is not statistically significant. Implications for the *M. niger* as a whole is that it appears to be undergoing a recovery and a population expansion after laws have been passed that protect this species from uncontrolled hunting.

Melanosuchus niger shows significant amount of population structuring and differentiation among populations analyzed. Differentiation among population was inferred from Wright's *F* statistics (Wright 1951) that revealed a significant F_{ST} values among all comparison, except between Purus and Janauacá (F_{ST}=-0.02, *P*=0.78); Purus and Janauacá are also the only two localities to experience any significant between locality gene flow. In order to estimating the extent of subdivision between samples we pooled the four populations into three larger groups according to macrogeographical areas and water type, where Solimões region comprised Purus and Janauacá, rio Negro comprised the Anavilhanas archipelago, and French Guiana comprised Kew Swamps. AMOVA tests indicate that there is significant structuring in the data at all hierarchical levels. The mean source of genetic variation (69.4%) was attributable to variation within populations, –2.79% of the total variance referred to variance among populations within regions, and 33.4% to variance among regions. The F_{ST} value for the combined data from all groups was significantly greater than zero (F_{ST}=0.31; *P*<0.05). The female variance effective population is on the order of ~ 2.1 x 10^5 individuals.

Melanosuchus niger is restricted to floodplain lakes that are isolated hydrologically to a large degree from main river systems, thus isolation by distance would seem to be likely mechanism responsible for structuring. However, gene flow among localities does exist as seen in the Purus and Janauacá Lake samples that are geographically approximately equidistant to the Anavilhanas archipelago that is significantly isolated. Other factors, such as water type may therefore play a role in the geographic structuring of *M. niger*. Based on these preliminary mtDNA results, the presence of a population structuring suggests that *M. niger* will have to be divided in to different management units and possibly comprises different evolutionary significant units.

Caiman crocodilus

A total of 24 individuals of *Caiman crocodilus* from two localities were scored. The final alignment comprised 1188 positions of nearly complete sequence of cytochrome *b* and a non-coding spacer between cytochrome *b* and $tRNA^{Thr}$ defining 11 haplotypes separated by 15 segregating sites (Fig. 2). The mean frequency base composition was 31% A, 25% T, 33% C and 11% G confirming a slight under representation of Guanine, as is normally observed in the mitochondrial genome (Zhang and Hewitt, 1996). One common haplotype predominates, while the other haplotypes are composed of rare haplotypes or singletons. Tajima´s D statistic test is significantly negative (D= -2.239, p<0.01), i.e. it shows a significant excess of the number of segregating sites compared to the average pair-wise sequence divergence. This pattern is frequently observed in populations undergoing a rapid demographic expansion (Hartl and Clark, 1997) as would be expected in a prolific species released from hunting pressure.

AMOVA tests indicate that there is no significant structuring (F_{ST}=0.04, *P*=0.04 and that gene flow between the two localities is high (Nm ~ 16). The mean source of genetic variation was attributable to variation within populations (96%) and only 4% of the total variance referred to variance between populations. These results are compatible with the life style of *C. crocodilus*; *C. crocodilus* is a habitat generalist and appears to disperse rapidly to newly available habitats while *M. niger* is a habitat specialist. While census populations sizes of *C. crocodilus* are higher than those of *M. niger*, the female variance effective population is only on the order of ~1.78 x 10^5 individuals.

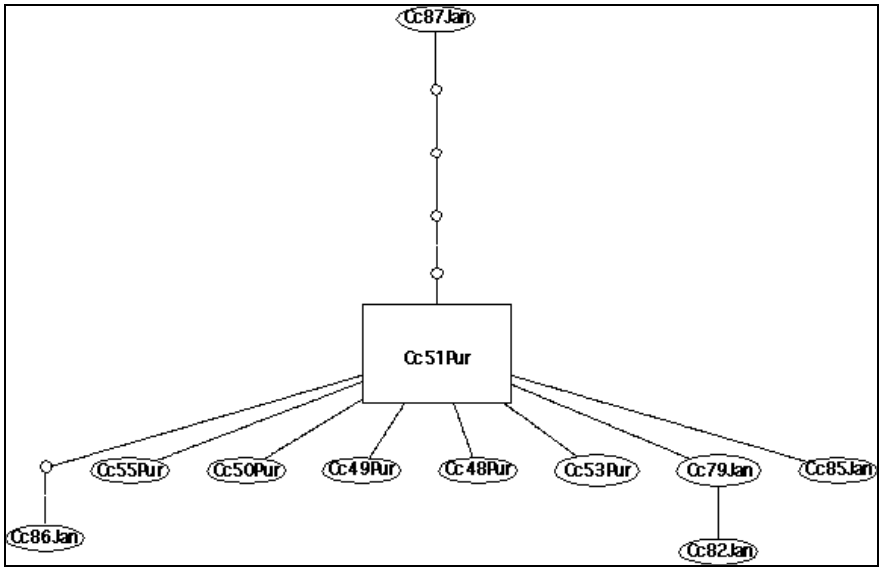

Figure 2. A minimum spanning network of *Caiman crocodilus* haplotypes. Cc51Pur is the most likely ancestral haplotype from which most other haplotypes are derived. Each oval represents a haplotype and the size of the oval is proportional to the number of individuals possessing that particular haplotype.

CONCLUSIONS

The cytochrome *b* gene has shown to be a good molecular maker to study population differentiation in the Amazon crocodiles. The two species analyzed in the present study are very different in terms of habitat use; *Melanosuchus niger* is a species restricted to floodplain lakes that are isolated hydrologically to a large degree from the main river systems while *Caiman crocodilus* is a habitat generalist. Our molecular data suggest that significant amount of genetic differentiation exists among *M. niger* populations, and populations inhabiting black water and white water regions of the Amazon basin may represent different evolutionary significant units, and certainly should be treated as separate management units. Additionally, *M. niger* appears to be undergoing a relatively slow demographic expansion, however, this result is not significant most likely due to its life history. *Caiman crocodilus* on the other hand is much more of a habitat generalist, and while some individuals may nest in floodplain lakes, the vast majority inhabit open lake systems and overflow channels whose water level fluctuates markedly during the year and facilitate dispersal. Molecular data suggest there is very little or no population structuring in this species. *Caiman crocodilus* appears to act as one large population that is currently undergoing a demographic expansion.

Based on this preliminary study, the prospects for the recovery of both species appear to be good. However, both species, and *M. niger* in particular, will need careful management as well as additional molecular studies to define appropriate units of management and conservation.

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Molecular Approaches for Evaluating Species Boundaries in Crocodilians

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ABSTRACT: We applied molecular sequencing and genotyping approaches to two questions concerning species boundaries in crocodiles. First, we evaluated population structure and validity of subspecies designations for wild populations of Nile crocodile (*Crocodylus niloticus)* from mainland Africa and Madagascar. We examined sequence data for unique molecular characters in both mitochondrial (mtDNA) and nuclear gene regions and then genotyped populations for unique allelic signatures. Results to date indicate substantial regional sub-structuring concordant with previously hypothesized biogeographical events. Second, we evaluated the southern Belize populations of the endangered American Crocodile (*C. acutus*) and Morelet's crocodile (*C. moreletii*) for evidence of hybridization using mtDNA and nuclear markers. These data were used to corroborate hybridization events inferred from behavioral and morphological data. The methods developed by these projects provide the basis for a more integrated approach to crocodile management and conservation.

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Conservation Genetics for *Caiman yacare* in Bolivia: Potential Forensic Applications

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ABSTRACT: *Caiman yacare* is a medium sized crocodilian of the family Alligatoridae. It occurs abundantly in variety of freshwater habitats in Bolivia, Brazil, Paraguay and Argentina. I took a total of 245 blood or tissue samples from caiman in the five principal lowland river drainages of Bolivia. Molecular analysis of the mtDNA cytochrome *b* gene using PCR techniques showed a distinct divergence between the caiman found in the Amazon basin (4 river drainages) and those from the Paraná River basin. Higher resolution was desired so genomic DNA was screened for microsatellites. Primers were developed and initial results showing population partitioning are presented.

All range states have commercial use programs for caiman hide export in various stages of development. Bolivia is in the process of conducting intial harvests of wild caiman populations. One of the main concerns is entry of illegal hides into the system during harvest, either from non-permitted areas or from a neighboring country. Use of molecular techniques with microsatellites has potential use by Wildlife authorities for proof of origin for caiman hides. A new technique, Amplified Fragment Length Polymorphisms (AFLP), is less complicated and less costly. AFLPs might potentially be used for forensic hide control and preliminary data are discussed.

Bioecological Aspects of *Crocodylus acutus* Liberated in the Tacarigua Reservoir (Falcon, Venezuela)

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ABSTRACT: In order to evaluate a program of repopulation of *C. acutus* in the Tacarigua reservoir, we undertook a comparative study of the bio ecological aspects of resident and released crocodiles. Among the results, we detected that the average growth rate of 7.60 mm/month in animals after release was significantly less than their prior growth in captivity of 24.93 mm/month. In resident crocodiles the average and instantaneous growth rates were greater: 10.03 mm/mo estimated from recaptures and 26.15 and 29.19 estimated by the models of von Bertalanffy and Richards. We used the Jolly-Seber method to estimate survival rates of 52.5% of the released crocodiles and 29.6% in residents. Indices of condition in in released male crocodiles were significantly greater than the resident males. Crabs (*Poppiana dentata*) occupy an important position in the released sample and fish (*Caquetaia kraussi, Hoplias* sp. and others) in the residents, in that their frequencies were similar. The released animals preferred positions in the openings with logs and branches, possibly as an instinctive response to caution produced in captivity, but the resdients preferred areas with aquatic vegetation. It can be concluded that released crocodiles have established themselves succesfully in the environmenatl structure of the reservoir, which would lead to sympatry with *Caiman crocodilus*.

Aspectos Bioecólogicos de *Crocodylus acutus* Liberados en el Embalse Tacarigua (Falcón, Venezuela)

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RESUMEN: Para evaluar el programa de repoblamiento de *Crocodylus acutus* en el Embalse Tacarigua, se realizó un estudio comparativo de aspectos bioecológicos de cocodrilos liberados y residentes. Entre los resultados destaca la tasa de crecimiento promedio de 7,60 mm/mes en liberados, significativamente menor a la tasa en cautiverio de 24,93 mm/mes. En residentes las tasas de crecimiento promedio e instantáneas fueron mayores: 10,03 m/mes por recapturas, y 26,15 y 29,19 mm/mes por modelos de von Bertalanffy y Richards. Por el método de Jolly -Seber se estimaron índices de sobrevivencia de 52,50 % en liberados y 29,60 % en residentes. Los índices de condición de cocodrilos machos liberados fueron significativamente mayores al de machos residentes. Los cangrejos (*Poppiana dentata*) ocupan un importante lugar en la dieta de liberados y el renglón peces (*Caquetaia kraussi*, *Hoplias sp* y otros) en residentes, a pesar que sus frecuencias son similares. Los liberados prefieren lugares en las orillas con palos y ramas, posiblemente como respuesta instintiva de cautela producto del cautiverio, mientras que residentes prefieren sitios de vegetación acuática. Se concluye que los cocodrilos liberados se han establecido exitosamente en el ambiente estructuralmente complejo del embalse, que a su vez favorece simpatría con *Caiman crocodilus*.

Ecology and Conservation of the American Crocodile in Florida

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ABSTRACT: The American crocodile (*Crocodylus acutus*) is primarily a coastal crocodilian that is at the northern end of its range in southern Florida. In Florida, habitat loss from human development has been the primary factor in this endangered species decline. Currently, they face new issues--Florida Bay has undergone a number of changes that have caused a great deal of concern for the ecological health of this ecosystem and primary crocodile habitat. Efforts have been, and continue to be, made to improve Florida Bay and Biscayne Bay. In south Florida we have the unique opportunity to integrate endangered species conservation with ecosystem restoration and management. American crocodiles thrive in healthy estuarine environments, and are particularly dependent on freshwater deliveries. Recovery of the American crocodile in Florida will require an integration of habitat enhancement for an endangered species with environmental education. As crocodiles benefit from a restored freshwater flow into estuaries, their numbers will increase. The challenge of integrating a recovering population of the American crocodile with an ever increasing use of coastal areas by humans will require a proactive educational program and will be the final challenge in the successful recovery of this once critically endangered species.

Monitoreo de Poblaciones Silvestres del Cocodrilo de Morelet *Crocodylus moreletii* en Varios Estados de la República Mexicana

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ABSTRACT: Durante 2002 hicimos tres viajes itinerantes en 8 estados de la república mexicana: Yucatán, Quintana Roo, Campeche, Chiapas, Tamaulipas, San Luis Potosí, Veracruz y Oaxaca. Monitoreamos 22 localidades potenciales de *Crocodylus moreletii* para conocer el estado actual que guardan sus poblaciones. Realizaron monitoreos nocturnos bajo las técnicas establecidas. Observaron 378 cocodrilos de Morelet y capturamos 80, En ningún sitio observamos neonatos (NN) ya que los muestreos se hicieron 6 meses posteriores a la eclosión. De las clases I, II, III y IV se observaron 144, 86, 70 y 78 ejemplares respectivamente. Las localidades donde se encontraron las densidades mas altas fueron: Lago El Caracol, Chiapas: 18.5, Hampolol, Campeche: 14.1, Arroyo San Vicente, Chiapas: 14,Lago El Aguacate, Chiapas: 13.3, Estero La Victoria, Veracruz: 10.24, Lago de Nixtamalapan, Veracruz: 10, Laguna de la Mancha, Veracruz: 10, Villa de Casas, Tamaulipas: 8.74 y Río Yalikín, Quintana Roo 8.33 cocodrilos/Km. En esta información se aportan las densidades poblacionales más altas registradas para la especie en México.

Population Size Structure of *Caiman latirostris* in Artificial Impoundments in Northern Uruguay

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ABSTRACT: Populations of *Caiman latirostris* are known from Uruguay but survey data is scarce. This species shows an apparent capability of colonizing a wide variety of habitats. As remaining habitats destruction and modification seems to be of major concern for this caiman conservation, we focused attention on its presence in artificial water bodies. Night-light surveys were performed in impoundments used for agricultural purposes in northern Uruguay. A total of 35 surveys were conducted over 26 sites corresponding to three stream basins of the Uruguay River drainage (Ñaquiñá, Lenguazo and Falso Mandiyú). Surveys were carried out between January and March 2001, and between December 2001 and March 2002. Caiman sizes were estimated from a slow moving boat for 153 individuals. Our results showed that juvenile, sub-adult and adult categories were represented in artificial impoundments. The most frequent sizes observed were included in juvenile categories up to 1 m total length, which comprised more than half of the individuals. The distribution of population size classes in this study is perhaps not atypical for crocodilian populations expected to be healthy. Accumulation of data is needed to assess the viability of populations in artificial habitats and their role in this species management and conservation strategies.

Status of the French Guianan Black Caiman (*Melanosuchus niger*) Population

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ABSTRACT: One of the largest remaining *Melanosuchus niger* population is located in the Kaw swamps Natural Reserve, French Guiana. Until a few years, this population focused a limited interest and remained substantially unknown. The habitat is a large flooded savanna (75 km_), bordered by marsh and gallery forests, and mangroves. The bordering rivers have been harvested during decades, until late eighties; alternatively the main part of the swamp remained inaccessible and rather pristine. Since 2000, monthly surveys are conducted on the 60 km-long river crossing the reserve and in surrounding areas; regular missions are undertaken in permanent ponds in the heart of the swamp. In these inaccessible ponds, *Melanosuchus* densities varied from 25 to 60 animals/km, the species accounting for 80-99% of caimans total sightings. On the river, densities are less than 1 individual/km, and dominant species are spectacled and dwarf caimans, with a strong habitat partionning. Current aditionnal developments are a capture/marking/recapture study on the Kaw River, and the study of 5 microsatellite DNA loci to assess the population efficiency and structuration; the recovery potential will now be followed-up in depleted areas. Our preliminary results confirm the presence of a large and viable black caiman population in French Guiana.

Monitoring wild populations of *Caiman crocodilus* (babas) in Guárico and Llanos Boscosos Ecological Regions, Venezuela

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ABSTRACT: The Cayman (*Caiman crocodilus*) program for commercial use in Venezuela is implemented in 7 ecological regions since 1983 to the present. Each ecological region is surveyed to determine the population status in terms of its abundance and sizes classes. During the year 2002, a total of 114,805 ha were censused in the Guárico Ecological Region and 117,840 ha in Llanos Boscosos. The density obtained in both regions resulted very inferior to the previous censuses, probably due to environmental factors, mainly the strong drought. However, sizes classes did not show differences in relation to previous censuses. It was recommended to suspend the implementation of the program in both ecological regions until carrying out a new census before the beginning of the next drought season, with the aim to determine the population's real situation.

RESUMEN: El programa de aprovechamiento comercial de la baba (*Caiman crocodilus*) en Venezuela se implementa en 7 Regiones Ecológicas desde 1983. Cada región ecológica es censada para determinar el status de las poblaciones en términos de su abundancia y estructura de tamaños. Durante el verano de 2002 se censaron 114.805 ha en la Región Ecológica Guárico y 117.840 ha en Llanos Boscosos. La densidad obtenida en ambas regiones fue muy inferior a los censos anteriores, problablemente debido a factores ambientales, principalmente la fuerte sequía. Sin embargo las estructuras de tamaños no mostraron diferencias en relación a los censos anteriores. Se recomendó suspender la implementación del programa en ambas regiones ecológicas hasta realizar un nuevo censo antes del inicio de la próxima temporada de sequía, a los fines de determinar la situación real de la población.

INTRODUCTION

Velasco and Ayarzagüena (1995) evaluated in 1991-92 the status of wild populations of baba in terms of abundance and sizes classes after 9 continuous years of harvesting. This work was the first one that study the effect caused by a sustainable program on wild populations of this species. Until then, PROFAUNA have not reliable records that allows evaluating the impact of harvesting.

Among the most important results of this work, it was the description and characterization of seven ecological regions, size classes and abundance, with an estimation of the capacity of sustainable crop in each one. A recommendation was proposed to PROFAUNA of suspending during four years the harvest in Guárico Region, due to the small proportion of Class IV individuals (mature males of great size), the hunted portion of the population. Finally, the study recommended continued population surveys in each ecological region.

In 1995, the region Guárico was evaluated, giving as results the characterization of six sub-regions in it and restarts the program in the sub regions Camaguán and Cazorla (Colomine *et. al*., 1996). In 1996, PROFAUNA took the decision to paralyze the program in Venezuela (Quero & Velasco, 1995), in order to execute another recommendation of the 1992 study: the evaluation of the harvest impact on the babas populations. In the 1995 study the ecological regions were ratified and the status of populations subject to annual harvest were qualified as good, as much in their abundance as in their size classes. A main

recommendation to PROFAUNA was the continuation of population surveys with a yearly schedule for ecological region, covering a wide surface during each study.

The present work is part of the aforementioned recommendation, with which the third survey of all the ecological regions is completed, coinciding with 20 years of application of the Sustainable Use Program.

OBJECTIVES

The objectives of the present study contemplate to establish the status of the populations in each ecological region, as much in their abundance as their size classes, and the characteristics of the habitats where the babas populations were located.

METHODS

Light-night counts were made accordingly to Chabreck (1966) and Woodward & Marion (1977), in order to determine population abundance and size classes (Ayarzagüena, 1983): Class I (hatchlings), Class II (juveniles), Class III (male adults and females) and Class IV (adults larger than 1.8 m of total longitude), in each one of the observed water bodies.

AREA OF STUDY

The fieldwork was carried out in Guárico and Llanos Boscosos ecological regions (Fig. 1), defining as study area a sample of farms visited previous censuses 1991-1992, 1995 and 1996 (Velasco & Ayarzagüena 1995; Colomine *et. al.* 1996 and Velasco *et. al*. 1997), according to the Ministry of Environment and Natural Resources (MARN) records.

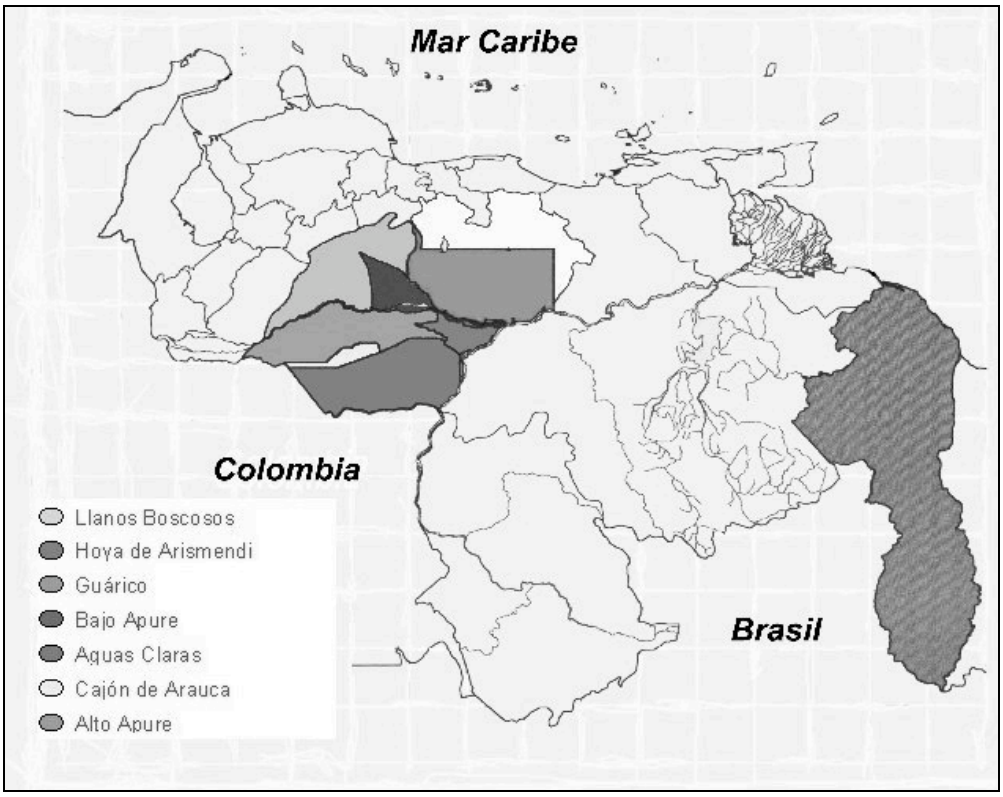


Figure 1. Ecological regions in Venezuela

The present study embraced a total surface of 232,645 ha, representing 4.06% of the total area in both regions, separated in 114,805 ha in Guárico Region and 117,840 ha in Llanos Boscosos Region with 42 farms visited (Table 1).

Compared with previous studies, the surface covered in Guárico Region was smaller than the observed in 1995-96 (Table 1), partly because census was carried on exclusively in those areas where the species was harvested since 1997. The areas of Cabruta, Santa Rita and Parmana, included in the total surface of the region, were not surveyed. In the case of Llanos Boscosos Region, the surveyed surface was similar to those of previous studies.

Table 1. Total and surveyed surfaces in 1991-1992, 1995-1996 and 2002

REGION	Total surface (ha)	Surveyed surface 1992 (ha)	%	Surveyed surface 1995-1996 (ha)	%	Surveyed surface 2002 (ha)	%
Guárico	2,620,800	69,297	2.64	288,420	11.01	114,805	4.38
Llanos Boscosos	3,114,384	193,873	6.23	125,515	4.03	117,840	3.78
TOTAL	**5,735,184**	**263,170**	**4.59**	**413,935**	**7.22**	**232,645**	**4.06**

Table 2 shows the aquatic surface observed in each region and survey. In 1991-1992 it covered 614.19 ha (0.23% of the total observed surface) in both regions. In the present work the observed aquatic surface was much smaller, 264.19 ha (0.11% of the total surface).

Table 2. Total and aquatic observed surfaces and their percentage in 1992, 1995 - 1996 and 2002

REGION	Total surface observed 1992 (ha)	Aquatic surface observed 1992 (ha)	%	Total surface observed 1995-1996 (ha)	Aquatic surface observed 1995-1996 (ha)	%	Total surface observed 2002 (ha)	Aquatic surface observed 2002 (ha)	%
Guárico	69,297	149.19	0.22	288,420			114,805	182.54	0.16
Llanos Boscosos	193,873	465.46	0.24	125,515	270.24	0.22	117,840	81.65	0.07
TOTAL	**263,170**	**614.65**	**0.23**	**413,935**			**232,645**	**264.19**	**0.11**

The aquatic surface reduction in both regions in 2002 indicates a strong drought in the moment of the census. In Guárico Region, the proportion of aquatic surface observed was inferior in comparison with previous censuses (0.22% in 1992 and 0.16% in 2002). The same situation is reported for Llanos Boscosos Region, where the drought effect was even more marked, with a drastic reduction of the observed aquatic surface (from 0.24% and 0.22% in the previous censuses to 0.07%).

GENERAL RESULTS

As a consequence of the described drought effects on both regions, the number of observed water bodies was lower than in previous censuses, surveying only 68 in Guárico and 36 in Llanos Boscosos (Table 3). The mean number of individuals observed in water bodies shown significant differences among both regions (_ =0.05), with Guárico reaching the highest value. Although these results reflected different conditions of the populations in each region, the statistical analysis is not supported by enough data and show high standard deviation.

Table 3. Results of ANOVA of the means of individuals for body of water in each region (_ =0.05).

REGION	CASES	MEANS	STD	Dif.
Guárico	68	90.65	106.04	X
Llanos Boscosos	36	40.50	121.59	X

The shortage of obtained data prevented to analyze statistically the abundance of babas in each type of water body. The sample obtained in Llanos Boscosos was 36 water bodies in total, conformed by 4 "caños" (natural drainage channels), 2 lagoons and 21 "préstamos" (man made excavations for construction of roads) with water, with other 9 water bodies completely dry. In Guárico 6 "caños", 35 lagoons, 22 préstamos and 3 artificial channels were observed, with other 2 water bodies completely dry.

These data confirms the strong influence of drought in both regions, reflected in the low aquatic surfaces observed under the prevalent environmental conditions at the moment of the census.

Most farms visited in Guárico and Llanos Boscosos were previously surveyed or appeared in MARN files of the Program. Many of these were currently abandoned, invaded, broken into fragments or even urbanized. That was a strong limitation for the realization of the census in this period.

GUÁRICO REGION

Density

The registered density was 0.05 babas per hectare, obtained from a total count of 6,266 individuals in 114,805 observed hectares, from which only 0.16% corresponded to aquatic surface. This density is inferior to the registered one in 1992 and 1995 of 0.13 babas/ha and 0.09 babas/ha, respectively (Fig. 3).

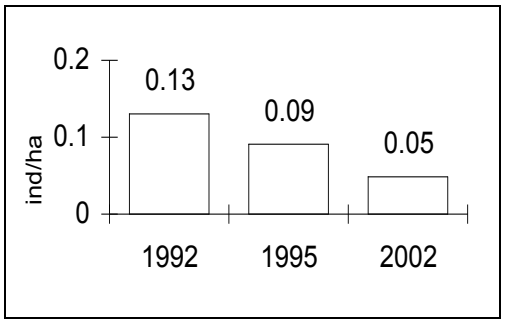


Figure 3. Density in Guárico Región (Baba/ha)

The values of density in the three censuses presented a sustained decline that can be related to the environmental conditions of extreme drought. These conditions caused a reduction of the aquatic surface and the number of observed bodies of water.

Size Classes

The size classes in this region did not show an important variation compared with the obtained in 1995 (Fig. 4), with a slight decrease of Classes IV and II, and an increase in proportion of Class III.

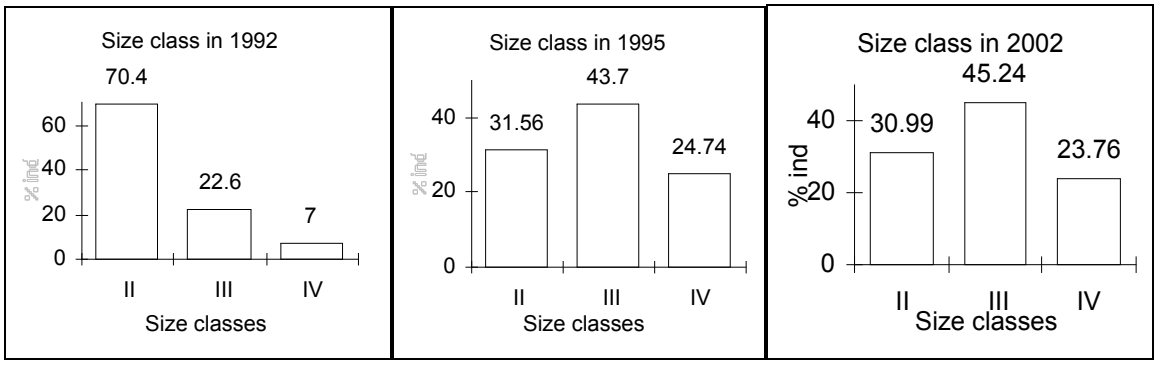


Figure 4. Sizes classes in Guárico

The histogram of size classes does correspond to wild populations subjected to sustained commercial exploitations, with pyramidal form and Class IV value superior to 18% (Velasco & Ayarzagüena 1995). The size structure also indicates the recovery reached by the populations after the suspension of the program between 1992 and 1995. A proportion of 23.76% of Class IV with 2,629 individuals is reported.

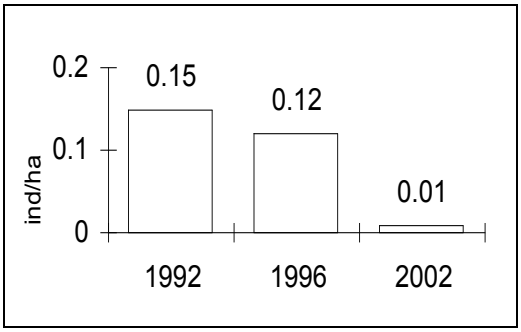


Figure 5. Density in Llanos Boscosos Region (Babas/ha)

LLANOS BOSCOSOS REGION

Density

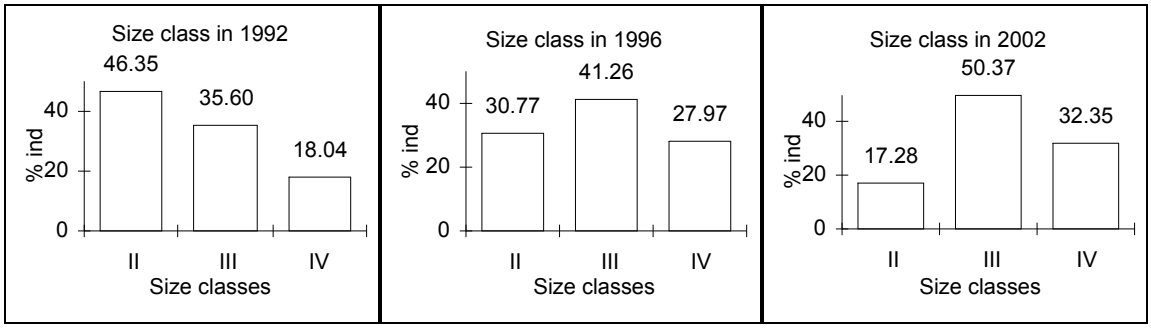
Global density in Llanos Boscosos was 0.01 ind/ha (Fig. 5), much lower that the previous results,

resulting from the count of 729 babas in 117,840 ha observed, with only 0.07% of aquatic surface.

As in Guárico, the density show a sustained tendency to decline in the successive censuses, being much more marked the reduction in the density in Llanos Boscosos, reaching the lowest data recorded in all censuses practiced to the present in ecological regions. However, it is necessary to point out that the environmental conditions in the moment of the present census were characterized by an intense drought, with a large amount of water bodies totally dried.

Size Classes

Size classes reflected increases in proportions of Classes III and IV in comparison to 1992 and 1996 (Figs. 6), with a reduction of Class II. The current structure presented a pyramidal type, corresponding to harvested populations coincident with the structure recorded in 1996. It is important to remark that PROFAUNA authorized the hunt of only 2,941 animals in 2001 while in 2000 no hunt was permit.



CONCLUSIONS

1. Both regions presented very low levels of density, showing a tendency to diminish in comparison to previous censuses. Values presently obtained should be related with the prevalent environmental conditions of extreme drought during the period of study. The scarce observed water bodies presented highly concentrated, abundant and stable populations, with size structures corresponding to not over cropped populations. This result indicates that the reduction registered in population density is not probably caused by on-exploitation or illegal hunt, but rather attributable to the drastic reduction of the availability of water bodies. However, extremely low density levels were registered in comparison with previous censuses in these two regions and densities obtained in other ecological regions.
2. Most of the visited water bodies included in MARN database or previously observed, were completely dried. It indicates that during the period of maximum drought the appropriate evaluation of the natural populations is affected, due to the promptness with which these water bodies disappear when the drought is advancing. Even several important rivers of the sector were segmented and their flow detained (i.e. Guariquito, Caballo and Apurito Rivers).
3. Together with the environmental condition of extreme drought, irregular condition of abandonment, invasion, fragmentation, disordered occupation and even urbanization in many properties registered in MARN records hindered the realization of censuses in both regions. Some properties registered in MARN simply did not exist at the present time and peasants irregularly occupy lands. This condition also affected the analyses on mean abundance of babas in each property type classified by the surface occupied by the farm.

RECOMMENDATIONS

1. The obtained values of density did not serve as base to allow sustainable harvest of babas in both regions. It is recommended to suspend the grant of licenses and maintain a strict surveillance of the illegal hunt in both regions, until a repeat of the population study under favorable environmental conditions to establish a confident evaluation of the wild population status.

2. The coincident of the survey with the months of maximum drought in both regions, did not allow a complete evaluation of the wild populations. It should be considered that these regions are located at north of the Apure River fluvial system. These areas keep natural water bodies flooded during a very short period in the dry season, due to their altitude above sea level, soil conditions and drainage capacity. It is advisable to perform population studies toward the end of rainy season and beginning of dry season (December-January), when natural water bodies still maintain enough flood levels and populations are more easy to evaluate.

3. The previous recommendation implies to study possible modifications of the Program calendar in the two regions, particularly the application lapses and grant of licenses, and the lapse of hunt. The situation of extreme drought and disappearance of water bodies in both regions also has a negative effect, because it favors that the granted skins has to be obtained in other ecological regions, due to the difficulty to locate animals in the few remaining water bodies during the lapse of hunt.

4. MARN database and files of farms and water bodies should be updated, evaluating the possibility to request again all the legal documentation to the users of the program in both regions. This can be an answer to the current nonexistence of a considerable number of farms or properties included in this database, and to the frequent situations of abandonment, invasion, fragmentation, deforestation, intervention, illegal and disordered occupation and even urbanization in the lands that were presented as legally registered properties participating in the Program. Equally, MARN should exhort to the competent organisms to achieve the regularization of the property of lands in both regions, as well as to promote the design of a special plan of surveillance and control in both regions, directed to the conservation and recovery of favorable primary and secondary habitats to the species and, in general, to the wild fauna.

5. It is recommended that MARN consider the possibility to restore at its previous levels the taxes collected through the Law of Tax Stamp by consideration of services. It has been proven the minimal influence on the economic activity of the decision took on last year of discharge 80% of the tax payment by users of the Program. There is a continuous rise of prices to the final consumers of the products obtained under this regime, although practically the tributary contribution was eliminated to the producers, middlemen and traders that have increased its earnings substantially thanks to the public consumers expenses. It is remarkable that the funds obtained with this tax were the only source to support population studies of the species subjected to commercial use. On the other hand, funds should be available at the end of each year.

6. The adoption of these recommendations requires the participation and wide consultation to all the involved stakeholders. A first step for this consultation will be the realization of a Workshop foreseen to evaluate the program, where regional and local governments, private sector integrated by producers, middlemen and industrial, ONG's, academic and research sectors, and the agencies involved in surveillance and control should participate. Technical meetings with National Council for Wild Fauna (CONAFASI), National Council for Baba, and Crocodile Specialist Group, should also be implemented in this consultation process.

7. MARN should develop a specific project to evaluate the hydrometeorological cycle rain/drought of the ecological regions, directed to settle down with more precision the flood characteristics and drying of the natural water bodies. This project should include the analysis of precipitation, evapo-transpiration and other climatic variables that can influence this cycle, and also the use of satellite images in environmental and geographical description of the water bodies in the ecological regions.

8. As indicated in previous reports, MARN should develop a specific activity of environmental education on the Program, focusing it as one of the most important practical activities in sustainable development applied to rural environment, working on a natural resource of wild fauna that has received recognition at international level for its quality. The main recommended issues for this educational activity should be the conservation of habitats, control of illegal hunt and participation of the communities in the wild fauna species management.

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Preliminary Results of a Population Study of American Crocodile (*Crocodylus acutus*) in Jalisco, Mexico.

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ABSTRACT: The American crocodile and its habitat in the coast of Jalisco are impacted by the tourist industry and the growing human population. Similar factors have extirpated or diminished *C. acutus* in most of its range. This research presents information about some aspects of population ecology in three populations in Jalisco.

During 1999-2002, three coastal locations in Jalisco (Boca Negra, Majahuas and La Manzanilla) were monitored to understand the population behavior and to provide a basis for making comparisons among the sites. Night surveys were made on two consecutive days in each site four times a year. The size of each crocodile sighted was estimated and classified into the following categories: (only eyes (OE), < 0.60, 0.60-1.5, 1.5-2.5, >2.5 m). The estimated size was calibrated by captures. Conversion of the number of animals sighted to a population estimate was made by a formula suggested by King et al. 1990 and Thorbjarnarson et al. 2000.

The estimation of population in Boca Negra estuary (28 ha) was 92 individuals (3.20 crocs/ha, p=28.56%), Majahuas (195 ha) 49 (0.26 croc/ha, p=46.63%) and La Manzanilla (153ha) 314 (2.05 crocs/ha, p=51.50%). Size structure in Boca Negra consisted of mostly hatchlings (<0.6 m) (38.6 %) and juveniles (0.6-1.5 m) (26.2%), in Majahuas of juveniles between 0.6 and 1.5 m (42%) and in La Manzanilla of adults (> 2.5m) (31%). The differences between these three sites and the effects on conservation of the species in Jalisco are discussed.

Key words: *Crocodylus acutus*, population size, size structure, nest, habitat use, Jalisco, Mexico.

RESUMEN

La industria turística y el incremento de la población humana han causado un impacto negativo en el hábitat y en las poblaciones de cocodrilo en la costa de Jalisco. Factores similares han provocado que la especie haya sido extirpada o reducida en la mayor parte de su distribución. Esta investigación presenta información acerca de algunos aspectos de la ecología de poblaciones en tres poblaciones silvestres en Jalisco.

Se censaron tres lagunas costeras (Boca Negra, Majahuas y La Manzanilla) en Jalisco de 1999 a 2002, para entender y comparar cada una. Se realizaron conteos nocturnos durante dos días consecutivos en cada sitio, cuatro veces al año. Se estimó el tamaño de cada cocodrilo avistado y se clasificó dentro de las siguientes categorías: (solo ojos (OE), < 0.60, 0.60-1.5, 1.5-2.5, >2.5 m). La estimación de las tallas se calibró mediante capturas. La conversión de los números de animales avistados a un estimado de la población se hizo mediante la fórmula sugerida por King et al. 1990 y Thorbjarnarson et al. 2000.

La estimación de la población en Boca Negra (28 ha) fue de 92 individuos (3.20 ind/ha, p=28.56%), Majahuas (195 ha) 49 (0.26 ind/ha, p=46.63%) y La Manzanilla (153ha) 314 (2.05 ind/ha, p=51.50%). La estructura por tallas en Boca Negra consiste, principalmente en animales menores a 0.6 m (38.6 %) y de la categoría 0.6-1.5 m (26.2%). En Majahuas de la categoría 0.6-1.5 m (42%) y en La Manzanilla