Anatomical Correlates Associated with the Bracing System of Extant Crocodilians: addressing the Locomotor Inadequacies of the Indian Gharial

Steven W. Salisbury[1] and Eberhard Frey[2]

[1]School of Life Sciences, The University of Queensland, Brisbane, QLD 4072, Australia (s.salisbury@uq.edu.au); [2]Staatliches Museum für Naturkunde Karlsruhe, Erbprinzenstrasse 13, D-76133 Karlsruhe, Germany (DinoFrey@aol.com)

All extant crocodilians brace their bodies against mechanical loading and during movement in a similar manner. This type of bracing mechanism is referred to as the 'eusuchian-type bracing system'. Differences in the configuration of the paravertebral shield and the length of the lateral epaxial muscles in the cranial half of the tail between *Gavialis gangeticus* and other extant crocodilians correlate with the former's apparent inability to high-walk at large sizes (> approx. 50 kg). These characteristics probably also preclude any capacity for galloping. A further correlation exists between: the configuration of the dorsal cervical muscles; the equivalence in height of the neural arches on the vertebrae in the trunk; tail base and caudal-most portion of the neck; the continuity between the nuchal and dorsal osteoderms in *Gavialis gangeticus*; and the inability of large, adult individuals of this species to flex the head ventrally against the neck to the degree observed in other taxa.

These differences mean that the operational repertoire associated with the bracing system of *G. gangeticus* is less diverse than that of other extant crocodilians. For this reason, it is proposed that two distinct forms should be recognised within the eusuchian-type bracing system: the *Gavialis*-form and the *Crocodylus*/*Alligator*-form.

The Mysterious Crocodylid Integumentary Sense Organs: What Are They For?

Kate Jackson

In all crocodylids and gavialids, a single dark dot is present on each of the ventral scales. Alligatorids lack this structure. Morphological study has shown this structure to be a sensory organ, similar in morphology to the mechanosensory "touch papillae" that are present on the facial scales of all crocodilians. Here I describe experiments completed and experiments in progress to test the possible function of these integumentary sense organs (ISOs). In particular I explore the possibility that they may be osmoreceptors, used for distinguishing hyperosmotic seawater from fresh water, similar to many other anatomical and physiological specialisations that make crocodylids better able to tolerate estuarine conditions than alligatorids.

Status of the American Alligator (*Alligator mississippiensis*) in Southern Florida, USA and its Role in Measuring Restoration Success in the Everglades

Kenneth G. Rice[1], Frank J. Mazzotti[2] and Laura A. Brandt[3]

[1]United States Geological Survey, Center for Water and Restoration Studies, University of Florida Field Station, 3205 College Ave., Fort Lauderdale, Florida 33314, USA (ken_g_rice@usgs.gov);
[2]University of Florida Fort Lauderdale Research and Education Center, 3205 College Ave., Fort Lauderdale, Florida 33314, USA (fjma@mail.ifas.ufl.edu);
[3]U.S. Fish and Wildlife Service, Arthur R. Marshall Loxahatchee National Wildlife Refuge, 10216 Lee Road, Boynton Beach, Florida 33437, USA (laura_brandt@fws.gov)

Abstract

The American alligator (*Alligator mississippiensis*) was abundant throughout the pre-drainage Everglades of Southern Florida, USA. Development and water management practices have reduced the spatial extent and changed the hydropatterns of these habitats. As a result of these activities, alligator populations have decreased. Currently, restoration of hydrologic pattern and ecological function is beginning in the Everglades. Due to the alligator's ecological importance and sensitivity to hydrology, salinity, habitat and system productivity, the species was chosen as an indicator of restoration success. A number of biological attributes (relative density, relative body condition, nesting effort, and nesting success) can be measured, standard methods for monitoring have been developed, and historical information exists for alligator populations in the Everglades. These attributes can be used as success criteria at different spatial and temporal scales and to construct ecological models used for predicting restoration effects.

Introduction

The American alligator once occupied all wetland habitats in south Florida, from sinkholes and ponds in pinelands to mangrove estuaries during periods of freshwater discharge (Craighead 1968; Simmons and Ogden 1998). Development and water management practices have reduced the spatial extent and changed the hydropatterns of these habitats (Fig. 1; Mazzotti and Brandt 1994). Less freshwater moves through the tributaries into the mangrove zone resulting in higher salinities and the filling in of creeks (Craighead 1971). In the marl prairies, the historic 6-9 month hydroperiod has been replaced by a hydroperiod of 3 months or less. In addition, water levels may drop 2 feet below the ground surface during the dry season. Because of these habitat changes, alligators are now less numerous in these habitats (Mazzotti and Brandt 1994).

Abundance, distribution, nesting success, and condition of the species are all expected to change in response to planned restoration of the Everglades. Alligator relative abundance is expected to increase as hydrologic conditions improve in overdrained marshes and freshwater tributaries. As canals are removed, densities of alligators in adjacent marshes and occupancy of alligator holes is expected to increase. As hydroperiods and depths approach more natural patterns, alligator nesting success, growth, and condition are all expected to increase in areas where they are currently lower than historic values.

In south Florida alone there are over 1000 miles of canals and nearly 700 miles of levees. These structures, initiated in the late 1800s, were initially designed to drain the Everglades and make it "usable." Later they became important for flood control and now they serve a third purpose of delivering water to urban and agricultural areas. During the 1970s and 1980s concern was raised that this extensive network of canals was having a negative impact on the environment and that the Everglades was, in fact, dying (Mazzotti 1999). In 1992, Congress authorized a study to reevaluate the network of canals and develop a plan to restore the hydrology of the Everglades. The result was a 7.8 billion dollar plan, the Comprehensive Everglades Restoration Plan (CERP; US Army Corps of Engineers 1999), that includes over 68 projects ranging from removing canals to creating alternative water storage areas both above and below ground. The goals of the plan include increasing the spatial extent of natural areas, enhancing the quality

Figure 1. Map of the historical habitats of the Everglades of South Florida, USA.

of existing natural areas, and improving the abundance and diversity of native plants and animals (US Army Corps of Engineers 1999).

In addition to their economic and ecological importance, the American alligator was selected as an indicator of restoration success because it is sensitive to hydrology, salinity, habitat and system productivity, all factors that are expected to change with the completion of CERP. In addition, there are a number of biological attributes that can be used as success criteria at different spatial and temporal scales, and there are standard methods for monitoring of alligators and their nests. Also, existing data for population densities, alligator condition and nesting ecology in some areas can provide baseline data for assessing changes and developing modeling tools (Jacobsen and Kushlan 1989; Kushlan and Jacobsen 1990; Dalrymple 1996a, 1996b; Barr 1997).

Several restoration performance measures (or targets) for the South Florida alligator population have been identified, including relative abundance, occupancy rates of alligator holes, nest success, and body condition. In the following discussion, we will outline the baseline conditions of the above restoration performance measures and suggest the role of monitoring alligator success during Everglades restoration.

Abundance and Distribution

Although we will never know accurately past numbers of alligators in the Everglades, the pattern of population changes is clear. Alligators were very abundant at the turn of the century. Hunting and wetland drainage caused alligator populations to decline through the 1930s. Coincident with statewide controls on alligator harvests, populations began to increase in to the 1950s, only to crash dramatically in the 1960s. Some poaching was occurring at this time but water management was the primary reason for this second decline.

Changes in land use and land cover resulting from water management also have affected alligator populations. Some locations that were alligator habitat have been converted to agricultural and residential development (Simmons and Ogden 1998). Other locations have not been developed, but are so over-drained that alligators only occur in permanent water bodies such as canals or ponds, or during periods of extremely high water. As Everglades restoration targets restoring the hydrology of these habitats, the reoccurrence of alligators in those areas will be an excellent indicator of success.

Salinity is a limiting factor for the distribution and abundance of reptiles in estuaries (Dunson and Mazzotti 1989). Freshwater flow into estuaries directly affects the location of alligators, as most alligators are found in areas of lowest salinity (Mazzotti 1983). The relationship of seasonal freshwater flow to the distribution and abundance of alligators in Everglades estuaries was noted by Craighead (1968), Brown (1993) and Simmons and Ogden (1998). Restoring a productive freshwater (oligohaline) zone with alligators of all size classes to Everglades estuaries would be a sign of successful restoration.

Alligator Holes

The Everglades alligator is an ecosystem engineer that physically influences the floral and faunal characteristics of the Everglades landscape through the construction and maintenance of small ponds (alligator holes) and associated underground caves (Craighead 1968). Alligator holes are excavated from the muck and peat that make up the Everglades soil, often down to the limestone bed. These depressions provide an aquatic refuge for alligators and other aquatic organisms, nest sites for other reptiles, and colonization sites for plants during frequent drying events in the Everglades (Craighead 1968; Kushlan 1972; Kushlan 1974; Loftus and Eklund 1994). Prior to drainage, alligators and alligator holes were abundant in the Everglades. Alligator hole occupancy was believed to be close to 100%, sometimes with several alligators occupying one hole (often a female with young).

Many holes were filled in with the development of areas to the east of Everglades National Park (Simmons and Ogden 1998). Those that were not destroyed experienced altered hydroperiods as a result of drainage. As early as the 1920s and 1930s, alligator hunters noted the drying out of alligator holes within some habitats. Occupancy of alligator holes in these fringe habitats is believed to have continued to decrease with continued hydrologic alterations that lead to the drying of alligator holes, especially in drought years (Craighead 1968).

The distribution and occupancy of alligator holes has been identified as a performance measure for restoration. As important as aquatic refugia are imagined to be, their ecology has remained an almost completely unstudied phenomena. This gap in information is becoming critical for making ecosystem restoration decisions.

Nesting

Current water management practices have resulted in a high and unpredictable rate of alligator nest flooding. Historically, maximum summer (nesting season) water levels were correlated with water levels in early summer when alligators were nesting. Alligators used the early summer water levels to determine nest placement and egg cavity height. In many areas, this natural predictability has been lost (Kushlan and Jacobsen 1990) and has resulted in mortality of eggs due to flooding.

While the historical record of alligator nest monitoring is not as extensive as that for population density or alligator hole occupancy rates, we do have information that flooding rates have increased due to water management practices (Kushlan and Jacobsen 1990) and that nest densities are extremely low in overdrained wetlands (Fleming 1991). Modified hydrologic conditions during restoration might be expected to decrease losses due to flooding, stabilize nesting effort in the long hydroperiod wetlands, and increase nesting effort and success of alligators in the aforementioned edge habitats. However, expansion of current monitoring is essential for assessment of restoration success.

Body Condition

The Everglades is a naturally oligotrophic system. Alligators in Everglades National Park weigh less than alligators of the same length from other parts of their range (Jacobsen and Kushlan 1989; Dalrymple 1996a; Barr 1997), do not get as large, and take longer to reach sexual maturity (Kushlan and Jacobsen 1990; Dalrymple 1996a). Changes in hydropatterns further contribute to the harshness of the south Florida environment. Jacobsen and Kushlan's (1989) model for growth in the Everglades of southern Florida predicted that alligators would reach a mere 1.26 metres in 10 years and requiring at least 18 years to reach sexual maturity. Dalrymple (1996b) analyzed the length to weight relationship of alligators in Shark Slough, Everglades National Park using a condition factor analysis and found that the Shark Slough alligators showed a slight reduction in mass as length increased. In addition, alligators tended to be in better condition during the dry months when food was concentrated and more available. Barr (1997) used a similar analysis on alligators in another portion of Shark Slough in ENP and showed that in very wet dry seasons alligators do not show this increase in condition, further supporting the influence that hydrology has on alligator condition. Our more recent analysis of alligator condition throughout the Everglades suggests that Everglades alligators exhibit lower body condition than populations in South Carolina and north Florida (Zweig 2002). It is currently suspected that the reason for this poor condition is a combination of low food availability due to hydrologic factors and high metabolic costs due to warm temperatures (Jacobsen and Kushlan 1989; Mazzotti and Brandt 1994; Dalrymple 1996a; Barr 1997; Percival *et al*. 2000).

Measures of body condition can provide a measure of restoration success through an examination of alligators throughout their range in the Everglades. Condition has been linked to reproductive success and long-term survival of alligators. Further, condition can be viewed as a measure of the quality and accessibility of prey species and provide a linkage to lower trophic levels and their success during restoration. Monitoring of condition in both newly restored habitats and in existing population centers is critical to an understanding of the effects of restoration.

Modeling

Everglades hydrologic patterns that result from the distribution, volume, and timing of water flow are major driving forces controlling the trophic dynamics of these systems. Hydrologic restoration alternatives are now being developed and proposed in response to the many decades of adverse water management practices that have impacted the Everglades with altered and intense drought and flooding events. Predicting and comparing the future effects of alternative hydrologic restoration scenarios on the biotic components of these systems is necessary. These forecasts can be accomplished only by ecological modeling. Several modeling efforts are underway including ATLSS (Across-Trophic Level System Simulation, 1999), Wildlife Suitability Indices and hydrology (SFWMD), and several others predicting hydrologic measures, water quality, and vegetation responses to restoration. Through the development of alligator population simulation models (see www.atlss.org) based on empirical data, we can evaluate restoration alternatives and assess restoration performance measures. By applying these models, we can provide information for making decisions on those alternatives that result in biotic characteristics that approximate historical conditions.

Conclusions

Future work on alligators in the Greater Everglades Ecosystem should focus on addressing the uncertainties identified while developing the components for the ecological models. Included in those are questions related to breeding female size, juvenile dispersal, and spatial and temporal variation in alligator distribution and abundance throughout the Everglades. Until recently, the only monitoring of alligators in Everglades National Park has been surveys for nests. Nesting surveys and limited nightlight surveys also have been conducted by the Florida Fish and Wildlife Conservation Commission in and near canals in the central Everglades as part of their public hunt and ranching programs. Though nest surveys provide important information and should be continued, it is not expected that changes in nesting in response to restoration will be measurable for 10 years, the time necessary for dispersing juveniles to become nesting animals. Evaluating the relative distribution, abundance, and demography of alligators using night light counts and condition analysis allows for a more rapid assessment of the impacts of CERP projects on target systems. We have recently begun developing an alligator survey network to collect the baseline population and demographic information necessary to evaluate restoration success on a 3 to 5 year time frame.

In this discussion, we have summarized what we know about alligators in the Greater Everglades Ecosystem. Baseline data are needed now to provide post restoration feedback to the policy making process. Using a combination of historic data and implementation of new monitoring programs, we will be better able to follow how alligators respond to restoration efforts. Night light surveys are a well-established, cost effective method for gathering the required information on abundance, distribution, and size class distribution (Bayliss 1987; Woodward and Moore 1990). Alligator hole distribution, densities, and occupancy rates have been monitored using a combination of mapping and survey (Mazzotti *et al*. 1999). Aerial nesting surveys have been used widely to monitor nest densities and distribution (Rice *et al*. 1999). Body condition indices have been used to monitor alligator population in several studies, and methods for more accurate comparisons between and among populations are being developed (Barr 1997; Dalrymple 1996a, 1996b; Rice *et al*. 2001). Ecological models used in evaluating restoration alternatives are constantly being refined and developed (ATLSS 1999). The methods required to monitor and predict alligator population responses to restoration of the Everglades exist, and in many cases are in place. Careful development of a monitoring and modeling program for an indicator of restoration success, such as the alligator, will insure that uncertainties and surprises about the system are incorporated during the adaptive process of Everglades restoration.

Literature

ATLSS *in* United States Army Corps of Engineers (1999). Central and Southern Florida Project Comprehensive Review Study (CD Version).

Barr, B. (1997). Food Habits of the American alligator, *Alligator mississippiensis*, in the southern Everglades. Unpublished PhD Thesis, University of Miami, Florida.

Bayliss, P. (1987). Survey methods and monitoring within crocodile management programmes. Pp. 157-175 *in* Wildlife Management: Crocodiles and Alligators, ed. by G.J.W. Webb, S.C. Manolis, and P.J. Whitehead. Surrey Beatty and Sons: Chipping Norton.

Brown, L.G. (1993). Totch: A Life in the Everglades. University Press of Florida: Gainesville.

Craighead, F.C., Sr. (1968). The role of the alligator in shaping plant communities and maintaining wildlife in the Southern Everglades. Florida Naturalist. 41: 2-7, 69-74, 94.

Craighead, F.C. Sr. (1971). The Trees of South Florida: The Natural Environments and Their Succession. University of Miami Press: Miami.

Dalrymple, G.H. (1996a). Growth of American Alligators in the Shark Valley Region of Everglades National Park. Copeia 1996(1): 212-216.

Dalrymple, G.H. (1996b). The effect of prolonged high water levels in 1995 on the American alligator in the Shark Valley area of Everglades National Park. Pp. 125-136 *in* Proc. Conf. Ecological Assessment of the 1994-1995 High Water Conditions in the Southern Everglades. USNPS, SFNRC: Homestead, Florida.

Dunson, W.A. and Mazzotti, F.J. (1989). Salinity as a limiting factor in the distribution of reptiles in Florida Bay: a theory for the estuarine origin of marine snakes and turtles. Bulletin of Marine Science 44: 229-244.

Fleming, D.M. (1991). Wildlife Ecology Studies. Annual Report. USNPS, SFNRC: Homestead, Florida. 51 pp.

Jacobsen, T. and Kushlan, J.A. (1989). Growth dynamics in the American alligator (*Alligator mississippiensis*). J. Zool., Lond. 219(2): 309-328.

Kushlan, J.A. (1972). An ecological study of an alligator pond in the Big Cypress Swamp of southern Florida. MS Thesis, University of Miami, Miami, Florida.

Kushlan, J.A. (1974). Observations of the role of the American alligator in the southern Florida wetlands. Copeia 1974: 993-996.

Kushlan, J.A. and Jacobsen, T. (1990). Environmental variability and the reproductive success of Everglades alligators. J. Herpetol. 24(2): 176-184.

Loftus, W.F. and Eklund., A.M. (1994). Long-term dynamics of an Everglades small fish assemblage. Pp. 461-483 *in* Everglades: the Ecosystem and its Restoration, ed. by S.M. Davis and J.C. Ogden. St. Lucie Press: Delray Beach, Florida.

Mazzotti, F.J. (1983). The ecology of *Crocodylus acutus* in Florida. PhD Thesis, Pennsylvania State University, State College, Pennsylvania. 162 pp.

Mazzotti, F.J. (1999). Everglades - South Florida Assessments, Science Review. Pp. 187-191 *in* Bioregional Assessments: Science at the Crossroads of Management and Policy, ed. by K.N. Johnson, F. Swanson, M. Herring and S. Greene. Island Press: Washington, D.C.

Mazzotti, F.J. and Brandt, L.A. (1994). Ecology of the American alligator in a seasonally fluctuating environment. Pp. 485-505 *in* Everglades: The Ecosystem and its Restoration, ed. by S. Davis and J. Ogden. St. Lucie Press: Delray Beach, Florida.

Mazzotti, F.J., Brandt, L.A., Campbell, M.R. and Palmer, M. (1999). An ecological characterization of Everglades alligator holes. Final Annual Report. University of Florida, Belle Glade, Florida. 82 pp.

Percival, H.F., K.G. Rice, and Howarter, S.H. (2000). American alligator distribution, thermoregulation, and biotic potential relative to hydroperiod in the Everglades. Fla. Coop. Fish and Wildl. Res. Unit, USGS Tech. Rep. 155 pp.

Rice, K.G., Percival, H.F., Woodward, A.R. and Jennings, M.L. (1999). Effects of egg and hatchling harvest on American alligators in Florida. J. Wildl. Manage. 63(4): 1193-1200.

Simmons, G. and Ogden, L. (1998). Gladesmen. University Press of Florida: Gainesville, FL.

US Army Corps of Engineers (1999). Final Integrated Feasibility Report and Programmatic Environmental Impact Statement. Central and Southern Florida Project Comprehensive Review Study. U.S. Army Corps of Engineers. Jacksonville District.

Woodward, A.R. and Moore, C.T. (1990). Statewide alligator surveys. Florida Game and Fresh Water Fish Commission Final Report, Tallahassee, Florida, USA.

Zweig, C.L. 2002. Body condition index analysis for the American alligator (*Alligator mississippiensis*). MS Thesis. University of Florida, Gainesville, Florida. 49 pp.

Captive Breeding and Reproductive Biology of the Indian Gharial *Gavialis gangeticus* (Gmelin)

Harry V. Andrews and Nikhil Whitaker

Madras Crocodile Bank Trust/Centre for Herpetology
Post Bag- 4, Mamallapuram- 603 104, Tamil Nadu, S. India
(mcbtindia@vsnl.net)

Abstract

Gavialis gangeticus females attain sexual maturity when they are close to 3 m and males when they are 3.5 m and above. The nesting season is during summer occurring between March and May and the maximum number of nests were laid between the 16th to 20th April. Mean clutch size was 24.53 ± 12.32 (range 2-44) and percentage of fertile eggs within a clutch averaged 75.31 ± 32.6% (range 0-100). Egg length averaged 85.89 mm (range of means 69.85-111.7 mm), width averaged 55.32 mm (range 45.8-63.6) and weight averaged 155.81 g (range 86.9-230). Total clutch weight averaged 3013.50 ± 1480.70 g (range 311.6-5640.0). Total clutch weight highly correlated with clutch size than with mean egg weight. Significant variation in clutch size and percentage of viable eggs within a nest was evident between nesting seasons in the 13 years of breeding. Age and clutch size were found to be highly correlated and total clutch weight is strongly correlated with mean clutch size. As in all other crocodilian species *G. gangeticus* has temperature- dependent sex determination (TSD) and the TSD pattern is female-male-female. Fertile eggs incubated at set constant temperatures from 29-31.5°C produced only females and at 32°C 89% were males. At 33 and 33.5°C, 20% and 15% males were produced, respectively. Incubation period averaged 70 days for eggs incubated at 31°C, which was is 1.20 times higher than eggs from 29°C and 1.17 times longer than 33°C (Lang and Andrews 1994).

Introduction

Gavialis gangeticus is endemic to the Indian sub-continent, occurring in India and Nepal, with Pakistan, Bhutan and Bangladesh having a few scattered individuals. Adults tend to inhabit slow flowing portions of large rivers at their bends and confluences, while smaller animals prefer sheltered backwaters (Singh 1978; Whitaker and Basu 1982). The GOI/FAO/UNDP Indian Gharial Project saved this species from the brink of extinction, when in the early 1970s they numbered less than a 100. Through major conservation efforts since 1974, the Indian gharial was brought back to safe numbers by the 1000s in several locations through out its range in India. Currently there are threats of extinction to some of the populations due to pressures on habitats and inadequate recruitment within populations (Whitaker and Andrews 2003; Ross 2003).

Several authors have previously discussed some aspects on the breeding biology of this species (Biswas *et al*. 1977; Kimura 1978; Singh 1978; Bustard 1979; Bustard 1980 a; Bustard and Maharana 1981, 1982; Chowdury *et al*. 1983; Whitaker and Basu 1982; Rao 1988; Andrews 1989b; Rao and Singh 1993; Acharjyo *et al*. 1996; Hussain 1999). Bustard (1980 b), Basu and Bustard (1981) and Maharana and Bustard (1981) have discussed maternal care in this species. Choudhury and Bustard (1983), Bustard and Maharana (1983) and Whitaker and Basu (1982) reported on the ecology and growth of this species. Bustard and Singh (1977) presented formulae for estimating total length of basking gharial from scute length, while Singh and Bustard (1977) reported on locomotory behavior and spoor formation of the species. Whitaker and Andrews (1988) also r eported on the locomotion for this species.

In the present study we examine trends in Gavialis reproductive biology in captivity from 1989 to 2002, save for 1997 in which no data was recorded. In addition to nest dynamics, ages at onset of sexual maturity, the age factors influence on clutch size and fertility, and mating and egg laying season, ambient temperature's effects relative to the same, temperature- dependent sex determination and relationships between morphometrics of eggs and hatchlings.

Materials and Methods

The Madras Crocodile Bank Trust/Centre for Herpetology (MCBT) is a non-profit charitable Trust, and has been involved in reptile breeding, conservation and research since its inception in 1976. MCBT is situated at 12º50'N, 80º10'E, 45 km south of Chennai city, along the Bay of Bengal. The facility receives an annual average rainfall of 1.2 m, occurring primarily during two seasons southwest monsoon (June-July) and the northeast monsoon (October-December). The annual mean temperature is 28°C and ranges from 20°C during December and January to 38°C in May and June.

The *Gavialis gangeticus* breeding enclosure is located at the lower end of the facility and at almost 1.5 m above sea level. Substratum of the enclosure is predominantly large grained sand, in addition to leaf litter. The enclosure contains a natural aquifer, within which depth varies from 1.5 to 2.5 m seasonally. The enclosure is irregular, roughly ovoid-rectangular in shape, measuring 42 m in length and 25 m in width. The aquifer, measuring 30 m long and 8 m wide, is at the base of gradually sloping banks. The bank on the northern side of the enclosure is almost 4 m above water level and only the upper reaches of this bank is used as a colonial-nesting bank. The banks on all sides of the enclosure are landscaped with trees and screwpine. In addition to the gharial, the enclosure pond contains, fish and freshwater turtle species comprising *Melanochelys trijuga trijuga*, *Aspideretes gangeticus*, *Lissemys punctata* and *Kachuga kachuga*.

The study animals 10 females and three males were acquired between 1974 and 1982 and two juvenile females in 1986. Of these a male at 1.3 m TBL and a female at 0.82 m TBL, originated from the wild, probably the lower eastern reaches of the River Ganga in Uttar Pradesh. Eight females and two males hatched from wild eggs These eggs acquired from the Kukrail Breeding Centre had been collected from the Chambal River in Uttar Pradesh. These females on arrival were in the age group of 2-6 years, the two females in the two years age group measured 1.2 and 1.3 m (TBL). The two females in the four and five year age group measured 1.45 and 2.47 m (TBL). The six large females in the six years age group measured 2.2-2.6 m and averaged 2.4 m (TBL). Two other captive-bred F1 females were acquired in 1986 from Nandankanan Biological Park in Orissa and these measured a little over 1 m (TBL). These were housed in separate enclosures till 1991 and then were included with the rest of the breeding groups. Upon arrival, animals were sexed, measured and designated individual code numbers by clipping a combination of scutes on the single and double caudal whorls. Until March 1995 the entire breeding group were held in the study enclosure. During March 1995, one of the males lost half of his upper jaw in male-male combat and was transferred to another enclosure. A second male died in June 1997 following capture, perhaps due to post-capture fatigue as discussed by Seymour *et al*. (1987). In January 1999 and December 1998 two females were transferred out of the study enclosure.

For the duration of the nesting season, *G. gangeticus* females were observed from 1900-0630 h. Eggs were collected from nests within 0-3 days by marking the upper surface of the egg to maintain orientation throughout handling and incubation. Nest parameters recorded were distance from nest to edge of pond, height above pond, nest cavity width and depth. Temperatures at different depths of each layer of eggs were also recorded. Measurements of animals were recorded by marking off, with a long stick, the snout and tail tips in the sand while females and males basked. The animals were then chased away into the pond and two ends were measured with a tape measure.

In a lab each egg was assigned individual clutch and egg numbers and then candled against a light to determine fertility by the presence or absence of the sub-embryonic fluid as discussed by Webb and Manolis (1987). Eggs were measured to the nearest ± 0.1 mm length and width with KWD type dial vernier calipers, and weighed to the nearest ± 0.1 g with an ACCULAB 226 digital weighing scale. Fertile eggs were either incubated in artificial nests buried in areas exposed or protected from sunlight (n= 26 nests), or incubated at various set constant temperatures (n= 20 nests) as previously described by Lang *et al*. (1989) and Lang and Andrews (1994). Of the 49 nests laid between 1989 and 2002 (1997 excluded), 44 of these (89.8%) have known laying dates and of 38 (77.6%) have known female IDs. Some nests that were not located at the time of nesting were ultimately discovered as females defended nest sites. In circumstances where oviposition date was unknown and a nest was discovered, band width of fertile eggs was recorded.

<u>Egg incubation</u>: Eggs were buried between 35-40 cm beneath the sand surface in an outdoor incubation facility, either as a whole clutch, or after halving the clutch in which case one half was exposed to sun and one half to shade. Temperature of each individual egg group was monitored with thermistor probes (YSI 400 series), digital thermometer, and the accuracy of absolute temperature measurements was 0.1ºC. Temperatures were recorded either two times a day, at 0800 and 1600 h, or three times a day at 0600, 1200 and 1700 h.

To demonstrate that incubation temperature determines sex, eggs were incubated in an insulated lab with an air-conditioner as discussed by Lang and Andrews (1994). The incubators were custom designed foam box incubators set at constant temperatures maintained within ± 0.1°C of the set. Eggs were assigned to different incubators at different constant temperatures, mainly 29, 30, 31, 31. 5, 32, 33 and 33.5°C.

Hatchlings: Hatchlings that resulted from both artificial nests and constant temperature incubation trials were coded and measured within 24 hours of hatching. Measurements recorded were standard total body length (TBL), snout to vent (SVL) and weight (wt).

Results

Sexual maturity

At MCBT first nesting occurred when a 10-year-old female laid eggs during April 1989, this female was measured on the following day, while she was basking and was 3.2 m TBL. Two other females nested during the same season and measured 2.8 and 3 m TBL (Andrews 1989 b). During the same season the three males were also measured and they ranged from 3, 3.5 and 3.8 m TBL. During the 1989 breeding season courtship and mating was observed and, only the two largest males were observed courting and mating.

Trial nests and temporal distribution of nests within the egg laying season

In all years, the first indication that nesting was imminent was the tracks of females around the nesting area (20 m x 10 m rough rectangle on the north bank) and by trial nests. Some females prior to nesting make trial nest holes probably trying to find optimum nesting sites. Trial nests were made on the same night of actual oviposition and

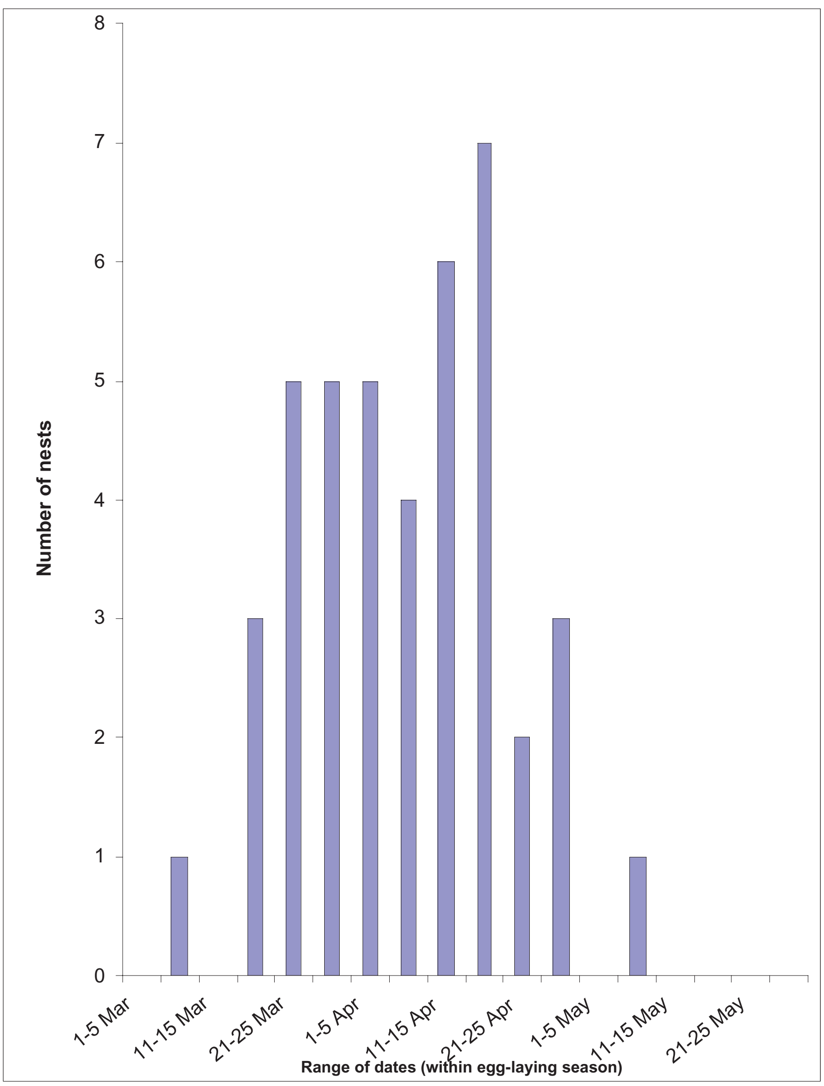

Figure 1. Spatial distribution of 44 *G. gangeticus* nests during 1989-2002.

maximum number of days before oviposition was seven days. Depths of trial nests varied between 10 and 36 cm. Nesting dates for the 44 nests (89.8% of the total) for which egg laying dates were known ranged from 10th March to 7th May. The maximum numbers of nests (n= 7) were laid between the 16th to 20th April (Fig: 1).

Clutch size, fertility and egg characteristics

Clutch size and clutch fertility were compared between the first laid clutch and last observed clutch. Significant difference in clutch size (Paired t-test, t= 2.91, df= 6) and fertility (t= 3.38, df= 6) between the first and last observed nest was evident. Mean clutch size (MCS) (n= 49) was 24.53 ± 12.32 (range 2-44). Percentage of viable eggs within a clutch (n= 49) averaged 75.31 ± 32.6% (range 0-100). Egg dimensions, given as the means of individual clutch means, were: length (n= 26 clutches) 85.89 ± 5.44 mm (range of means 69.85-111.7 mm); width (n= 26 clutches) 55.32 ± 3.51 mm (range 45.8-63.6 mm); and weight (n= 24 clutches) 155.81 ± 25.14 g (range 86.9-230 g). Total clutch weight (TCW) (n= 21) averaged 3013.50 ± 1480.70 g (range 311.6-5640.0 g). TCW correlated more highly

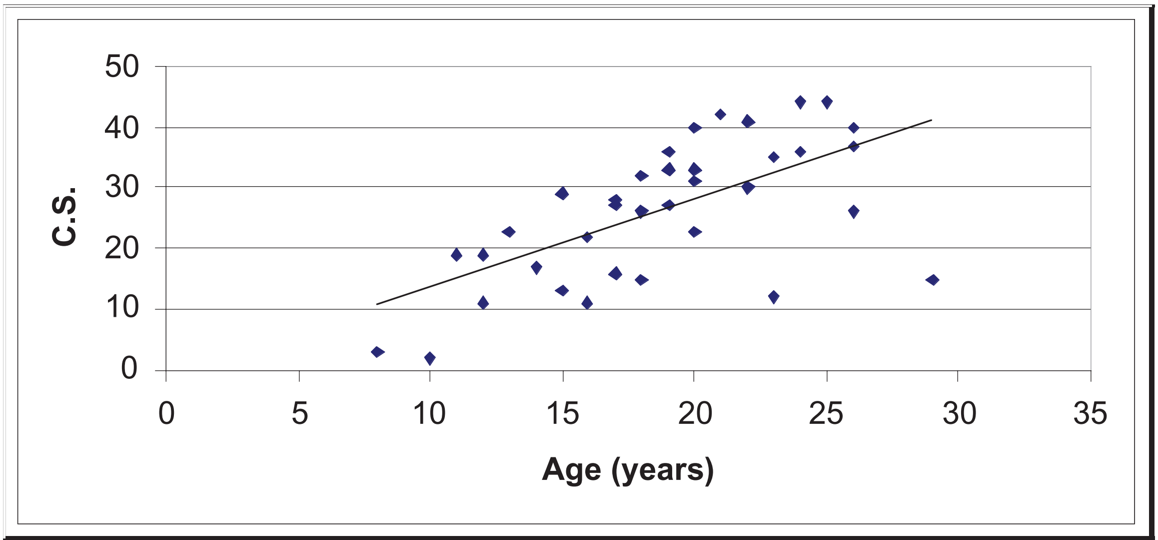

Figure 2. Relationship between age and clutch size in *G. gangeticus* (Age= 11.67 + 0.27CS ± 3.79; n = 37, r^2= 0.39).

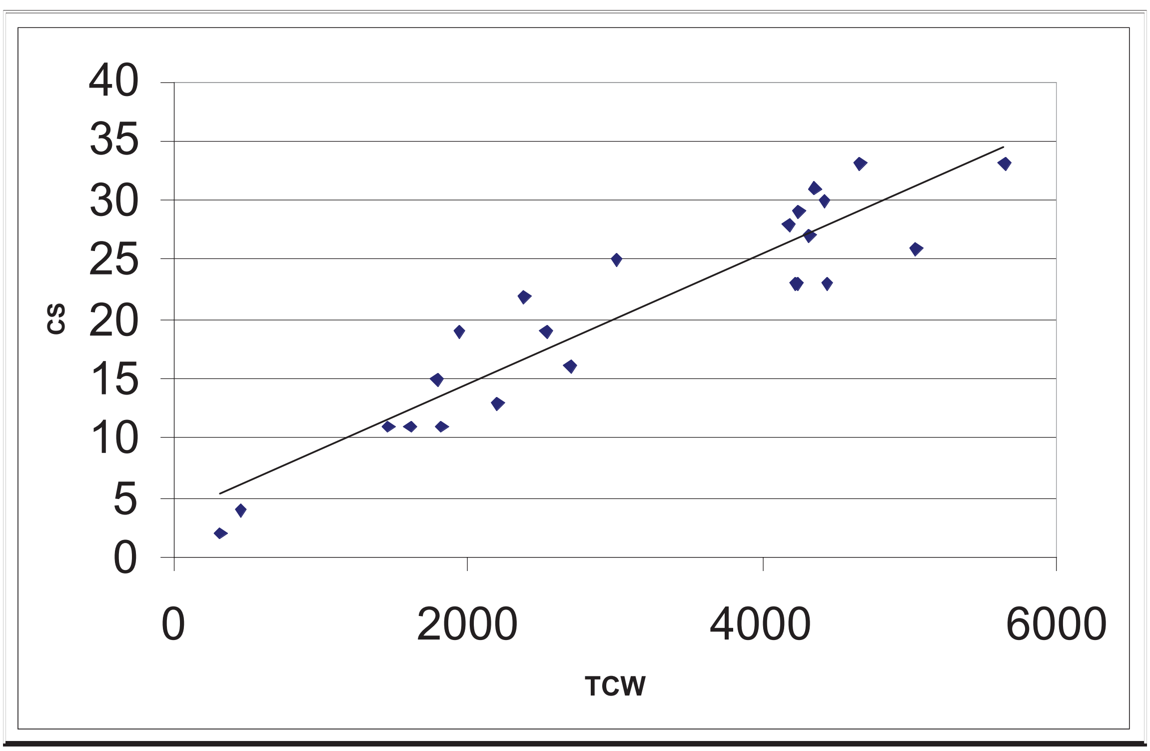

Figure 3. Relationship between total clutch weight and clutch size in *G. ganeticus* (TCW= -125.17 + 157.82CS ± 545.78; n= 23, r^2= 0.86).

with clutch size (Product Moment Correlation, n= 23 nests, r= 0.93) than with mean egg weight (r= 0.37, n= 23 nests). Age and clutch size were found to be highly correlated (Fig. 2). TCW is strongly correlated with MCS, and may be predicted from the formula as shown on Figure 3.

Reproductive effort

For the first six years of nesting (1989-1994), three males were present in the breeding enclosure; clutch size (CS) averaged 19.44 ± 7.0 eggs, viability averaged 50.42 ± 38.17%, and average number of females nesting per year was 3.16 ± 1.60 (n= 18 clutches for X CS & %Via, n= 19 clutches for X number of females nesting/year). Between 1995 and 1996, two males were present in enclosure and CS averaged 25.92 ± 11.07 eggs, viability averaged 84.69 ± 17.23%, and average number of females nesting per year was 6 ± 0.0 (n= 12 clutches). For the four years between 1998 and 2002, when only one male was in enclosure 13, CS averaged 32.88, average viability was 91.64% and average number of females nesting per year was 2.4 (n= 16 clutches for X CS and %Via, n= 12 clutches for X number of females nesting/year). Measured as a combination of average clutch size, number of clutches produced during the 13 years the animals were studied, and average fertility per clutch, reproductive effort of the eight females observed nesting are given in Table 1. The highest number of nests produced over the years by a female was eight clutches and the lowest was a single clutch - this female was very old and finally died trying to lay eggs and after she extruded her oviducts (Andrews and Whitaker 1988). We could not identify factors affecting variation in the number of clutches produced by individual females and social hierarchy and too much of male-male interactions could have influenced this.

Table 1. Summary of ages, clutch sizes and fertility (expressed as % of fertile eggs) of nesting female *G. gangeticus*. (*= one clutch excluded from C.S. and fertility summary).

ID	Mean Age	Age Range (First to last clutch)	SD (Age)	No. of Clutches (1989-2002)	Mean CS	CS Range	SD (Clutch)	Mean % Via.	% Via. Range	SD % Via.	Total Eggs Prod.
1	22.1	18-26	3.13	7	35.9	15-44	10.09	81.49	0-100	36.34	251
2	20.5	17-26	3.87	4	32.3	28-37	3.77	92.5	85.7-100	6.04	129
3*	21.43	16-29	5.06	7	17.16	11.26	6.05	84.8	56.3-100	17.99	108
7	20.5	17-26	3.87	4	28.3	26-33	3.2	76.2	48.0-92.3	19.96	113
9	19.8	16-24	3.19	5	31.2	22-36	5.76	82.6	22.7-100	33.6	156
10	13.5	12-15	2.1	2	24	19-29	7.07	84.3	78.9-89.7	7.64	48
12	14.75	10-22	4.46	8	21	2-42	14.1	64.7	0-100	43.5	168
109	8	-	-	1	3	-	-	33.3	-	-	3

The number of eggs produced in the course of the 13 years is highly correlated to the number of clutches (Table 1), (Product Moment Correlation, n= 8, r= 0.81), average clutch size (n= 8, r= 0.76), average nesting age (n= 8, r= 0.73), but weakly related with the percentage of fertile eggs within a clutch (n= 8, r= 0.49). Significant variation in clutch size (ANOVA; F= 2.41, N= 45) and percentage of fertile eggs within a nest (ANOVA; F= 4.60, N= 45) was evident between nesting seasons in the 13 years of breeding.

Re-nesting intervals and sequence of laying

Re-nesting intervals of seven females is presented in Table 2. As mentioned previously, it must be noted that there is a margin of error as not all nests had females identified with them. Sequence of laying, that is the order in which a said female laid her eggs in relation to the other seven females, was ranked for all the females analysed; Female 12 (n= 8 nests) frequency averaged 1.25 ± 0.46 (range 1-2), female 3 (n= 7) averaged 2.57 ± 0.79 (range 2-4), female 9 (n= 5) averaged 2.60 ± 1.14 (range 1-4), female 01 (n= 7) averaged 2.43 ± 0.79 (range 2-4), female 7 (n= 4) averaged 4.0 ± 0.82 (range 3-5), female 2 (n= 4) averaged 3.5 ± 2.89 (range 1-6), while female 10 (n= 2) averaged 2.0 ± 1.41 (range 1-3)

Table 2. Re-nesting intervals (years) of 7 female *G. gangeticus*.

ID	N1		N2		N3		N4		N5		N6		N7		N8
12	G1-89	1	G1-90	1	G1-91	1	G2-92	1	G1-93	1	G1-94	6	G1-00	1	G2-01
3	G2-89	1	G2-90	1	G2-91	2	G3-93	3	G2-96	4	G3-00	2	G4-02	-	-
9	G2-93	2	G3-95	1	G4-96	3	G1-99	2	G3-01	-	-	-	-	-	-
1	G2-94	1	G2-95	1	G3-96	3	G2-99	1	G2-00	1	G4-01	1	G2-02	-	-
7	G4-93	2	G4-95	1	G5-96	6	G3-02	-	-	-	-	-	-	-	-
2	G6-93	2	G1-95	1	G6-96	6	G1-02	-	-	-	-	-	-	-	-
10	G3-89	3	G1-92	-	-	-	-	-	-	-	-	-	-	-	-
Mean	-	1.71	-	1	-	3.5	-	2	-	2	-	3	-	1	-
SD ±	-	0.76	-	0	-	2.07	-	0.82	-	1.73	-	2.65	-	0	-
Range	-	1-3	-	1	-	1-6	-	1-3	-	1-4	-	1-6	-	1	-

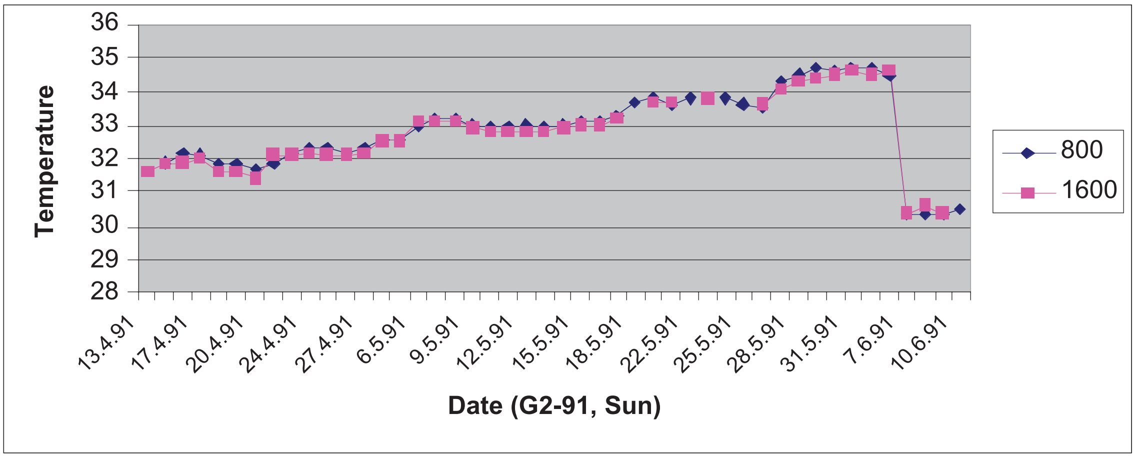

Figure 4. Temperature profile of natural nest (G2-91; exposed to sun); 45 of 59 incubation days recorded. Incubation period = 59 days; X at 0800 h = 32.84 ± 1.18°C (range 31.7-34.7°C) ; X at 1600 h =32.74 ± 1.12°C (range 30.4-34.6°C); Pooled X = 32.79 ± 1.15°C (range 30.4-34.7°C).

Artificial nest temperatures

Incubation temperatures remained relatively constant throughout the incubation period, drops and peaks occurred when rainfall occurred and nests dried out again respectively (Fig. 4). Rainfall effectively lowered nest temperature within eight hours. The incubation period ranged between March-July, the latter trimester coinciding with the south-west monsoon. Pooled temperatures averaged 31.41 ± 0.79°C, range 30.43-32.79°C (n= 6) for treatments at sunny locations, and averaged 30.70 ± 1.22, range 29.43-32.32°C (n= 5) for treatments at shaded locations.

Hatchlings from artificial nests

Hatchlings from nests located in sunny and shady locations collectively pooled and measured within a day of hatching, had the following dimensions; mean TBL 35.52 ± 1.30, ranging between 33.8-37.45 cm (n= 185), mean SVL 17.27 ± 0.73, range 16.13-18.50 cm (n= 185) and mean weight of 104.02 ± 14.19, range 75.5-124.05 g (n= 151). No significant difference was found in the TBL (ANOVA, F ratio= 0.0056; N= 19), SVL (ANOVA, F ratio= 0.0; N= 19), and wt. (ANOVA, F ratio= 0.55; N= 15) was observed between clutches exposed to sunlight, and shaded clutches. Hatchling morphometrics were found to be highly correlated to each other, regression equations are presented in Table 3.

Temperature- dependent sex determination

Lang and Andrews (1994) have previously discussed temperature-dependent sex determination for this species. As in all other crocodilian species *G. gangeticus* has TSD and the pattern is female-male-female. Fertile eggs incubated at set constant temperatures from 29-31.5°C produced only females and at 32°C 89% males were produced. At 33 and 33.5°C resulted in 20% and 15% males respectively. Incubation period averaged 70 days for eggs incubated at 31°C, which was 1.20 times higher than eggs from 29°C and 1.17 times longer than 33°C.

Table 3. Natural nests ("sunny" and "shady" hatchlings pooled); Correlation coefficients and linear regression equations for predicting hatchling parameters from each other. 151 hatchlings from nine clutches analysed for all wt. related equations, while n= 185 hatchlings from 11 clutches analysed for non-wt. related equations; for equations 1-6, values calculated from average of the average; for Hwt/Ewt equation 7, n= 100 hatchlings, direct correlation between parameters; hatchlings resulted from eggs laid by five different females in addition to two nests of unknown origin. (SEE= standard error of the estimate).

Equation	Formula	SEE	r2
1	TBL = 5.99 + 1.71SVL	0.13	0.92
2	SVL = -1.56 + 0.53TBL	0.06	0.92
3	HWt = -2.35.18 + 9.62TBL	54.26	0.71
4	TBL = 27.56 + 0.07HWt	0.60	0.71
5	HWt = -223.21 + 19.17SVL	59.91	0.69
6	SVL = 13.33 + 0.036HWt	0.11	0.69
7	HWt = 17.937 + 0.525Ewt	6.17	0.66

Discussion

Sexual maturity

Size and age at the onset of sexual maturity for several species has been previously discussed to an extent. McCann (1940) and Joanen and McCann (1975) have established that sexual maturity is size, not age, dependent. Bustard and Singh (1981) have reported for *C. palustris*. McCann (1940), Whirworth (1971), Joanen and McCann (1975) and Nichols and Chabreck (1980) have reported the onset of sexual maturity for *A. mississippiensis*. Andrews (1986, 1989a, 1989b, 2000) has reported for *C. palustris*, *C. simensis*, *C. niloticus*, *Camain crocodilus crocodilus*, *C. moreletti* and *G. gangeticus*. Hornaday (1885) first reported a female *G. gangeticus* that he had shot measuring 2.7 m and had 15 developing eggs. Another 2.97 m long female shot in the Sutlej River had 56 eggs (Parshad 1914). Whitaker and Basu (1982) have reported that the minimum breeding size of females as 2.6 m TBL, and Biswas *et al*. (1977) reported that males attain sexual maturity when they are over 3 m. Acharjyo *et al*. (1990) confirmed that females attained sexual maturity when they are close to 3 m TBL and when they are 8.5-9 years old in captivity. Bustard and Maharana (1982) reported 3-3.17 m TBL for *G. gangeticus* females and Maharana (2001) reported that females in captivity (n= 7) attained sexual maturity when they reached 3 m and from 10-17 years of age.

At MCBT first nesting occurred when a female 3.2 m TBL and 10 years laid eggs. Two other females nested during the same season and these two females measured 2.8 m (17 years) and 3 m (11.8 years) (Andrews 1989b). During the same season the three males were also measured and they ranged from 3, 3.5 and 3.8 m TBL, and were aged 9.6, 12.6 and 18.6 years of age. During the 1989 breeding season courtship and mating were observed and only the largest males was observed courting and mating.

Of the three males in the breeding group, the largest male's ghara development has been reported (Whitaker and Whitaker 1989); the snout looked like a female's snout until 1982, when the first protuberance became obvious. Ghara growth proceeded steadily with a definite back folding tendency which eventually covered the nostrils in 1988. This male's ghara was first evident at 11 years of age, and covered the nostrils by 18.6 years (in 1989). Coincidentally, nesting occurred for the first time in the same year. Biswas *et al*. (1977) noted that a male at Nandankanan Biological Park, Orissa, could be distinguished from females at the age of 12.5 years on the basis of the development of the ghara.

Temporal distribution of nests within the egg laying season

Nesting coincided with the period of minimal rainfall, and increasing ambient temperatures, a trend parallel to that observed in *C. palustris* at the same study site (Lang *et al*. 1989). Span of the nesting season (accumulative range) varied from 57 days at lower latitudes (MCBT) to 22 days at higher latitudes such as the Narayani River in Nepal (Bustard 1980). Maximum deposition of clutches at MCBT occurred between the 16th and 20th April (51.9% of the total) (Table 4). In the Narayani River, Nepal, nesting occurred during 1-5 April (42.4% of the total) (Bustard 1980a). Rao (1988) reported 51.5% between 26-30 March at Baroli in the National Chambal Sanctuary, Madhya Pradesh.

Table 4. Average temperatures during courtship and egg-laying seasons of *Gavialis gangeticus* (excluding 1997).

	Courtship and mating season (December-February)		Egg laying season (March-May)	
Year	Mean Max.	Mean Min.	Mean Max.	Mean Min.
1989	29.13	21.25	33.55	25.36
1990	29.23	22.62	33.59	27.38
1991	32.60	23.05	35.84	27.20
1992	29.01	22.57	33.19	26.01
1993	29.24	22.67	33.11	26.81
1994	29.06	23.79	34.10	27.28
1995	31.90	21.12	34.72	23.34
1996	31.03	21.77	32.93	25.06
1998	31.19	23.51	35.74	23.96
1999	29.91	22.11	32.94	25.38
2000	30.49	22.29	33.04	25.40
2001	32.41	21.47	34.65	25.32
2002	32.36	21.95	33.30	25.09
Average	30.58	22.32	33.90	25.66
SD	1.41	0.83	1.03	1.25
Range	29.01-32.60	21.12-23.79	32.93-35.84	23.34-27.38

Clutch size and egg characteristics

Clutch size averaged 25.9 (n= 49 nests) for the entire study period, however when calculated for the last four years the clutch size averaged 32.9. Rao and Singh (1993) reported 38 eggs as the average for 12 nests at the Baroli communal nesting site in the National Chambal Sanctuary. Hussain (1999) reported 36.52 eggs for 124 nests in the National Chambal Sanctuary and this is from the largest sample size. Maharana (2001) reported 32.6 (n= 69 clutches) ranging 2-57 in captivity at the Nandankanan Biological Park. In the current study, *G. gangeticus* clutch size (CS) gradually decreased from 39 eggs in the 1-5 March timeframe to the 26-30 March timeframe (Mean CS= 22.8 eggs). It then escalated in the 1-5 April timeframe (Mean CS= 38.25 eggs), then showed a general gradual decrease till the 26-30 April timeframe (Mean CS= 15.33 eggs), and finally a second escalation in the 1-5 April timeframe (X CS= 28 eggs). Thus, there is a trend of decreasing clutch size through the oviposition period, and this in turn indicates that larger females lay clutches first in a given nesting season. This trend is confounded by the varying number of clutches for each time frame under consideration. Webb *et al*. (1983) noted that in *C. johnstoni*, larger clutches tend to be laid first in a given nesting season. At variance to our observations is that provided by Hall (1991), with respect to *C. novaeguinae* where clutch size was found to remain nearly constant throughout the oviposition period.

Egg morphometrics from the current study are similar to that reported from previous studies. In our study, egg length averaged 85.89 mm (range 69.85-111.7), egg width averaged 55.32 mm (range 45.8-63.6) and weight averaged 155. 8 g (range 146.9-230). Rao (1988) recorded dimensions of eggs for 60 nests and egg length averaged 86 mm (range 77-94), width averaged 61.3 mm (range 59-64), and weight averaged 185.5 g (range 145-210). Srivastava (1981) recorded egg dimensions for 15 nests laid over four consecutive years, egg length averaged 84.8 mm (range 71.5-88.7), width averaged 59.5 mm (range 50.3-64.5), and weight averaged 145.2 g (range 102-185). Maharana (2001) reported egg weights from 69 clutches laid in captivity and mean clutch weight was 155 g (range 105-195).

Acknowledgements

Most is due to Jeff Lang for initiating this program at MCBT and for his continued encouragement over the many years of the project and after. We also thank the staff of the Madras Crocodile Bank/Centre for Herpetology for their assistance at every stage. Grahame Webb and Rom Whitaker provided valuable comments and reviewed an earlier draft of this manuscript. We are grateful to the Trustees of the Madras Crocodile Bank Trust/Centre for Herpetology for their continued support and encouragement. Last but not least, we are indebted to Seema Mundoli for her patience in reviewing this manuscript three times and for all her editorial help.

Literature

Acharjyo, L.N., Singh, L.A.K. and Patnaik, S.K. (1990). Age at sexual maturity of gharial, *Gavialis gangeticus* (Reptilia: Crocodilia). J. Bombay Nat. Hist. Soc. 87(3): 458- 459.

Acharjyo, L.N., Kar, S.K. and Patnaik, S.K. (1996). Studies on captive breeding of the gharial, *Gavialis gangeticus* (Gmelin) in Orissa. J. Bombay Nat. Hist. Soc. 93 (2): 210-213.

Andrews, H.V. (1986). Variation in egg and clutch size of mugger in different age groups. Hamadryad 11(3): 13- 14.

Andrews, H.V. (1989a). An unusual record of *Crocodylus moreletii* nesting. Hamadryad 14 (1): 11-13.

Andrews, H.V. (1989b). *Gavialis gangeticus* nest at the Madras Crocodile Bank. Hamadryad 14 (1): 13-14.

Andrews, H.V. (2000). Breeding, growth and reproductive effort of the Morelet's crocodile (*Crocodylus moreletii).* Pp. 182-190 *in* Crocodiles. Proceedings of the 15th Working Meeting of the Crocodile Specialist Group. Varadero, Cuba. IUCN-The World Conservation Union: Gland, Switzerland.

Andrews, H V. and Whitaker, R. (1988). A note on gharial oviduct extrusion while nesting at the Madras Crocodile Bank. Hamadryad 13 (2): 2.

Basu, D. and Bustard, H.R. (1981). Maternal behavior in the gharial [*Gavialis gangeticus* (Gmelin)]. J. Bombay Nat. Hist. Soc. 78: 390- 392.

Biswas, S., Acharjyo, L.N. and Mohaputra, S. (1977). A note on the protuberance or knob on the snout of male gharial *Gavialis gangeticus* (Gmelin). J. Bombay Nat. Hist. Soc. 74 (3): 536- 537.

Bustard, H.R. (1979). A note on the nesting behavior in the Indian gharial *Gavialis gangeticus* (Gmelin) (Reptilia: Crocodilia). J. Bombay Nat. Hist. Soc. 16: 519- 520.

Bustard, H.R. (1980a). Clutch size, incubation, and hatching success of gharial *Gavialis gangeticus* (Gmelin) eggs from Narayani River, Nepa, 1976-1978. J. Bombay Nat. Hist. Soc. 77: 100- 105.

Bustard, H.R. (1980b). Maternal care in the gharial, *Gavialis gangeticus* (Gmelin). British J. Herpetol. 6: 63-64.

Bustard, H.R. and Maharana, S. (1981). Fatal male-male conflict in the gharial *Gavialis gangeticus* (Gmelin) (Reptilia: Crocodilia). J. Bombay Nat. Hist. Soc. 78 (1): 1- 3.

Bustard, H.R. and Maharana, S. (1982). Size at first breeding in the gharial (*Gavialis gangeticus* (Gmelin)) (Reptilia: Crocodilia) in captivity. J. Bombay Nat. Hist. Soc. 79 (1): 206- 207.

Bustard, H.R. and Maharana, S. (1983). Growth rates in sub-adult gharial *Gavialis gangeticus* (Gmelin) (Reptilia: Crocodilia). J. Bombay Nat. Hist. Soc. 80 (1): 224-226.

Bustard, H.R. and Singh, L.A.K. (1977). Studies on the Indian gharial *Gavialis gangeticus* (Gmelin) (Reptilia:Crocodilia): Estimation of body length from scute length. Indian Forester 103 (2): 140-149.

Bustard, H.R. and Singh, L.A.K. (1981). Age at onset of sexual maturity in male Indian mugger (*Crocodylus palustris*, Lesson) in captivity. J. Bombay Nat. His. Soc. 78 (3): 607- 669.

Choudhury, B.C. and Bustard, H.R. (1983). Stunted growth in captive reared gharial. J. Bombay Nat. Hist. Soc. 80 (2): 423- 425.

Chowdury, S., Bustard, H.R. and Tandan, B.K. (1983). Incubation and hatching of eggs of *Gavialis gangeticus* in hatcheries. British J. Herpetol. 6: 337- 342.

Hall, P.M. (1991). Estimation of nesting female crocodilian size from clutch characteristics: Correlates of reproductive mode, and harvest implications. J. Herpetol. 25 (2): 133- 141.

Hornaday, W.T. (1885). Two Years in the Jungle. Charles Seribner's Sons: NewYork.

Hussain, S.A. (1999). Reproductive success, hatchling survival, and rate of increase of gharial, *Gavialis gangeticus* in the National Chambal Sanctuary, India. Biological Conservation 87: 261- 268.

Joanen, T. and L. McCann, L. (1975). Notes on the reproductive biology and captive propagation of the American alligator. Proc. 29th Annual Meeting Southeast Assoc. Game & Fish Comm. 29: 407- 415.

Kimura, W. (1978). Artificial incubation of gharial eggs. Bulletin of Atagawa Institute of Tropical and Alligator Garden: 16.

Lang, J.W. and Andrews, H.V. (1994). Temperature-dependent sex determination in crocodilians. J. Experimental Zool. 270: 28- 44.

Lang, J.W., Andrews, H. and Whitaker, R. (1989). Sex determination and sex ratios in *Crocodylus palustris*. American Zoologist 29: 935- 952.

Maharana, S. 2001. Nandankanan gharial captive breeding program. Crocodile Specialist Group Newsletter 20 (4): 79- 80.

Maharana, S. and Bustard, H.R. (1981). Behavior of the male gharial during the nesting and post-hatching period. J. Bombay Nat. Hist. Soc. 79 (3): 677- 681.

McCann, C. (1940). A reptile and amphibian miscellany. J. Bombay Nat. Hist. Soc. 41(4): 742- 747.

Nichols, J.D. and Chabreck, R.H. (1980). On the variability of alligator sex ratio. Amer. Nat. 116: 125- 137.

Parashad, B. (1914). The gharial (*Gavialis gangeticus*). J. Bombay Nat. Hist. Soc. 23: 369.

Rao, R.J. (1988). Nesting ecology of the gharial in National Chambal Sanctuary. Study Report, August 1988, Wildlife Institute of India, Dehra Dun, India.

Rao, R.J. and Singh, L.A.K. (1993). Communal nesting by gharial *Gavialis gangeticus* (Gmelin) (Reptilia: Crocodilia) in National Chambal Sanctuary. J. Bombay Nat. Hist. Soc. 90 (1): 17- 22.

Ross, P. (Ed). (2003). India- Gharial reach a crisis in Chambal River. Crocodile Specialist Group Newsletter 22(4): 12- 13.

Seymour, R.S., Webb, G.J.W., Bennett, A.F. and Bradford, D.F. (1987). Effects of capture on the physiology of *Crocodylus porosus*. Pp. 253-7 *in* Wildlife Management Crocodiles and Alligators, ed. by G.J.W. Webb, S.C. Manolis and P.J. Whitehead. Surry Beatty and Sons: Sydney.

Singh, L.A.K. (1978). Ecological studies on the Indian gharial *Gavialis gangeticus* (Gmelin) (Reptila: Crocodilia). Ph.D. Thesis, Utkal University, Orissa, India.

Singh, L.A.K. and Bustard, H.R. (1977). Locomotory behavior during basking and spoor formation in the gharial (*Gavialis gangeticus*). British J. Herpetol. 5: 673- 676.

Srivastava, A.K. (1981). Biology of the Indian gharial *Gavialis gangeticus* with special reference to its behavior. Ph.D. Thesis, University of Lucknow, Up.

Webb, G.J.W. and Manolis, S.C. (1987). Methods for retrieving crocodilian embryos. Pp. 423-6 *in* Wildlife Management Crocodiles and Alligators, ed. by G.J.W. Webb, S.C. Manolis and P.J. Whitehead. Surry Beatty and Sons: Sydney.

Webb, G.J.W., Buckworth, R. and Manolis, S.C. (1983). *Crocodylus johnstoni* in the McKinlay River, N.T. VI. Nesting biology. Aust. Wildl. Res. 10: 607- 637.

Whitaker, R. and Andrews, H.V. (1988). Notes on crocodilian locomotion. J. Bombay Nat. Hist. Soc. 85 (3): 621-622.

Whitaker, R. and Andrews, H.V. (2003). Crocodile conservation, Western Asia Region: An update. J. Bombay Nat. Hist. Soc. 100 (2 & 3): 432- 445.

Whitaker, R. and Basu, D. (1982). The gharial (*Gavialis gangeticus*): a review. J. Bombay Nat. Hist. Soc. 79 (3): 531- 548.

Whitaker, R. and Whitaker, Z. (1989). The ghara of the gharial. Hamadryad 14 (2): 2- 3.

Whirworth, J. (1971). Notes on the growth and mating of American alligator (*Alligator mississippienisis*). Int. Zoo Year Book 11: 144.

Conservation Genetics of Chinese Alligator, *Alligator sinensis*

Wu Xiaobing[1] and Gu Changming[2]

[1]College of Life Sciences, Anhui Normal University, Wuhu 241000, China;
[2]Anhui Forestry Department, Hefei 231001, China

Chinese alligator is one of the most endangered species among 23 species of crocodiles. Only single population, of no more than 200 individuals, remains in the wild at Xuanzhou, Anhui Province in China. To save this species, captive propagation programs were established, which consist of 2 captive subpopulations at Xuanzhou and Changxing breeding farms. The first crocodile farm, The Anhui Research Center of Chinese Alligator Reproduction (ARCCAR), is the largest captive subpopulation of this alligator, was set up at Xuanzhou, Anhui Province in 1979. 212 alligators came from wild as foundation stock for ARCCAR, and subsequently raised in a natural pond, where the survivors (about 60-70 individuals) remain today. From then on, about 9000 individuals including first (F_1) and second filial generation (F_2) were bred in ARCCAR.

A major concern among conservation biologists is loss of genetic diversity in small or captive populations through genetic drift and inbreeding. Because of their restricted population size, inbreeding is virtually unavoidable in captive populations (Pray *et al*. 1994). The molecular approach has been proved an increasingly valuable tool in the identification of animal genetic variation. Genetic markers, such as Random Amplified Polymorphic DNA (RAPD), mitochondrial control region sequences and microsatellites, have been applied to assess genetic structure of Chinese alligator captive population. The results show that very low genetic variation was revealed in captive population of Chinese alligator. Especially, only one haplotype of control region sequence was found in Chinese alligator population. Recently, we continued to analyze the mitochondrial control region sequences variation using the enlarged samples of Chinese alligator. Total 2 variable loci were found in the control region sequences in skull samples from history population. But also, no sequence variation was detected in captive population. In another study, a pair of degenerate primers was used to amplify polymorphic segments of MHC class II genes from the genomic DNA of *Alligator sinensis*. Ten different nucleotide sequences, which were divided into two groups (A, B), were obtained from cloning and sequencing. There were 38 variable sites among ten nucleotide sequences, and 23 variable sites among amino acid sequences. So, we think that, the loss of genetic variation may not occur in the whole genome of Chinese alligator, the high polymorphism of some functional genes, such as MHC in *A. sinensis*, was of great benefit to genetic conservation in the captive population of Chinese alligator.

Crocodilian Genome Projects: Status and Resources

Travis C. Glenn[1,2]

[1]Savannah River Ecology Laboratory, PO Drawer E, Aiken, SC 29802, USA (glenn@srel.edu);
[2]Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA

Many new tools to analyze the genetics of crocodilians are being developed. Standard genetic tools to identify individuals, family groups, populations, and species are now reasonably advanced. In addition to these more traditional tools, additional approaches are advancing rapidly. The number of genes described from crocodilians will go from a few dozen in 2003 to thousands within the next year. The vast majority of the new data will be from American alligators (*Alligator mississippiensis*). American alligators will also be included in a Reptilian Genome Project proposal to the U.S. National Human Genome Research Institute. Leveraging the data from American alligators and other new comparative genomic data sets will present new opportunities for both applied and basic research of crocodilians. 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 (http://www.uga.edu/srel/CrocDNA/CrocDNAindex.htm).

Crocodilian Gene Hunting with Expressed Sequence Tags (ESTs)

Nancy A. Schable[1], Travis C. Glenn[1,2] and Roger H. Sawyer[2]

[1]University of Georgia, Savannah River Ecology Laboratory, Georgia, USA;
[2]University of South Carolina, USA (schable@srel.edu)

Increasing our knowledge about genome organization and expressed genes for the American alligator (*Alligator mississippiensis*) will provide important information about the basic biology of crocodilians, enhance captive propagation success, and increase the commercial value for this species. Developing an Expressed Sequence Tag (EST) library is the most direct and efficient way to examine the type and number of active genes that are expressed in a particular tissue. We are currently developing EST libraries for brain, liver, kidney, skin, ovary, testes, embryonic tissues, as well as "whole animal" for the American alligator. We will present information about how these libraries are constructed, how they can be used, and how other researchers can access the libraries, clones, and information generated. Several newly discovered American alligator genes will be revealed along with a brief discussion of the significant research that may result from their identification.

Captive Animals with Known Pedigrees Are Needed for Genetic Maps of Crocodilians

Cris Hagen[1] and Travis C. Glenn[1,2]

[1]Savannah River Ecology Laboratory, PO Drawer E, Aiken, SC 29802, USA (hagen@srel.edu); [2]Department of Biological Sciences, University of South Carolina, Columbia SC 29208, USA (Travis.Glenn@sc.edu)

Genetic maps are an important resource for biologists with interests ranging from evolution to production agriculture. Unfortunately, no genetic maps exist for crocodilians. We are interested in developing genetic linkage maps for American alligators (*Alligator mississippiensis*), as well as other crocodilians. To accomplish this, our immediate goal is to obtain the necessary blood samples from captive crocodilians with known pedigrees. In general, samples from families with 3 generations (ie grandparents, parents, and offspring) are desirable. Family groups with large numbers of offspring as well as those from interspecific crosses (eg golden crocodiles) are particularly valuable. We welcome information from anyone with knowledge of or access to such samples.

Stochastic Simulation Model of the Population Dynamics of the *Caiman yacare* in the Brazilian Pantanal

Marcos Coutinho, Milani Chaloupka and Zilca Campos

marcos.coutinho@ibama.gov.br; m.chaloupka@uq.edu.au; zilca@cpap.embrapa.br

A heuristic simulation model was developed to foster a better understanding of the complex nature of caiman population dynamics in the southern Pantanal wetlands. The model was based on systems of finite difference equations linked with demographic rates characterized by nonlinear, feedback and stochastic properties. The model is age- and sex-structured with significant environmental stochasticity included through the relationship between female breeding likelihood and mean maximum water level in the wetlands. Water level fluctuates significantly from year to year and is a major environmental function which also displays long periods of low water levels, resulting in complex nonlinear population dynamics. Demographic stochasticity was included in the model by sampling vital rates such as age-specific survival probabilities from normal probability distribution functions reflecting the estimated measurement error. The model was used to analyse the behaviour of the population and to support risk evaluation of each of following management strategies: a) Egg harvesting: presently, this is the only legal possibility of exploiting natural populations of caimans in the Pantanal, even though the infrastructure and logistic support required to rear the hatchlings is unavailable in the large majority of the farms within the wetlands; b) Harvesting individuals five to nine years of age (50 to 90 cm SVL); c) Harvesting individuals >10 years of age (>90 cm SVL): scenarios *b* and *c* are likely to be economically feasible given that only a low infrastructure and small capital investments are required. It is important to assess the biological feasibility of these approaches. However, changes in the Brazilian federal environmental legislation would be necessary before they could be implemented, given that commercial use of wildlife by hunting is presently prohibited; d) Headstarting and harvest age classes five to nine and/or >10 years: headstarting the population with hatchlings and harvesting young-adults and/or adults are management activities which require relatively low investments and are technically possible to be implemented, despite the lack of infrastructure and logistic support that prevails in most farms in the Pantanal.

Evidence for Long-Distance Migration by Wild American Alligators

Ruth M. Elsey[1], Valentine A. Lance[2] and Phillip L. Trosclair II[1]

[1]Louisiana Department of Wildlife and Fisheries,
Rockefeller Wildlife Refuge, Grand Chenier, Louisiana 70643, USA;
[2]Graduate School of Public Health, San Diego State University, San Diego, California 92128, USA

As part of an ongoing study on growth and sexual maturation of the American alligator on Rockefeller Refuge, 3004 specimens ranging in total length from 28 to 361 cm were captured from June 2001 through January 2004. Each animal was tagged, measured, the sex recorded and released at the site of capture. A large number of these marked animals were recaptured outside the refuge at the annual alligator hunts during the month of September. Of the recaptures identified, 69 were males, 22 females and six of undetermined sex. From each recaptured alligator, total body length and number of days since initial capture were recorded, and minimum distance from initial capture site estimated. From these preliminary data we calculated growth rate and plotted minimum distance moved. The number of days between first capture and ultimate capture ranged from 29 to 1151 days. To calculate growth rate we used only the days between April 1 and September 30. Males grew significantly faster than females (3.55 ± 0.15 vs. 1.94 ± 0.23 cm/growing month). Distance from initial capture site to final capture site ranged from 0.33 to 90.17 km. Three alligators moved more than 30 km, seven alligators moved between 20 and 30 km and 19 moved between 10 and 20 km. There was no significant bias in the compass direction moved. These results greatly extend previous estimates of long-distance movement by alligators and demonstrate that both sub-adult and sexually mature animals move considerable distances. Suggested reasons for such dispersal will be discussed.

Characterization of the Innate Immune System of the American Alligator (*Alligator mississippiensis*)

Mark Merchant[1], Ruth M. Elsey[2] and Phillip Trosclair III[2]

[1]McNeese State University, Department of Chemistry, PO Box 90455, Lake Charles, Louisiana 70609, USA (mmerchan@mcneese.edu);
[2]Louisiana Department of Wildlife and Fisheries, Rockefeller Wildlife Refuge,
5476 Grand Chenier Hwy, Grand Chenier, Louisiana 70643, USA (Elsey_RM@wlf.state.la.us)

Recent studies in our laboratory have shown that serum of the American alligator exhibits potent and broad-acting antibacterial properties. In addition, alligator serum exhibits strong amoebacidal properties. The antimicrobial character of the serum has been attributed to the innate immune system of the alligator. The innate immune system is the first line of defense against infection. The serum complement protein system comprises an important component of innate immunity. We have identified one alligator complement protein component and characterized much of the biochemistry of the complement system, including kinetic analyses, thermal dependence, and the role of Mg^{2+} and Ca^{2+}. Other studies have focused on the role of leukocytes in innate immunity. We have described the antifungal effects of crude granulocyte extracts. In addition, we have measured complement-mediated opsonization and phagocytosis of bacteria and the generation of oxidative bursts by alligator granulocytes using advanced flow cytometeric techniques. This presentation will focus on an overview of our recent and current studies on the innate immune system of the American alligator.

Isolation and Identification of Microorganisms in the Eggs of *Caiman crocodilus fuscus* from Three Farms on the Atlantic Coast of Colombia

Iván D. Palacios R.[1] and Monica Sick G.[2]

[1] Wildlife Management Adviser, Colombia (crocofauna@hotmail.com);
[2] Estudent M.V.Z. Universidad San Martín, Barranquilla, Colombia

Abstract

During the incubation process of the crocodylia, embrionary mortality occurs due to different effects, one of them being contamination by microorganisms. Our sample material came from three farms that carry out industrial zoobreeding programs in a closed cycle with the *C. c. fuscus* species, located on the Colombian atlantic coast (from the Colombian Caribbean, San Francisco and Colombian Crocodilia) and have automated incubation systems and calibrated at 32°C and 99% of relative humidity. In order to evaluate this situation 29 eggs (shell, inside) were analysed, three water samples, four samples composed by vegetal material, three samples composed by vermiculita. Microorganisms were is dated and identified, from these. The most frequent bacterial on shells were *Pseudomonas* spp., *Klebsiella pneumoniae*. Fungi were only found on shells and less than bacteria, *Trichophyton rubru* and *Aspergillus fumigatus* being the most frequent. The latter microorganisms were also found on vermiculita samples and vegetal material from nests, the latter presumably being the contaminating origin. It is fundamental to carry out disinfection techniques for eggs and incubation equipment

Introduction

In the closed cycle production systems of *Caiman crocodilus fuscus* the incubation process places a fundamental role for the productive success of the system, therefore, embrionary mortality occurred during this process, may affect the production. For such effect and in order to find causes that may by corrected through the implementation of and adequate handling, the Colombian Caribbean, San Francisco and Colombian Crocodilia farms collaborated with the logistic and economic support to carry out this investigation.

In these farms, the maximum length observed for males is 225 cm (88.58'') and the females reach up to 185 cm (72.83''). The females of largest size normally lay a big number of eggs and of great size, which makes the newborn be equally big.

For the area of study (Caribbean zone of Colombian), the mating occurs after the first symptoms of courtship starting at the end of January which last until the end of June. The nests are constructed by the female piling vegetation material, an event that starts in the first week of April and ends by the middle of August. Hatching occurs after an average of 72 days of incubation at a temperature of 32°C and 99% relative humidity, starting by the middle of June and ending during the first week of November. In these three farms the physical characteristics of the eggs of *C. c fuscus* have been found to be: weight that can vary from 29 to 69 g, length from 5 to 6.4 cm and width of 3.1 to 4.1 cm.

The shell of these eggs is hard and calcified stuck to a fibrous membrane. The calcified portion of the shell is rough and contain groups of fine pores that may not be seen by the human eye. The pores are vital for gaseous interchange and are also structure that permit maintaining hydration balance of the egg according to the level of humidity that surrounds it.

Microorganisms can also go through these pores the same way and affect the immune system of the eggs and cause the mortality of the embryo because of this. The incubation systems used in these three farms are electronic, reason why they have the necessary equipment to offer the conditions that permit maintaining a constant temperature during the whole incubation process.

Objectives

The main objectives was to identify the types of bacteria and fungi present in *C. c. fuscus* eggs and the materials involved in their incubation.

Materials and Methods

Basic laboratory equipment was used for the sample taking as for their analysis, each one of them well sterilized. The egg samples were taken from three farms, inside the group of incubated nest, having as a selections criterion, visual characteristics such as fungi presence, unfertile eggs and fertile eggs.

Procedure

Initially, through ultrasound, females were detected that presented sonogram characteristics such as presence of structures compatible with eggs, eggs in abdominal cavity and females with no eggs. From these samples were taken of ovaries, oviducts, follicles and eggs found in oviduct as in abdominal cavity, to be analysed in laboratory which determined the absence of microorganisms in the different samples.

There are proceeded to take the egg samples, identifying the nest, taking these with gloves and depositing them in sterile bags, the same with the vermiculite, vegetative material, from the nest and water samples, to be transported this way to the laboratory.

Where it proceeded to analyse the external and internal material of the egg following the corresponding protocol to do the planting using Agar blood as a cultivation means and EMB for anaerobiosis (non-oxygen) at 37°C for 24 hours and the means for fungi at laboratory room temperature from 8 to 10 days.

Samples

29 eggs were used, analysing the shell and the inner content, 4 samples of compound vegetative material, three of water and three of compound vermiculite, this means a total of 39 samples were analysed.

Global Analysis of Samples

Samples were taken from 19 lakes of reproducers of a total 30,that exist on the three farms, which corresponds to a 63.3%.

For the global analysis of samples the number of isolations was established of each type of bacteria and kinds of fungi, in each one of the three farms and its percent relation.

Results

Bacteria

Fifteen (15) different types of bacteria were detected on the shell and 13 in the inner part. Among the isolated bacteria on the shell and the inner part of the eggs, the most common ones were: *Pseudomonas*, *Klebsiella pneumonidae* and *Enterobacter aglomerans*.

Fungi

Five different kinds of fungi were identified on the shell, and interestingly they weren't found in the inner part of the eggs. The fungi that was isolated in a greater number of times was *Trichophyton rubrum* and *Aspergillus fumigatus*, each one 16 times.

Discussion

When making a comparison between the samples taken and the bacteria and fungi detected in each one of them, we can notice that a high relationship exists between the most isolated microorganisms in eggs, with the ones found in vegetation material. The bacteria and fungi that were isolated in this procedure, coincide greatly with the ones reported by Mayer (1998).

Births and Deaths Per Farm

The rate of embryonic mortality and the percentage of hatchings were calculated using only the viable eggs taken from the nests used for this evaluation - broken eggs were not taken into account. In the case of farm one, we used eggs from 11 nests, in which embryonic mortality was 73.5%, and the hatching rate was 26.5%; the newborn mortality rate was 11%.

In the case of farm 3, the embryonic mortality in the nest was 47% (hatching rate of 53%), and the newborn mortality rate was 1.7%. In farm two, embryonic mortality was 55% (hatch rate of 45%), with a newborn mortality rate of 34%. The average embryonic mortality rate for all incubated eggs in the three farms was 16.3% (hatching success of 83.7%).

This comparison could reflect that the bacteria and fungi in the eggs and the environment that surrounds them, influence notably so that the eggs don't culminate in a successful birth.

We have to take into a account that the initial samples of eggs were taken under specific circumstances like: infertility, microscopic presence of fungi and normal eggs. This is why these percentages are valid for the total sampling done for this study, which isn't a totally real situation for each one on the farms at a global level, nevertheless it those reflect the high embryonic and newborn mortality caused by these microorganisms, which is a warning to take measures to better this situation.

Conclusions

The isolated bacteria and fungi are microorganisms natural to the environment in which the samples were and because of the conditions the *C. c. fuscus* eggs were incubated in, such as a 99% relative humidity and 32°C temperature, made the environment good for the growth of these microorganisms. Specially for the fungi, which managed to grow to such a point the manifested microscopically so well as it they were in an environment of enriched plantation special for them, like the ones in the laboratory are.

The eggs and the newborn have their own defence mechanisms, which are able to fight a certain number of microorganisms nevertheless when the number of these microorganisms is such bigger, they overcome the defences of the eggs and newborn managing to cause embrionary mortality, the newborn deaths and postbirth sicknesses.

The presence of bacteria in the inner content of the eggs, reflected that some of then had the chance to penetrate the shell causing damages nevertheless the possibility that some were the result of contamination isn't discarted eventhough the measure taken to prevent the contamination in the planting in the laboratory.

About the fungi, these weren't isolated from the inner content of the eggs, which makes us presume they didn't mange to penetrate the shell, these doesn't mean they don't cause harm, due to the data of obtained, we observed that in the nests where microscopic growth took place, was where a 100% of embryonic mortality of the nesting was present. These fungi are presumed to act two ways: one is that when forming an extense longer over the surface of the egg, the manage to stop the normal gas exchange between the egg and the external environment, causing the embryonic mortality this way; or that the fungi can generate toxins and these penetrate the shell, can be the ones causing the embryonic mortality, surely these are only suppositions and to confirm them, they must be studied specifically and with the means for it.

Suggestions

To be able to diminish the microbial load on the fertile eggs for incubation and in general in all equipment and incubation infrastructure, its advisable to practice disinfection techniques on these.

Acknowledgements

We specially thanks the croc farms: From the DEL CARIBE COLOMBIANO, SAN FRANCISCO, CROCODILIA COLOMBIANA, located in the Atlantico department Colombia for the unconditional cooperation for the development of this investigation. Thanks to croc farm REPTICOSTA for their support, and Miguel Rodriguez for his comments.

Literature

Carter, G.R. and Chengappa, M.M. (1994). Bacteriología y Micología Veterinaria. 2a Ed. Santafé de Bogotá.

Fraser, C.M. (1991). Manual de Merck. 4a ed. Océano Grupo.

Hutton, J.M. and Webb, G.J.W. (1994). Introducción a la cría de cocodrilianos.

Joklik, W. and Amos, W. (1995). Microbiologia Zinsser. 2a ed. Panamericana.

Koneman, A. and Sommers, D. (1993). Diagnóstico Microbiológico Texto y Atlas Color. Panamericana. pp. 447-450.

Ladds, P.W. (1998). Management and diseases of farmed crocodiles.

Larriera, A. and Verdade, L.M. (1998). La conservación y el manejo de los caimanes y cocodrilos de América Latina. Vol. 1.

Mason, B.M. (1997). The bibliography of crocodilian biology.

Mayer, R. (1998). Crocodile Farming. Rural Industries Research and Development Corporation.

Pachón, E. (1982). Algunos aspectos relativos a la conservación y manejo de los Crocodylia en Colombia. Inderena. pp. 1.

Palacios, R.I.D. (1999). Ministerio Del Medio Ambiente. Zoocría en Colombia. Evolución y perspectivas.

Youngrapakorn, P. and Ousavaplanechai, L. (2000). A Color Atlas Of Diseases of the Crocodile. Style Creative House Co. Ltda.: Thailand.

Ross, R. (1984). The bacterial diseases of reptiles.

Sierra D.C.L. (1994). Caracterización del desarrollo embrionario del *Caiman crocodilus fuscus*, incubado bajo tres regímenes de temperatura. Tesis. Santa fé de Bogotá: Universidad Nacional de Colombia, Departamento de Biología.

Vergel, G. (1997). Un manual para la elaboración de diseños y proyectos de investigación. 3a ed. Mejoras Ltda.: Barranquilla.

Using Bioelectrical Impedance Analysis (BIA) to Determine the Body Condition of Farmed Estuarine Crocodiles

Stephen Peucker[1], Rob Jack[1], Bob Mayer[1], Beth Symonds[2], Leigh Ward[2], David Booth[2], Peter Fisher[3] and David Wilson[3]

[1]Agency for Food and Fibre Sciences, Department of Primary Industries and Fisheries, PO Box 1085, Townsville, QLD 4810, Australia (steve.peucker@dpi.qld.gov.au); [2]University of Queensland, Brisbane, QLD 4072, Australia (bsymonds@zen.uq.edu.au); [3]Melaleuca Crocodile Farm, PO Box 1298, Mareeba, QLD 4880, Australia (pwfisher@bigpond.com)

Bioelectrical Impedance Analysis (BIA) technology has been used to assess the body composition of humans in nutritional-related studies for over 30 years. BIA is safe, rapid, accurate, non-invasive, portable and inexpensive. It works on the principal that the body is essentially composed of two compartments: a lean compartment that contains all the water and conducting electrolytes, and a fat compartment that contains little water and is non-conducting. In this study, a low-frequency, fixed current was passed through the body and a value for impedance (Z) is calculated. This value, combined with the crocodile's length and weight, allowed the calculation of the animal's total body water (TBW). However, BIA does not measure actual fat levels in the body and instead uses TBW, which is a good estimate of lean body mass. The BIA spectrometer was calibrated for use with crocodiles using twenty crocodiles that ranged in age and size. An independent measurement of TBW using tritiated water, combined with impedance measurements, the animal's length and weight and carcass analysis, was used to develop a prediction formula that was then programmed into the BIA spectrometer. BIA could be an effective tool in developing manufactured feed for crocodiles by assessing the condition of animals trialling different feeds, since this technology is capable of measuring changes in body condition of individuals or groups of animals over time.

Development of Electrical Stunning Equipment for Farmed Crocodiles

Stephen K.J. Peucker[1], Rob H. Jack[1], Bernie M. Davis[1] and Ian Graham[2]

[1]Agency for Food and Fibre Sciences, Department of Primary Industries and Fisheries, PO Box 1085, Townsville, QLD 4810, Australia (steve.peucker@dpi.qld.gov.au); [2]Electrician, PO Box 858, Atherton, QLD 4883, Australia

The development of electrical stunning equipment to capture farmed estuarine crocodiles has occurred over the last five years. The equipment was developed to address two primary considerations, the safety and well-being of farm workers and the welfare of the crocodile. Initially the equipment comprised of two units, one unit that operated off mains power, the other a portable unit in the form of a belt pack. The stunning wand was made from PVC piping which had a rubber belt running through the middle of the wand, which allowed the crocodiles to be dragged out of the water once they had been stunned. The equipment has been refined over time with the original operating units being replaced with a backpack unit housed in a clear Perspex box. This unit is fully waterproof and will float if dropped into water. Crocodiles easily damaged the original wands during capture. In an attempt to remedy this, wands have been manufactured from stainless steel pipe. A junction box at the top of the wand delivers power via two 3/16 shrink-wrapped stainless steel rods connected to electrodes at the head of the stunning wand. A push button switch supplies power to the equipment.

CrocProfit

Bill Johnston[1], Steve K.J. Peucker[2], Rob H. Jack[2] and Bernie M. Davis[2]

[1]Rural Industry Business Services, Department of Primary Industries and Fisheries, GPO Box 46, Brisbane, QLD 4001, Australia (bill.johnston@dpi.qld.gov.au); [2]Agency for Food and Fibre Sciences, Department of Primary Industries and Fisheries, PO Box 1085, Townsville, QLD 4810, Australia (steve.peucker@dpi.qld.gov.au)

CrocProfit is a complete information package for crocodile farmers and potential investors. The CD also contains reference material and a comprehensive list of contacts for related State Governments and industry associations for any questions or assistance that you may require.

In conjunction with the reference material, the farm model provided allows you to evaluate the economics of crocodile farming, using your own input parameters, before any investment or construction occurs.

The model covers all aspects of farming in a comprehensive fashion. The model is based upon the cost-benefit analysis technique. Cost-benefit analysis is a conceptual framework for the economic evaluation of projects, in this case, crocodile farming projects. This approach differs from financial appraisal in that it considers all gains and losses. The basic premise of cost-benefit analysis is to assist you to make a decision in regard to the allocation of resources. In particular, *CrocProfit* helps you to make decisions about whether or not to invest in crocodile farming.

Existing farmers can also use *CrocProfit*. Once the data is entered into the model a farmer can use the computer version of his farm to determine the impact of different management decisions. For example, the farmer may wish to know how introducing new diets will effect annual production and profitability.

The model is easy to operate. It is simply a matter of entering data into the input cells of the model you have selected. It is better to be as accurate as possible with data entry in order to get the best possible results.

Risk analysis is also included to ensure that all possible economic outcomes are considered. The model provides a static figure on the summary page (annual return). When probabilities (uncertainty) are added to production and price the range of economic outcomes are delivered (cumulative probability distribution). Investors or potential farmers must accept that they can receive a wide range of returns (including negative ones).

Once all the data is entered into the model you can view the summary statistics for the farm. All the statistics used are explained in the model itself. Basically, the farm is run over a 20-year period. The output includes the expected annual returns, when the farm is paid off and the interest rate at which you can borrow funds to invest in the project. The summary statistics will also provide a break down of costs on a per animal basis.

The models are set up in Microsoft Excel as a spreadsheet. All the sheets contained in the model are labelled for easy reference and there are buttons in the menu for easy movement between sections.

The basic premise for the operation of the model is that there are two types of cells; the "data entry cell" and the "data secured cell". The yellow cells represent data entry cells that allow you to enter data. The red cells are locked because they contain calculated answers.

Data entry cell

Data secured cell

CrocProfit is available from DPIF at a cost of $AU220 (GST inclusive) by phoning the DPI Call Centre on 13 25 23 (local call within Queensland) or 1800 816 541 (within Australia) or +61 7 3239 3163 (overseas), email "books@dpi.qld.gov.au" or go to the DPI website www.dpi.qld.gov.au.

A Parentage Determination Kit for Saltwater Crocodiles

Sally R. Isberg[1], Y. Chen[1], Stuart G. Barker[2] and Chris Moran[1]

[1] ReproGen, Faculty of Veterinary Science, University of Sydney, NSW 2006, Australia (sisb7192@mail.usyd.edu.au);
[2]Janamba Croc Farm, PO Box 496, Humpty Doo, NT 0836, Australia

Fifteen microsatellite loci were evaluated in farmed saltwater crocodiles for use in parentage testing. One marker (C391) could not be amplified. For the remaining fourteen, the number of alleles per locus ranged from 2 to 16, and the observed heterozygosities ranged from 0.219 to 0.875. The cumulative exclusion probability for all fourteen loci was 0.9988. The eleven loci that showed the greatest level of polymorphism were used for parentage testing with an exclusion probability of 0.9980. Using these eleven markers on 107 juveniles from 16 known-breeding pairs, a 5.6% pedigree error rate was detected. This level of pedigree error, if consistent, could have an impact on the accuracy of genetic parameter and breeding value estimation. The usefulness of these markers was also evaluated for assigning parentage in situations where maternity and/or paternity may not be known. In these situations, a 2% error in parentage assignment was predicted. It is therefore recommended that more microsatellite markers be used in these situations. The use of these microsatellite markers will broaden the scope of a breeding program allowing progeny to be tested from adults maintained in large breeding lagoons for selection as future breeding animals.

Developing Crocodilian Education in Public Schools

Harold E. Nugent

Paynes Prairie Preserve , State Park and Lower Suwannee and Cedar Keys, National Wildlife Refuges, 6406 NW 36th Terrace, Gainesville, Florida 32653, USA

The overriding purpose of this poster presentation is to help presenters increase public school students' understanding and appreciation of crocodilians by making effective classroom presentations. This poster consists of two panels presenting an overview of the following concepts: The first panel presents the process by which three entities: the local, the scientific, and the school communities can collaborate in constructing and presenting meaningful environmental education. The Local Community with its special needs determines the content and approach. The Crocodile Specialist Group provides the scientific knowledge. The State Department of Education contributes the appropriate standards, objectives, and curriculum format. Venn diagrams illustrate how the Interpretive Approach and the Cognitive Approach can be integrated using the student's four Learning Styles. The second panel presents examples of twelve intellectual strategies as they are applied to the study of crocodilians. These strategies are basic to effective writing, critical thinking, and problem solving. Each strategy on the poster is accompanied with a crocodilian photo illustrating its use. A number of handouts and resource bibliographies will be available as well as model environmental curriculum guides from successful programs found in Refuges and Parks around the U.S. and Cuba.

Improved Quality of Australian Crocodile Skins (*C. porosus*)

Stephen C. Hawkins and Chi P. Huynh
CSIRO Textile and Fibre Technology

Crocodile skins can be fairly accurately graded at all stages of processing, hence faults such as brown spot and scarring that impact upon the final leather value result in a large reduction in the raw skin value. Faults arise from poor husbandry, butchering and preservation. The opportunity for improvement in skin quality ceases at the point of slaughter so husbandry should aim to have quality peaking at that point. Animals with skins that are less than first grade should be assessed for their potential and retained, if suitable, until first grade is achieved. Competent butchering and effective preservation will then ensure maximum return.

Faults due to poor preservation are very common within the industry, resulting in scale slip, grain damage and loss of strength. Salt alone is not sufficient to protect skins for an extended period of time from bacteria, including red heat, and fungi. As an aid to determining the significance of faults seen on the live animal, marks and damage that are observable on the raw skin have been photographed and tracked through to finished leather. It has been found that some apparently minor brown spot infections can cause major pitting of the leather grain, whilst severe scale erosion may not affect the finished leather. Partially regrown scales often appear as crescents (double scaling') on the leather, and faint scars may also show up very clearly.

This project, to improve the quality of Australian Saltwater Crocodile (*Crocodylus porosus*) skins is jointly supported by CSIRO and RIRDC (Rural Industries Research and Development Corporation), in collaboration with Koorana Crocodile Farm, Rockhampton, Queensland.

Persistent Organic Pollutants in Eggs of the Saltwater Crocodile from Tropical Australia: a Preliminary Survey

Bob Packett[1], Phillip Ford[2], John Lever[3], Adam Britton[4], Charlie Manolis[4], Rob Bredl[5] and Steve Watson[6]

[1] Cooperative Research Centre for Coastal Zone, Estuary and Waterway Management, PO Box 1762, Rockhampton, QLD 4700, Australia (robert.packett@nrm.qld.gov.au); [1,2] CSIRO Land and Water, PO Box 1666, Canberra, ACT 2601, Australia (phillip.ford@csiro.au); [3]Koorana Crocodile Farm, 65 Savages Road, Coowonga, QLD 4700, Australia; [4]Wildlife Management International, PO Box 530, Sanderson, NT 0812, Australia; [5]Wildlife Park, Shute Harbour Road, Airlie Beach, QLD 4802 Australia; [6]Proserpine River Eco Tours, PO Box 568, Airlie Beach, QLD 4802, Australia

Abstract

Nonviable eggs (n= 23) of the Saltwater Crocodile (*Crocodylus porosus*) were collected from three sites across the tropics of Northern Australia and analysed for persistent organic pollutants (POPs) over the 2002 to 2004 summer breeding seasons. The p,p-DDT metabolite p,p-DDE was found to be the most prevalent contaminant for all sites. Residues of p,p-DDE in eggs collected from Queensland nests ranged from a mean of 45 ppb for Proserpine River (n= 2) to 156 ppb for the Fitzroy River (n= 6), while residues in Northern Territory eggs had a mean of 31 ppb (n= 6). All eggs analysed (from all sites) had detectable residues of p,p-DDE. A number of eggs was also contaminated by other persistent pollutants at trace levels. The survey was conducted to gain an understanding of the bioaccumulation of POPs in several Australian coastal ecosystems and compliments similar recent studies for the Central American region where comparable concentrations were detected in crocodilian eggs. The results will provide information for wild crocodile management issues concerned with toxicology and infer that the species is a valuable long-term indicator of ecosystem health and contaminant exposure. Suggestions for future Australian work are given.

Introduction

Persistent organic pollutants (POPs) are those chemicals that are resistant to degradation in the environment under ambient conditions and bioaccumulate in food chains via their solubility in the lipids of organisms. In addition, many are capable of long-range transport due to their vapor pressure and partitioning characteristics. Concerns over the global distribution of POPs has led to recent studies aimed at bioaccumulation of PCBs and organochlorines (OCs) in particular (Kalantzi *et al*. 2001; Smith 1999). The discovery and widespread application of the long-lived OC pesticides such as DDT, Dieldrin, Aldrin, and persistent non-organochlorine compounds such as Endosulfan has been of great benefit to agriculture and public health. However, the particular properties of these materials such as their persistence, and their fat solubility which contribute, in part, to their utility has lead to them being almost universally distributed throughout the ecosphere.

Recent work on bioaccumulation in top level predators suggest that some of these materials have pseudo endocrine activities in alligators (Guillette *et al*. 1995, 2000; Lind *et al*. 2004). Much of this work relies, in part, on specimens taken from Lake Apopka in Florida, USA. This Lake was highly contaminated in 1980 by a chemical spill of dicofol, DDT and sulphuric acid (Matter *et al*. 1998) and the question arises whether comparable levels of OC contamination and bioaccumulation occur in other environments, and if species other than the American alligator (*Alligator mississippiensis*) are subject to the same effects. Alligators, with crocodiles, caimans, and gharials, make up the crocodilians. They all share the same characteristics of being long-lived top-level predators in tropical and semi-tropical aquatic systems and are frequently highly territorial.

Many species of crocodilians seem to be undergoing a population decline worldwide, though this may merely reflect habitat destruction or increased hunting pressures rather than a widespread impact of endocrine disrupting chemicals. Morelet's crocodile (*C. moreletti*) occurring in Belize, Guatemala, and Mexico is considered to be an endangered species by the IUCN - World Conservation Union and is listed on Appendix I of CITES. Using crocodile eggs collected from two lagoons in northern Belize, Wu *et al*. (2000a) showed that Morelet's Crocodiles in these two environments had been exposed to organochlorine compounds and that such an " exposure may represent a health threat to populations of crocodiles in central America". In a closely related examination of eggs from Morelet's Crocodile (*C. moreletii*) and the American Crocodile (*C. acutus*) from southern Belize and the coastal zones of Belize respectively, Wu *et al*. (2000b) demonstrated the presence of *p,p*-DDE, a degradation product of DDT as well as DDT and p,p-DDD another metabolite of DDT.

In contrast, in Australia, where the non-traditional hunting pressure on the Saltwater Crocodile (*C. porosus*) has been removed, the population has rebounded, and they can at times constitute a nuisance species that has to be relocated from areas where the potential for fatal human-crocodile contact (for the humans) exists. Worldwide there have been at least 6 investigations into the occurrence of organochlorines in the flesh of various crocodilians (Best 1973a, 1973b; Vermeer *et al*. 1974; Wheeler *et al*. 1977; Matthiesen *et al*. 1982; Delany *et al*. 1988; Phelps *et al*. 1989) and more limited investigations of the organochlorine content in crocodile eggs (Wessels *et al*. 1980; Phelps *et al*. 1986; Heinz *et al*. 1991; Skaare *et al*. 1991; Wu *et al*. 2000a, 200b).

The only measurements on OCs in both Freshwater and Saltwater Crocodile eggs from Australia (Best 1973) predate the prohibition on the widespread use of DDT which was phased out for general use around the early 1980s. We report on persistent organic pollutants in wild and farmed crocodile eggs collected in Queensland and the Northern Territory of Australia in order to contribute to the knowledge base for these chemicals in crocodilians in the southern hemisphere. In addition, we point to the advantages of using nonviable *C. porosus*. eggs as a convenient, low impact and reliable method of assessing bioaccumulation of POPs in the environment.

Materials and Methods

Egg Collection

Nonviable *C. porosus* eggs were collected during 2002, 2003 and 2004 from 3 sites across tropical Australia. Not all sites were sampled in all years, farmed eggs from Crocodylus Park in the Northern Territory and Koorana Crocodile Farm in Queensland were only sampled during 2002. Collection sites are shown in Figure 1.

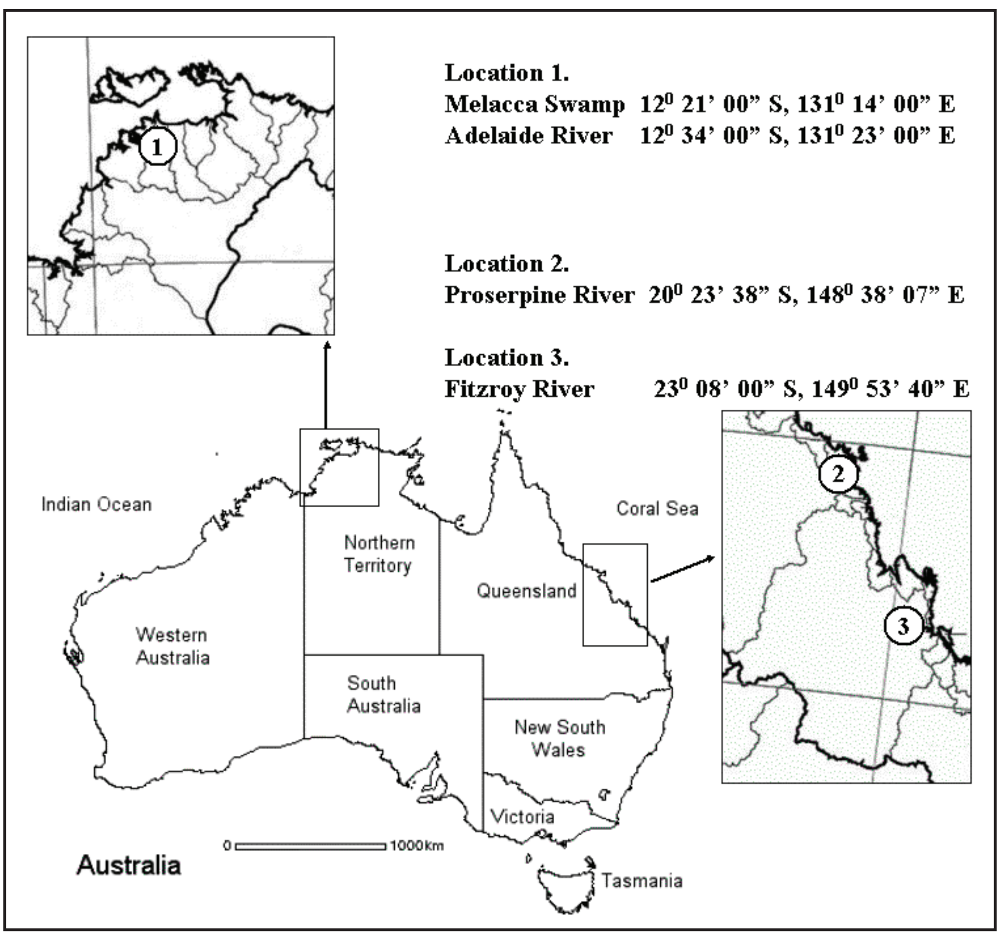

Figure 1. Collection locations of nonviable *C. porosus* eggs during 2002-2004 survey.

Location 1 included collections from Melacca Swamp (mostly fresh - near coast) and from the Adelaide River system (tidal). The Adelaide system has an approximate area of 4400 km^2 and the major land use is cattle grazing with limited areas of agriculture. The Proserpine River location (tidal) has a drainage area of around 550 km^2 with grazing in the upper catchment and sugar cane cultivation in the lower reaches.

The Fitzroy River location consists of a series of large water holes in the fresh water system around 100 km upstream of tidal influences, it has a catchment area of around 142,600 km^2 and has grazing as the main land use with substantial dry land and irrigated agriculture.

The Fitzroy River in Queensland is considered the southern breeding limit for *C. porosus*. All the above sites are listed in the Department of Environment and Heritage directory of Important Wetlands in Australia. Eggs were labeled, frozen, packaged and transported (chilled) to the Queensland Health Scientific Services laboratories in Brisbane, Queensland, for analysis a range of persistent and non-persistent pollutants.

<u>Extraction and Analysis of Egg Residues</u>

Analysis of shell-free wet egg contents were performed at the National Association of Testing Authorities Australia (NATA) certified laboratories of Queensland Health Scientific Services in Brisbane via quality assured procedures. The laboratory regularly conducts analyses for contaminant residues in food products including hen eggs as part of an ongoing Australian standards program. Basically, the contents of an egg were blended and approximately 5 g taken for analysis and accurately weighed, results are calculated on a wet basis. The sub-sample was mixed with anhydrous sodium sulphate until a free flowing powder was obtained. Extraction was via acetone solvent. Finally, Gel Permeation Chromotography (GPC) and Florisil cleanup steps followed by Gas Chromatography/Mass Spectrometry (GC/MS) were used for determination of contaminant types and amounts. The analysis covered a wide spectrum of common environmental contaminants with 184 chemical species including OCs, OP's, and other insecticides, herbicides, fungicides (i.e. common pesticides) included. In addition, some eggs (2003 collections) were screened for the following PCB congeners (confined to those commonly associated with Arachlor residues) IUPAC 1, 5, 18, 28, 31, 52, 44, 66, 101, 87, 110, 118, 151, 149, 153, 141, 138, 183, 180, 170, 194 and 206. A similar and more in-depth description of the methodology for the isolation of organochlorine and organophosphorus residues from hen eggs (Schenck and Donoghue 2000) can be readily applied to crocodile eggs. Eggs were not analysed for heavy metals or dioxins.

Results

A total of 23 eggs from both captive and wild Saltwater Crocodiles (*C. porosus*) were examined . Organochlorine residues were found in all eggs (Table 1). The concentrations of DDE ranged from 2 to 350 ppb and the highest concentrations were in the eggs from the freshwater reach of the Fitzroy River upstream of Rockhampton, Queensland. DDE concentrations in eggs taken from captive animals were at the lower end of the range and were comparable to the lower concentrations seen in the Northern Territory wild samples from the Adelaide River and Melacca Swamp in 2003. The concentrations of DDD another metabolite of DDT, and of DDT itself were always lower than the DDE concentrations in the same egg. Analysis of nest material (Proserpine Site 2003) showed nil concentrations (by our methods) of DDT metabolites thus providing confidence that the eggs had not been contaminated by nest material (Canas and Anderson 2002) and that the measured concentrations in the eggs reflected the maternal body burden of DDE and were not an artefact. A comparison of mean concentrations of DDE found in eggs from the three locations is shown in Figure 2.

Concentrations of Heptachlor epoxide and Dieldrin were always less than 10 ppb (Table 1). Numerous PCB congeners were detected in the various samples though they seemed to be heterogeneous with respect to their content of the various congeners. That is, there was no constant ratio between the different congeners suggesting that there must be, at least several different sources each with its own characteristic mixture of congeners. Tri-n-butyl phosphate was detected in the Northern Territory samples together with DEET. This is the active ingredient of various insect repellent products and suggests that the whole area has been contaminated by this widely used material.

Discussion

Our results show a wide range of DDE concentrations in the eggs of the Saltwater Crocodile collected in a range of habitats in Queensland and the Northern Territory, Australia. The average concentration (56 ppb; standard deviation 97 ppb) does not differ significantly from the average value (68 ppb; 86 ppb) for the most extensive other set of data

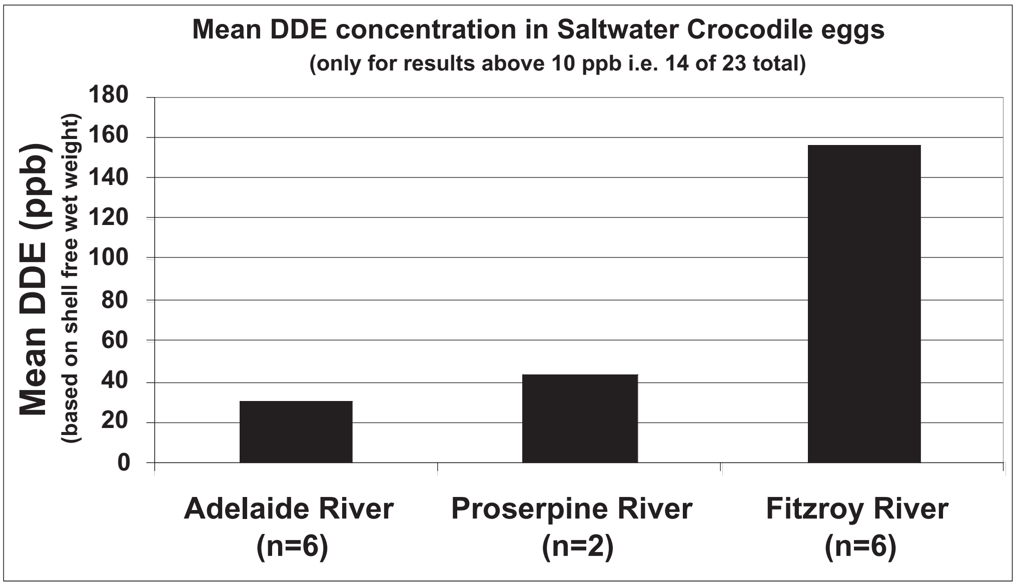

Figure 2. Mean DDE concentration in eggs by collection location.

Table 1. Persistent pollutant residues detected in *C. porosus* eggs. NA= not analysed for contaminant; ND= not detected; Trace = below level of reporting (5 ppb) but detected; RP= results pending.

Contaminant residues in *C. porosus* eggs expressed as ppb													
Location - site - Date								PCB congeners					
	DDT	DDD	DDE	Heptachlor Epoxide	tri n-butylphosphate	Dieldrin	DEET	118	138	141	149	153	180
Northern Territory (2002)													
Captive	ND	ND	**Trace**	ND	ND	ND	ND	NA	NA	NA	NA	NA	NA
Wild	ND	ND	**7**	ND	ND	ND	ND	NA	NA	NA	NA	NA	NA
Northern Territory (2003)													
Adelaide River nest 1	ND	ND	**12**	ND	ND	**5**	**Trace**	NA	NA	NA	NA	NA	NA
Adelaide River nest 2	ND	ND	**17**	ND	ND	ND	ND	NA	NA	NA	NA	NA	NA
Adelaide River nest 3	ND	ND	**12**	**7**	ND	ND	ND	NA	NA	NA	NA	NA	NA
Adelaide River nest 4	ND	ND	**9**	ND	ND	ND	ND	NA	NA	NA	NA	NA	NA
Adelaide River nest 5	**Trace**	ND	**Trace**	ND	ND	**Trace**	ND	NA	NA	NA	NA	NA	NA
Adelaide River nest 6	ND	ND	**Trace**	ND	ND	ND	**8**	NA	NA	NA	NA	NA	NA
Adelaide River nest 7	ND	ND	**Trace**	ND	**Trace**	ND	ND	NA	NA	NA	NA	NA	NA
Melacca swamp nest 1	ND	ND	**Trace**	ND	ND	ND	**Trace**	NA	NA	NA	NA	NA	NA
Melacca swamp nest 2	ND	ND	**86**	ND	ND	ND	ND	NA	NA	NA	NA	NA	NA
Melacca swamp nest 3	ND	ND	**17**	ND	ND	ND	ND	NA	NA	NA	NA	NA	NA
Melacca swamp nest 4	ND	ND	**41**	ND	ND	ND	ND	NA	NA	NA	NA	NA	NA
Queensland													
Proserpine River (2003)													
Nest 1a	ND	ND	**45**	**Trace**	ND	ND	ND	ND	**Trace**	ND	ND	**Trace**	**Trace**
Nest 1b	ND	ND	**42**	ND	ND	ND	ND	ND	ND	ND	ND	**Trace**	ND
Nest material	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fitzroy River													
Koorana Farm (2002)	ND	ND	**7**	**8**	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fitzroy nest 1 (2003)a	ND	ND	**74**	ND	ND	ND	ND	ND	ND	**Trace**	**Trace**	**Trace**	**Trace**
Fitzroy nest 1 (2003)b	ND	ND	**81**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fitzroy nest 2 (2003)a	**Trace**	**Trace**	**310**	ND	ND	ND	ND	**Trace**	ND	ND	ND	**Trace**	**Trace**
Fitzroy nest 2 (2003)b	**Trace**	**Trace**	**350**	ND	ND	ND	ND	**Trace**	ND	ND	ND	**Trace**	ND
Fitzroy nest 1 (2004)	RP	RP	**60**	ND	ND	ND	ND	RP	RP	RP	RP	RP	RP
Fitzroy nest 2 (2004)	RP	RP	**60**	ND	ND	ND	ND	RP	RP	RP	RP	RP	RP

covering both the American Alligator and Morelet's Crocodile in Belize (Wu *et al*. 2000b). The range of concentrations was virtually identical in the two cases. DDT concentrations were always much less than DDE concentrations in the same eggs when DDT could be detected. This suggests that the primary route for DDE contamination of Saltwater Crocodile eggs in Australia is via maternal consumption of DDE contaminated prey rather than by ingestion of prey which is primarily contaminated by DDT, and this is subsequently metabolised within the mother to DDE. The DDE in the environment arises from bacterial and other metabolism of DDT released some time in the past and the results imply that there are few if any actual fresh DDT releases. The highest concentrations of DDE were found in eggs from the Fitzroy River system downstream of several irrigated agricultural areas, the DDE was most likely transported attached to soil particles from these areas.

There appears to be a high correlation of DDE concentration in eggs sampled from the same nest. Further work to verify this may lead to some confidence in sampling a single egg from an individual nest leading to more efficient survey strategies in the future.

A survey of the global distribution of POPs (Smith 1999) found that Australian butter (n= 9 samples) had a DDE concentration of around 12 ppb. In a similar study into DDT in human breast milk Kalantzi *et al*. (2001) found that whole breast milk from Australia (n= 14) had around 42 ppb DDE. Both studies suggest that long range atmospheric transportation of DDT and DDE could be involved in the concentration found in milk fat. Long range transport and deposition from other countries where DDT is still in use may be a source for DDE in river sediments via fluvial concentration in large catchments similar to the Fitzroy basin.

Therefore there may be two separate sources for DDE contamination in *C. porosus* eggs, a supply from historical use in the catchment via agriculture and grazing, and another via aerial deposition originating from other countries. We suggest that further work is required concerning contaminants in nonviable crocodile eggs in tropical Australia. A more general sampling approach aimed at assessing levels over the entire range for *C. porosus* and *C. johnstoni* (freshwater crocodile) in Australia will provide valuable information on the bioaccumulation of persistent organic pollutants in the environment. It would also be useful to develop an international strategy for sampling various crocodilian eggs on a global scale using common protocols. This would enable comparisons to be made between geographically isolated populations in regard to persistent pollutant exposure and bioaccumulation. The information gained in such an exercise may be important for crocodilian conservation in the future and for ecotoxicology in general.

Acknowledgements

We gratefully acknowledge the assistance of Queensland National Parks and Wildlife officers in Rockhampton and Shute Harbour in securing the various permits required for the collection of eggs. We would also like to thank the staff of Queensland Health Scientific Services Laboratories in Brisbane for their assistance and professional service.

Literature

Best, S.M. (1973). Some organochlorine pesticide residues in wildlife of the Northern Territory, Australia, 1970-71. Australian Journal of Biological Sciences 26: 1161-1170.

Canas, J.E. and Anderson, T.E. (2002). Organochlorine contaminants in eggs: influence of contaminated nest material. Chemosphere 47: 585-589.

Delany, M.F., Bell, J.U. and Sundlof, S.F. (1988). Concentrations of contaminants in muscle of the American alligator in Florida. Journal of Wildlife Diseases 24: 62-66.

Guillette, L.J. Jnr., Pickford, D.B., Cain, D.A., Rooney, A.A. and Percival, H.F. (1996). Reduction in penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment. General and Comparative Endocrinology 101: 32-42.

Guillette, L.J. Jnr., Crain, D.A., Kools, M.P., Milnes, M.R., Orlando, E.F., Rooney, A.A. and Woodward, A.R. (2000). Alligators and endocrine disrupting contaminants: A current perspective. American Zoologist 40: 438-452.

Heinz, G.H., Percval, H.F. and Woodward, A.R. (1991). Contaminants in American alligator eggs from Lakes Apopka, Griffin, and Okeechobee, Florida. Environmental Monitoring and Assessment 16: 277-285.

Kalantzi, O.I., Alcock, R.E., Johnston, P.A., Santillo, D., Stringer, R.L., Thomas, G.O. and Jones K.C. (2001). The global distribution of PCBs and organochlorine pesticides in butter. Journal of Environmental Science and Technology 35(6): 1013-1018.

Lind, P.M., Milnes, M.R., Lundberg, R.D., Bermudez, Orberg, J. and Guillette, L.J. Jr. (2004). Abnormal bone composition in female juvenile American Alligators from a pesticide-polluted lake (Lake Apopka, Florida). Environmental Health Perspectives 112(3): 359-362.

Matter, J.M., Crain, D.A., Sills-McMurray, C., Pickford, D.B., Rainwater, T.R., Reynolds, K.D., Rooney, A.A., Dickerson, R.L. and Guillette, L.J. Jnr. (1998). Effects of endocrine disrupting contaminants in reptiles: alligators. *In* Principles and Processes for Evaluating Endocrine Disruption in Wildlife, ed. by R.J. Kendall, R.L. Dickerson, Giesy, J.P. and W.K. Suk. SETAC Press: Pensacola, Florida.

Matthiessen, P., Fox, P.J., Douthewaite, R.J. and Wood, A.B. (1982). Accumulation of endosulfan residues in fish and their predators after aerial spraying for the control of tsetse fly in Botswana. Pesticide Science 13: 39-48.

Phelps, R.J., Focardi, S., Fossi, C., Leonzio, C. and Renzoni, A. (1986). Chlorinated hydrocarbons and heavy metals in crocodile eggs from Zimbabwe. Transactions of the Zimbabwe Scientific Association 63: 8-15.

Phelps, R.J., Toet, M. and Hutton, J.M. (1989). DDT residues in the fat of crocodiles from Lake Kariba, Zimbabwe. Transactions of the Zimbabwe Scientific Association 64: 9-14.

Schenck, F.J. and Donoghue, D.J. (2000). Determination of organochlorine and organophosphorus residues in eggs using a solid phase extraction cleanup. Journal of Agricultural and Food Chemistry 48: 6412-6415.

Skaare, J.U., Ingebrigtsen, K., Aulie, A. and Kanui, T.I. (1991). Organochlorines in crocodile eggs from Kenya. Bulletin of Environmental Contamination and Toxicology 47: 126-130.

Smith, D. (1999). Worldwide trends in DDT levels in human breast milk. International Journal of Epidemiology 28: 179-188.

Vermeer, K., Risebrough, R.W., Spaans, A.L. and Reynolds, L.M. (1974). Pesticide effects on fishes and birds in rice fields of Surinam, South America. Environmental Pollution 7: 217-236.

Wessels, C.L., Tannock, J., Blake, D. and Phelps, R.J. (1980). Chlorinated hydrocarbon insecticide residues in *Crocodilus niloticus* Laurentius eggs from Lake Kariba. Transactions of the Zimbabwe Scientific Association 60: 11-17.

Wheeler, W.B., Jouvenaz, D.P., Wojick, D.P., Banks, W.A., van Middelem, C.H., Lofgren, C.S., Nesbit, S., Williams, L. and Brown, R. (1977). Mirex residues in nontarget organisms after application of 10-5 bait for fire ant control, northeast Florida 1972-74. Pesticides Monitoring Journal 11: 146-156.

Wu, T.H., Rainwater, T.R., Platt, S.G., McMurry, S.T. and Anderson, T.A. (2000a). Organochlorine contaminants in Morelet's crocodile (*Crocodylus moreletii*) eggs from Belize. Chemosphere 40: 671-678.

Wu, T.H., Rainwater, T.R., Platt, S.G., McMurry, S.T. and Anderson T.A. (2000b). DDE in eggs of two crocodile species from Belize. Journal of Agricultural and Food Chemistry 48: 6416-6420.

Population Status and Future Management of *Crocodylus niloticus* (Nile Crocodile) at Lake Sibaya, South Africa

Xander Combrink[1], J. Perran Ross[2] and Jan L. Korrûbel[3]

[1]Ezemvelo KZN Wildlife, Private bag X1, St Lucia, 3936, and Centre for Environment and Development, University of KwaZulu-Natal, South Africa; [2]Florida Museum of Natural History, University of Florida, Box 117800, Gainesville, FL 32611, USA; [3]Centre for Environment and Development, University of KwaZulu-Natal, Pvt. Bag X01, Scottsville 3209, Pietermaritzburg, South Africa

Introduction

The Greater St Lucia Wetland Park (GSLWP) is one of the most important areas in South Africa for the conservation of *Crocodylus niloticus*. The largest freshwater system within the GSLWP, Lake Sibaya, hosts the second largest crocodile population in the Park. Research from the late 1950s suggested that crocodiles were abundant at Lake Sibaya; yet only 67 were counted during the first aerial survey in 1985. Subsequent aerial surveys indicated an increase in the population during the following five years, with some 107 crocodiles counted in 1990. Concerns over the current population status, breeding component and future of crocodiles at Lake Sibaya, led to aerial and spotlight counts, nesting surveys and the identification of potential breeding areas from February 2003 to January 2004.

Study Area

Lake Sibaya, South Africa's largest freshwater lake is located between Kosi Bay and the St Lucia estuary, on the seaward margin of the low-lying Moçambique Coastal Plain (refer to Figure 1). It also forms part of the Greater St Lucia Wetland Park, South Africa's first World Heritage Site. The area is a transitional zone between a tropical and sub-tropical climate, forming the southernmost limit for a number of tropical species, resulting in an area rich in biological diversity.

Methodology

- Literature review of crocodilian survey and monitoring techniques
- Aerial surveys (microlight)
- Spotlight surveys (boat and foot)
- Nesting surveys for the 2002/3 and 2003/4 breeding seasons
- Identification of potential nesting areas

Results

Figure 2 indicates the aerial (1985-2003) and spotlight (2003) counts for the total number of crocodiles counted at Lake Sibaya and surrounding wetlands. The combination of clear water with sparsely vegetated and exposed shorelines, favours aerial surveys for large parts of the lake, although most of the channels with dense reed beds are subjected to high levels of visibility bias if only surveyed from the air. This is reflected in the spotlight counts, which resulted in a 72% increase in the density of crocodiles (excluding hatchlings), as well as a better representation of smaller size classes. Please note that during the following years no surveys were conducted: 1989, 1991, 1992, 1994-2002.

Figure 3 indicates the decrease in the population at Lake Sibaya is accompanied by an apparent decrease in breeding success. During the 1970 nest survey, 30 crocodile nests were found. This decreased to three nests found during the 2002/3 survey and not a single nest was found during the 2003/4 survey. Please note that during the following years no surveys were conducted: 1972-1975, 1977-1985 and 1992-2002.

Figure 4 illustrates the spatial distribution of 36 crocodiles counted during the aerial survey, 65 counted during the spotlight surveys, three nests found during the 2002/3 survey (no nest were found during the 2003/4 survey) and potential nesting areas in the Lake Sibaya system. Due to the low number of crocodiles and their uneven distribution in the Lake Sibaya system, all surveys were "total surveys", covering the total 137.3 km shoreline.

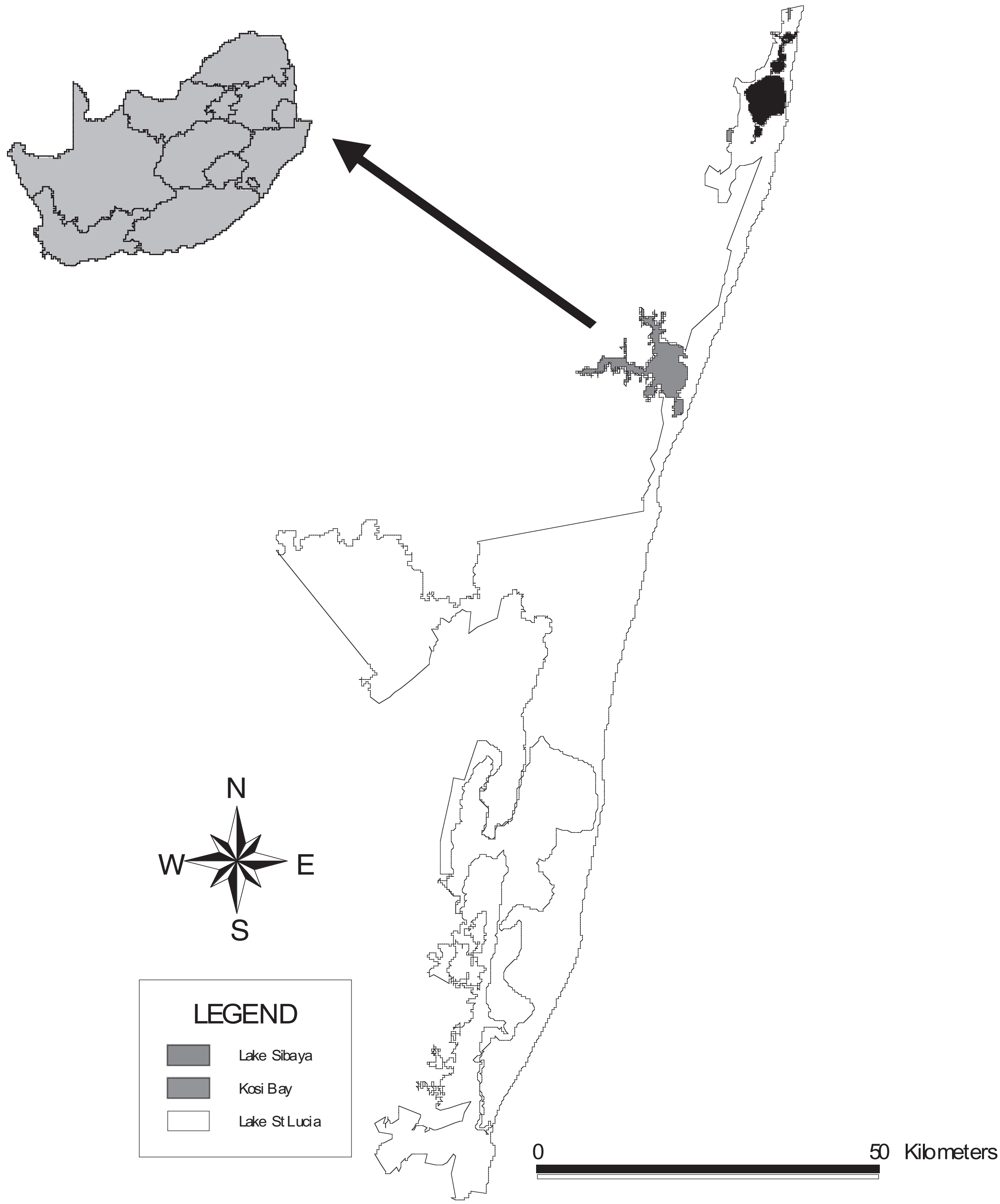

Figure 1. The Greater St Lucia Wetland Park. The Greater St Lucia Wetland Park, South Africa's first World Heritage Site, is situated in the province of KwaZulu Natal in the northeastern corner of South Africa. The Park consists of five distinct ecosystems and Lake St Lucia is also the most southern breeding population of Nile crocodiles in the world.

Discussion

The population appears to have declined significantly during the past decade with only 36 crocodiles counted during the 2003 aerial survey. The spotlight counts (65 crocodiles) reflects a 72% increase in density and reflects the importance of having a combined survey approach, since accuracy, and not precision, was the key objective of the survey. A

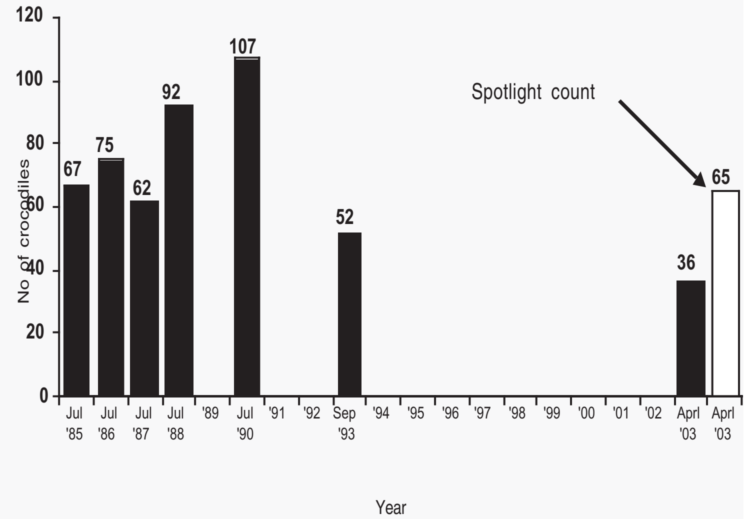

Figure 2. Aerial (1985-2003) and spotlight counts (2003) at Lake Sibaya.

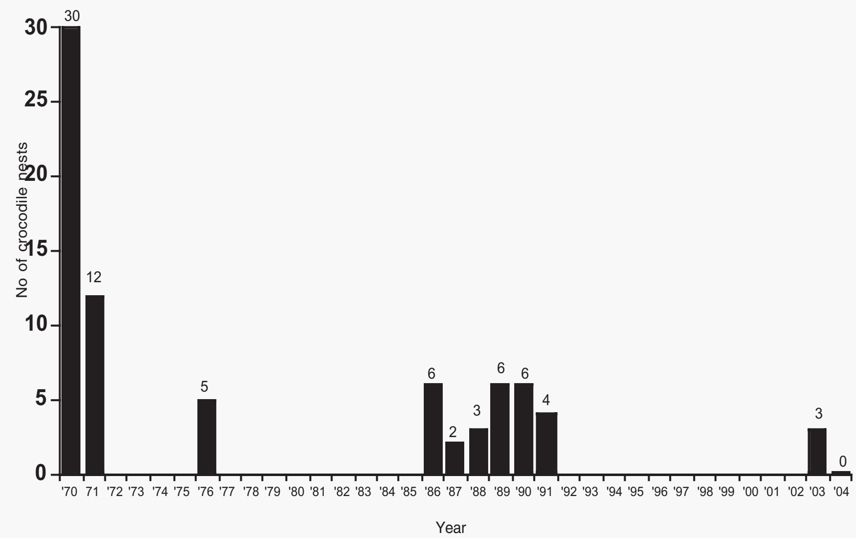

Figure 3. Nesting surveys (1974-2004) at Lake Sibaya.

correction factor of 1.72 was calculated for future aerial surveys, based on the spotlight counts. Using Magnusson *et al.* (1977) double-count technique for two independent counts (in this case aerial and spotlight), the population is estimated to be 112 crocodiles with a variance of 22.49 and standard error of 4.47.

It seems like the decrease in breeding at Lake Sibaya could be a result of high levels of disturbance by subsistence fisherman and pressure from herds of drinking cattle at historical as well as potential nesting areas. Sixty-five potential nesting areas have been identified (refer to Figure 4) and evaluated in terms of their importance both from a species (Nile Crocodile) and community perspective. These areas will form part of an overall strategy to actively conserve some key breeding areas at Lake Sibaya.

Conclusions

Despite legal protection and World Heritage Site status, the population is clearly under threat as a result of poaching and increase disturbance of nesting areas. The survival of crocodiles at Lake Sibaya might only be possible through the establishment of a joint management partnership between the conservation agency and the local community where the protection of crocodiles and nesting areas will benefit the community. The alternative is the likely extinction of this top predator from the largest freshwater ecosystem in South Africa's first World Heritage Site.

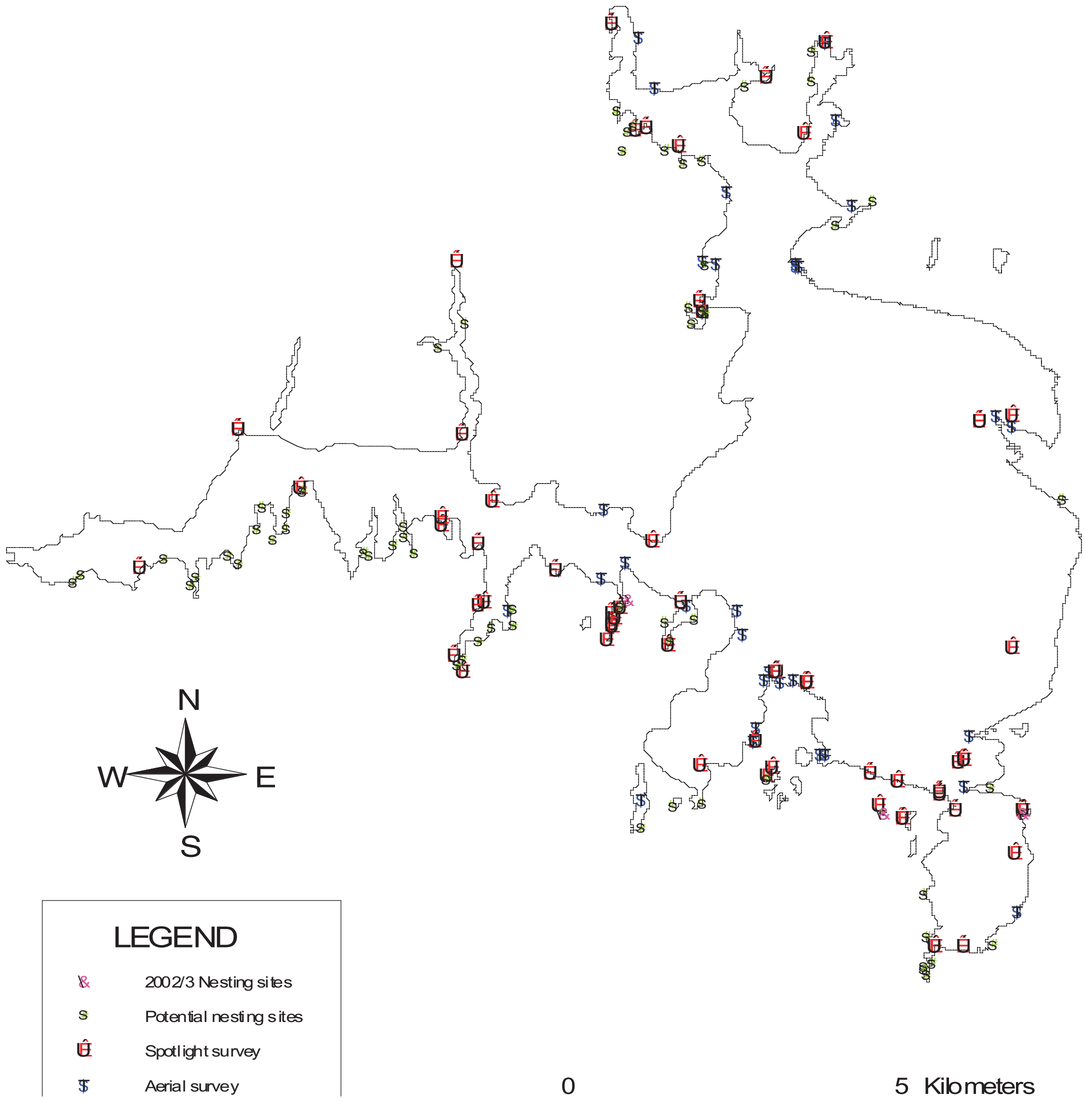

Figure 4. Spatial data (GPS) for aerial, spotlight and nest surveys at Lake Sibaya. The Lake Sibaya system is a unique independent microcosm with 18 fish, 22 frog, 8 reptile and 279 bird species recorded in the area. Numerous rare or threatened species occur in the lake system. Lake Sibaya was designated a Ramsar Wetland of International Importance in 1991 and although the area surrounding the lake is tribal land, the water surface was proclaimed a protected area in 1994 in terms of the KwaZulu Nature Conservation Act. In December 1999, the surface area of the lake was included in the formation of the Greater St Lucia Wetland Park, Word Heritage Site.

Literature

Bruton, M.N. (1979). The amphibians, reptiles, birds and mammals of Lake Sibaya. In: Lake Sibaya, ed. by B.R. Allanson. Monographiae Biologicae Vol. 36: 246-285. Dr. W. Junk bv Publishers: The Hague.

Hearne, J. and McKenzie, M. (2000). Compelling reasons for game ranching in Maputaland. Pp. 417-438 *in* Wildlife Conservation by Sustainable Use, ed. by H.T. Prins, J.G. Grootenhuis and T.T. Dolan. Kluwer Academic Publishers: Boston, Dordrecht, London.

Kyle, R. and Ward, M.C. (1995). Lake Sibaya - South Africa. Information sheet for the site designated to the list of wetlands of international importance. *In* The State of the Environment Report - South Africa.

Magnusson, W.E., Caughley, G.J. and Grigg, G.C. (1978). A double survey-estimate of population size from incomplete counts. J. Wildlife Management 42(1): 174-176.

Effects of Two Different Incubation Media on Hatching Success, Body Mass and Length in *Caiman latirostris*

Carlos I. Piña[1,2], Melina Simoncini[1] and Alejandro Larriera[1]

[1]Proyecto Yacaré. Bv. Pellegrini 3100. CP: 3000, Santa Fe, Argentina (yacare@arnet.com.ar); [2]C.I.C y T.T.P. - CONICET/Fac. Cs. y Tec., UAdER. Dr. Matteri y España. CP: 3105, Diamante, Entre Ríos, Argentina (cidcarlos@infoaire.com.ar)

Since 1990, Proyecto Yacaré (MAGIC/MUPCN) has been developing new technologies and improving/adapting old ones to reduce production costs for the ranching programs in South America. Previous works evaluated growth rates of *Caiman latirostris*, the effect of time collection on hatching success, depredation and temperature effects on incubation, but there is no information about incubation media for the species. In this experiment we tested the effects of two incubation media, vermiculite and grass ('nesting material'), on hatching success and hatchling size. We collected nine nests (350 eggs) from natural *C. latirostris* populations in Santa Fe, Argentina, soon after oviposition. In Proyecto Yacaré facilities, each nest was divided in two groups and each one received one of two incubation treatments (vermiculite or nesting material). We found no difference in hatching success among the treatments (0.89 for vermiculite and 0.87 for nesting material). We observed, but did not measure, that incubation with vermiculite tended to be longer, and that hatchlings from this treatment had more unabsorbed abdominal yolk, in all nests used in the experiment. Hatchlings from nesting material treatment were bigger than vermiculite treatment in length, but we found no differences between treatments in body mass. As we found similar hatching success in both treatments (but possibly overestimated in vermiculite), a larger size of hatchlings (which could enhance survivorship as was reported in lizards and turtles) and lower cost, nesting material is the recommended incubation media. Future investigations should address how incubation media might modify hatchling performance.

Desde 1990, e Proyecto Yacaré (MAGIC/MUPCN) está desarrollando nuevas técnicas y mejorando o adaptando otras para reducir los costos de los programas de ranching en Sur América. Trabajos previos evaluaron tasas de crecimiento, el efecto del momento de cosecha en el éxito de eclosión, la predación y el efecto de la temperatura en la incubación, pero no hay información sobre los medios de incubación para *Caiman latirostris*. En este experimento analizamos el efecto de dos medios de incubación, vermiculita y pasto en el éxito de eclosión y el tamaño de los neonatos. Cosechamos nueve nidos (350 huevos) de poblaciones naturales de *C. latirostris* en Santa Fe, Argentina luego de la postura. En las instalaciones del proyecto yacare, cada nido fue dividido en dos grupos y a cada uno se le asigno un tratamiento de incubación (vermiculita o pasto). No encontramos diferencias en el éxito de eclosión entre los tratamientos (0,89 en vermiculita y 0,87 en pasto). Observamos, pero no medimos, que la incubación con vermiculita fue más larga, y que los pichones de este tratamiento tenían más yema sin absorber en todos los nidos. Los pichones del tratamiento de pasto fueron más largos pero del mismo peso. Debido a que encontramos un éxito de eclosión similar entre ambos tratamientos (pero posiblemente sobre estimado para la vermiculita), un tamaño mayor de los pichones (que puede mejorar la supervivencia tal cual se reportó para lagartos y tortugas) y el menor costo, el pasto es el medio de incubación recomendado. Próximas investigaciones deberían estudiar como puede modificar el medio de incubación el crecimiento y la supervivencia de los neonatos.

Size of *Caiman latirostris* Eggs and Hatchling Body Size Coming from Three Different Environments in Santa Fe, Argentina

J.P. Montini[1], Alejandro Larriera[1], Carlos I. Piña[1, 2] and Pablo Siroski[1]

[1]Proyecto Yacaré. Bv. Pellegrini 3100. CP: 3000, Santa Fe, Argentina (yacare@arnet.com.ar); [2]C.I.C y T.T.P.–CONICET/Fac. Cs. y Tec., UAdER. Dr. Matteri y España. CP: 3105, Diamante, Entre Ríos, Argentina (cidcarlos@infoaire.com.ar)

It is known that *Caiman latirostris* (n.v. broad snouted caiman) has a great capacity to make use of different environments for nesting; but females could select microhabitats, potentially affecting hatchlings sex ratios. The factors that would lead females to make that selection are unknown. Regarding this aspect, it was proposed as the objective of this work to evaluate the reproductive parameters related to egg size and hatchling size in three different environments (forest, floating vegetation and savannah) in Santa Fe Province (working area includes San Cristóbal and San Javier Districts). Eggs were harvested during the 2001-02 season and incubated until hatching in the Proyecto Yacaré incubator. Based on our results, we discarded that all reproductive variables be different among the environments. Only egg width and hatchling SVL (both related to female body size) were different among environments. Hatchling body mass in the three environments was always better associated to egg volume than to length. Finally, we could infer that floating vegetation is the environment that has the best performance.

Según trabajos previos, *Caiman latirostris* (n.v. yacaré overo) posee una gran capacidad para utilizar diversos ambientes para nidificar. Recientemente se ha planteado la hipótesis que las hembras de *Caiman latirostris* podrían seleccionar los micro-hábitats, afectando potencialmente la proporción de sexos de los pichones; pero los factores que conducirían a las hembras a dicha selección son desconocidos. En este aspecto; nuestro trabajo tuvo como objetivo específico evaluar parámetros reproductivos relacionados al tamaño de los huevos y tamaño de los pichones de yacaré overo en tres ambientes (sabana, embalsado y bosque) de la provincia de Santa Fe. Los nidos recolectados para tal fin provienen de áreas de muestreo relevadas por el equipo del Proyecto Yacaré en los departamentos de San Javier y San Cristóbal, durante la temporada 2001-02. A partir de los resultados obtenidos, se descarta que todas las variables reproductivas de esta especie sean diferentes entre los ambientes, ya que de todas las ellas, solamente el ancho de los huevos y la longitud desde el hocico hasta la cloaca en los pichones (ambas variables asociadas al tamaño de la hembra), registraron diferencias entre los mismos, demostrando un efecto del ambiente sobre dichas variables. Por otra parte, en los tres ambientes el peso de los pichones estuvo siempre mejor asociado al volumen de los huevos que la longitud. Finalmente, de acuerdo a los resultados, el embalsado resultó ser el ambiente de mejor performance.

Food Conversion Rates of *Caiman latirostris* Reared at Two Different Temperatures

V. Parachú[1], Carlos I. Piña[1,2] and Alejandro Larriera[1]

[1]Proyecto Yacaré (MAGIC/MUPCN), Bv. Pellegrini 3100, (3000) Santa Fe, Argentina (yacare@arnet.com.ar); [2]CIC y TTP-CONICET, Proyecto Yacaré, Fac. de Cs. y Tec., UAdER. Dr. Matteri y España, 3105, Diamante, Entre Ríos, Argentina (cidcarlos@infoaire.com.ar)

Temperature and diet have influence over growth of caimans and crocodilians. Animals maintained at higher temperatures obtained a greater growth and increase of the real food consumption. The objective of this work is to evaluate the efficiency of food conversion in *Caiman latirostris* at two different temperatures: 29°C and 33°C. In the experiment we used 68 animals from four different nests, of two month of age, bred in captivity. All the treatments were kept under similar conditions of captivity, but temperature. We found differences in body mass and total length between groups, being bigger those maintained at higher temperature. The greater growth of animals raised at 33°C was probably due to the relationship between increasing temperature and its consequently reduction of time to process the food, promoting repeated food ingestions. Also, we found a more efficient nutritional conversion rate in animals kept at 33°C. Lang (1987) reported that the increase of temperature did not increase the amount of energy extracted from food. Nevertheless, that is a possible explication of our results.

Gender Discrimination in Hatchling Broad-Snouted Caiman (*Caiman latirostris*): an Alternative Technique

Carlos Piña[1,2], Alejandro Larriera[1], Pablo Siroski[1] and Luciano M. Verdade[3]

[1]Proyecto Yacaré, Bv. Pellegrini 3100, (3000) Santa Fe, Argentina (yacare@arnet.com.ar);
[2]FCyT/UAdER; CIC y TTP – CONICET. Proyecto Yacaré, Dr. Matteri y España, (3105) Diamante, Entre Ríos, Argentina(cidcarlos@infoaire.com.ar);
[3]Laboratório de Ecologia Animal /LEA/ESALQ, Universidade de São Paulo Caixa, Postal 09, Piracicaba, SP 13418-900, Brazil (lmv@esalq.usp.br)

Broad-snouted caiman (*Caiman latirostris*) hatchlings present a consistent sexual dimorphism in their cranial form not only size. Hatchling males present smaller cranium than females. Using multivariate statistical analyses it is possible to discriminate sex in broad-snouted caiman hatchlings by their cranial form with a relatively high efficiency. Sex discrimination of crocodilian hatchlings might possibly be improved by experimental manipulation of both genetic and phenotypic variables such as incubation environment and genealogy.

Los neonatos de yacaré overo (*Caiman latirostris*) presentan dimorfismo sexual en la forma del cráneo y en su tamaño. Con el uso de técnicas multivariadas es posible discriminar el sexo de los pichones de yacaré overo, basado en su forma craneal con una aceptable eficiencia. Los pichones macho tienen un cráneo menor que las hembras. La discriminación sexual de los pichones de cocodrilos podría mejorarse por la manipulación experimental de variables genéticas y fenotípicas tales como el ambiente de incubación y la genealogía.

The Effect of Three Different Experimental Salinity Levels on *Caiman latirostris* Growth

Luciana Santucho[1], Alba Imhof[1,2] and Alejandro Larriera[1]

[1]Proyecto Yacaré, Pje. Pvdo. 4455, Santa Fe 3000, Argentina (yacare@arnet.com.ar);
[2]Facultad de Humanidades y Ciencias, UNL, Santa Fe, Argentina

In this experiment we evaluated the effect of three different salinity levels (0, 7 and 20 ppt marine salt) on the growth of 36 *Caiman latirostris* hatchlings from three different nests, over a period of 52 days. All treatments were kept under similar conditions of captivity, except for salinity.

We found differences in food consumption, growth, fitness and survivorship between treatments. At 20 ppt salinity, no food was taken by the animals, there was no growth and mortality rate was 33%. At 7 ppt survivorship was 100%, and bodyweight was similar to the animals kept at 0 ppt, but the fitness and the morphology of the animals was affected because they have showed evidence of subcutaneous oedema.

Growth of *Caiman latirostris* Reared at Three Different Stocking Densities

Gisela Poletta[1, 2], Pablo Siroski[1] and Alejandro Larriera[1]

**[1]Proyecto Yacaré, Aristóbulo del Valle 8700, (3000) Santa Fe, Argentina (gisepoletta@hotmail.com);
[2]Facultad de Humanidades y Ciencias (UNL)-Ciudad Universitaria, Paraje "El Pozo",
(3000) Santa Fe, Argentina**

In this study we have evaluated the effects produced by stocking density over growth of juveniles *Caiman latirostris*. Thirty-six captive-reared juvenile caimans were used in the experiment. They were randomly assigned to three different treatments consisting on stocking densities of 0.12, 0.06 and 0.04 m^2/individual that were maintained in environmentally controlled chambers for 3 months. The juveniles were weighed and measured at the beginning and at the end of the experiment to determine the increase in growth for each group. The results showed that caimans maintained at the lower and intermediate stocking densities gained more weight and showed a greater increase in total length than those of the higher stocking density group. The results obtained indicated that crowding of juvenile caimans inhibits optimum growth. These data agree with results found in similar studies for other crocodilians.

Population Genetic Analysis of *Caiman latirostris* in Santa Fe Province, Argentina, by RAPD

Patricia Amavet[1, 2], E. Rosso[1], P. Siroski[2] and Rosa Markariani[1]

[1]Universidad Nacional del Litoral, Ciudad Universitaria, Paraje "El Pozo" (3000) Santa Fe, Argentina (pamavet@arnet.com.ar);
[2]Proyecto Yacaré, Arist. del Valle 8700, (3000) Santa Fe, Argentina (latirostris@arnet.com.ar)

Abstract

Caiman latirostris is a species scarcely studied to genetic level in our country, which is essential to complement strategies for management and conservation. In the present paper the preliminary results of a population analysis performed in Santa Fe province are described. Random Amplified Polymorphic DNA markers (RAPD) are used in order to determine variability and genetic structure of 3 distant wild populations. Genomic DNA isolation of 31 individuals were performed by a method that does not require the use of proteinase K, cheaper and effective in its results.

At the moment, 20 primers were tested, out of which 8 have been selected for analysis. All samples of DNA have been amplified with these primers, and amplification products from one population were analyzed on agarose gels. Registered results show that all primers examined produced different RAPD fragment patterns and could find no differences between banding patterns of individuals of the same population.

Introduction

Wild populations of Santa Fe Province of *Caiman latirostris* (broad-snouted caiman) are subjects of management of the Proyecto Yacare (MAGIC-MUPCN) program, whose object is the sustainable use of this species, together with its habitat conservation. Since 1997, the *Caiman latirostris* population of Santa Fe is listed in Appendix II of CITES due to an obvious numeric recovery, for which the regulated trade is allowed (Larriera 1998), and is turned into an important species at both national and international levels.

Particularly in crocodilians (Forstner and Forstner 2002), molecular methods apported valuable data about reproductive mechanisms, gene flow, population effective size, geographic distribution, and genetic variability measures, essential to complement management strategies. More important molecular studies in crocodilians implicate proteins analysis (Gartside *et al.* 1976; Lawson *et al.* 1989), DNA mitochondrial (Densmore and White 1991; Janke and Arnason 1997; Glenn *et al*. 2002; Ray and Densmore 2002), and microsatellites in wild populations (Davis *et al.* 2000, 2001, 2002; Glenn *et al.* 1998, 2002), existing limited antecedents of the application of the technique chosen for the present work (Glenn *et al*. 1998; Wu *et al.* 2002, in genus *Alligator*).

Population genetic studies in broad snouted caiman using microsatellites (Verdade *et al.* 2002; Zucoloto *et al.* 2002) have started in Brazil, but in our country we do not have data about variability neither about structure in wild populations of *Caiman latirostris* due to the little previous work at the genetic level. One of them is a comparative analysis of the karyotypic structure between *Caiman latirostris* and *C. yacare*. Results indicate similarities between them, with a diploid number of 42 chromosomes, with equal karyotypic structure and two microchromosomes. Both lack sexual chromosomes and show very similar C and NOR banding patterns (Amavet *et al.* 2000, 2002, 2003). Due to the remarkable similarities found, added to the absence of data in relation to their population structure, the present study intends to gather some data about the genetic structure of wild populations of *Caiman latirostris* in our country.

In addition, an alternative technique for DNA isolation is described, that implies lower costs and a higher biosecurity in relation to traditional methods, equally achieving a considerable concentration of DNA for further analysis by RAPD.

Materials and Methods

Sixty samples were processed. They were taken from 31 individuals of *Caiman latirostris* (19 females and 12 males) coming from of 3 distant populations of Santa Fe province: San Justo State (S 30° 43' 587'', W 60° 17' 458''), San Cristóbal State (S 29° 42' 844'', W 60° 50' 404'') and San Javier State (S 30° 03' 341'', W 59° 58' 746'').

Entire blood samples are used, obtained by puncture of intern jugular vein at the level of the cervical vertebrae (Tourn *et al.* 1993) with EDTA as anticoagulant. The technique used for DNA isolation (modified by Murray and Thompson 1980) consisting in the following steps: Suspend 500 mL entire blood in 3 mL TE (10 mM Tris-HCl, pH 7.5, 10mM Na_2EDTA 2 H_2O). Wash 3 times. Eliminate the last wash's supernatant. Add extraction solution (CTAB-NaCl-Mercaptoethanol-EDTA-Tris HCl). Homogenize and then carry to thermostatized bath to 60ºC for 3 or 4 hours. Wash 3 times in chloroform. Precipitate the last supernatant with isopropyl alcohol. Eliminate the alcohol by means to vaporization. Hydrate in 1 mL sterile double distilled water.

A set of 20 decamer primers from Promega - (Nº Cat B050-10 and B051-10) were tested in this study. Then 8 primers were selected taking into account which of them showed a band mean number (between 5 and 10 bands). The samples of all individuals of three populations were amplified with these primers (Table 1).

Table 1. Sequence and Promega codes of the random primers selected to study variation in *Caiman latirostris*.

Selected Primers Codes	Sequence (5'to 3')
A01	CCC AAG GTC C
A02	GGT GCG GGA A
A03	AAG ACC CCT C
A05	CAC CAG GTG A
A06	GAG TCT CAG G
B04	TGC CAT CAG T
B05	GCG CTC ACG C
B07	AGA TCG AGC C

Amplification reactions are carried out in a final volume of 25 mL with 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl2, 0.01 per cent Gelatin, 200 pM de dATP, dTTP, dGTP and dCTP, 5 pmoles of 10-base primer, 1.25 unit *Taq* DNA polimerase (Promega, Biotec.) and 50 ng genomic DNA. A negative control containing all reagents except genomic DNA was included in each reaction.

DNA amplification was performed in a thermal cycler (PTC-100 Peltier Thermal Cycler) with a cycle program the 45 cycles of 2 min to 94°C, 1 min to 36°C and 2 min to 72°C, with a final extension to 72ºC to 10 min. Aproximately 15 mL of amplification products were separated on 2% agarose gels in TBE buffer (0.89 M Tris, 0.89 M boric acid and 0.11 M EDTA, pH 8.3). Gels were stained with ethidium bromide and photographed under UV light.

RAPD markers are dominants and their presence or absence in them is analyzed (Lynch and Milligan 1994). Genetic distances will be calculated following different measures (Nei and Li 1985; Lynch 1991; Bardakci and Skibinsi 1994).

Preliminary Results

After processing of 60 samples, genomic DNA was extracted with a mean concentration of 1.84 mg/mL and a mean purity of 1.667 (l260.0/l280.0). DNAs extract were obtained in a native, not degraded, of high molecular weight form, as the presence of band unique in a electrophoresis in agarose gels shows (Fig. 1).

In relation to band analysis, at the moment, registered results are preliminary and refer only to one population. All the primers examined produced different RAPD fragment patterns. The number of fragments generated per primer varied between five and 12. Generated bands are between 500 and 1200 bp indicate with 100 bp ladder. Electrophoresis on agarose gels of amplificated products with 8 primers shows no differences between the banding patterns of the individuals of the same population (Fig. 2).

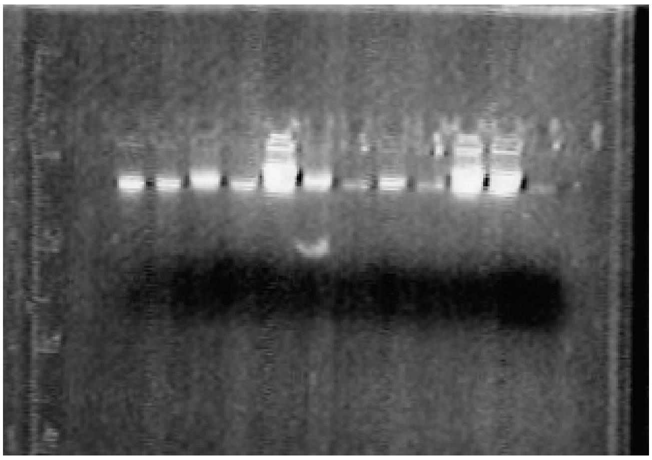

Figure 1. 12 DNA samples at different concentrations on agarose gels stained with ethidium bromide under UV light.

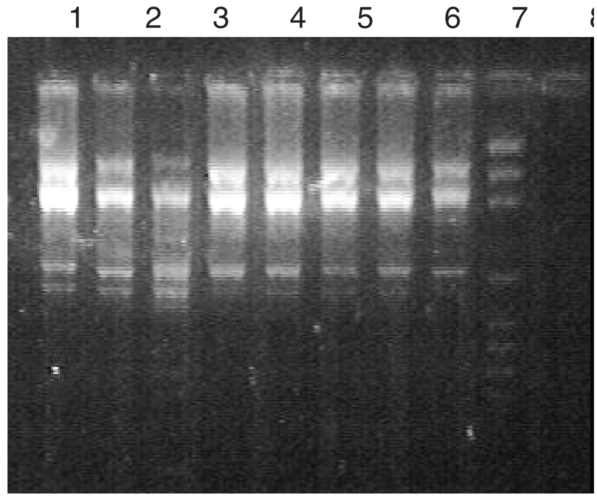

Figure 2. 1-8: Amplification products with primer A05 from 8 individuals of the same population. L: 100 bp ladder.

Discussion

The technique used for DNA isolation by CTAB has shown good results in relation to the requirements of DNA concentration for further analysis by RAPD, although the purity was not high.

For a higher purity DNA isolation precipitate again with phenol the extracts obtained allowing that material to be used for other specific analysis.

In relation to RAPD analysis, preliminary results , at the moment, allow us to assume low levels of polymorphism with low per cent of heterozigosity, in agreement with previous studies using other methods, except microsatellites (Gartside *et al.* 1976*;* Lawson *et al.* 1989; Densmore and White 1991; Dessauer *et al*. 2002). That fact was explained by bottle necks that were experimented by crocodile species, together with big size and longevity, add to the low sensivity of this technique to detect polimorphism.

After registering results of last samples, it will amplify all samples with others primers to expand series.

Acknowledgements

This study is supported by the Proyecto Nº 102, Programa Nº 14 CAI+D 2002, Universidad Nacional del Litoral and Proyecto Yacaré (MAGIC-MUPCN), Santa Fe, Argentina. The authors thank other members of Proyecto Yacaré, especially Dr. C. Piña, for their comments and suggestions. The authors are also grateful to G. Poletta and L. Lucero for their help with processing of samples.

Literature

Amavet, P., Siroski, P., Donayo, P. and Medina, M.(2000). Karyotype of *Caiman latirostris* and *Caiman yacare* (Reptilia, Alligatoridae). Pp. 135-138 *in* Crocodiles. Proceedings of the 15th Working Meeting of the IUCN-SSC Crocodile Specialist Group. Varadero, Cuba, 17-20 January 2000. IUCN: Gland, Switzerland.

Amavet, P., Markariani, R. and Siroski, P. (2002). Caracterización citogenética de *Caiman latirostris* y *Caiman yacare* (REPTILIA, ALLIGATORIDAE). Pp. 21-25 *in* Conservação e Manejo de Jacarés e Cocodrilos da America Latina - La Conservación y el Manejo de Caimanes y Cocodrilos de América Latina. Vol II, ed. by A. Larriera and L. Verdade. C.N. Editoria: Piracicaba, Brazil.

Amavet, P., Markariani, R. and Fenocchio, A. (2003). Comparative cytogenetic analysis of the South American Alligators *Caiman latirostris* and *Caiman yacare* (Reptilia, Alligatoridae) from Argentina. Caryologia 56(4): 489-493.

Bardakci, F. and Skibinsi, O.F. (1994). Application of the RAPD technique in tilapia fish: species and subspecies identification. Heredity 73: 117-123.

Davis, L.M., Glenn, T.C., Elsey, R.M., Brisbin Jr.,I.L., Rhodes, W.E., Dessauer, H.C. and Sawyer, R.H. (2000).Genetic structure of six populations of American alligators: a microsatellite analysis. Pp. 38-50 *in* Crocodilian Biology Evolution, ed. by G. Grigg, F. Seebacher. and C.E. Franklin. Surrey Beatty and Sons: Chipping Norton.

Davis, L.M., Glenn, T.C., Elsey, R.M., Dessauer, H.C. and Sawyer, R.H. (2001). Multiple paternity and mating patterns in the American alligator, *Alligator mississippiensis*. Molecular Ecology 10: 1011-1024.

Davis, L.M., Glenn, T.C., Strickland, D.C., Guillette, L.J. Jr, Elsey, R.M., Rhodes, W.E., Dessauer, H.C. and Sawyer, R.H. (2002). Microsatellite DNA analyses support an east-west phylogeographic split of American alligator populations. J. Exp. Zool. 294(4): 352-72.

Densmore, L.D. and White, P.S. (1991). The systematic and evolution of the Crocodylia as suggested by restriction endonuclease analysis of mitochondrial and nuclear ribosomal DNA. Copeia 1991(3): 602-615.

Dessauer, H.C., Glenn, T.C. and Densmore, L.D. (2002). Studies on the molecular evolution of the Crocodylia: footprints in the sands of time. J. Exp. Zool. (Mol. Dev. Evol.) 294: 302-311.

Forstner, M.R.J. and Forstner, J.M.K. (2002). La utilidad del ADN en la Conservación de los Crocodylia. Pp. 99-117 i*n* Conservação e Manejo de Jacarés e Cocodrilos da America Latina - La Conservación y el Manejo de Caimanes y Cocodrilos de América Latina. Vol II, ed. by A. Larriera and L. Verdade. C.N. Editoria: Piracicaba, Brazil.

Gartside, D.F., Dessauer, H.C. and Joanen, T. (1976). Genic homozigosity in an ancient reptile (*Alligator mississippiensis*). Biochemical Genetics 15(7/8): 655-663.

Glenn, T.C., Dessauer, H.C. and Braun, M.J. (1998). Characterization of microsatellite DNA loci in American alligators. Copeia 1998(3): 591-601.

Glenn ,T.C., Staton, J.L., Vu, A.T., Davis, L.M., Bremer, J.R., Rhodes, W.E., Brisbin, I.L. Jr and Sawyer, R.H. (2002). Low mitochondrial DNA variation among American alligators and a novel non-coding region in crocodilians. J. Exp. Zool. 294(4): 312-24.

Janke, A. and Arnason, U. (1997). The complete mitochondrial Genome of *Alligator mississippiensis* and separation between recent Archosauria (Birds and Crocodiles). Mol. Biol. Evol. 14(2): 1266-1272.

Larriera, A. (1998). The *Caiman latirostris* ranching program in Santa Fe, Argentina : The first commercial rearing 1998. Pp. 379-385 *in* Crocodiles. Proceedings of the 14th Working Meeting of the IUCN-SSC Crocodile Specialist Group, IUCN: Gland, Switzerland.

Lawson, R., Kofron, C.P. and Dessauer, H.C. (1989). Allozyme variation in a natural population of the Nile Crocodile. Amer. Zool. 29: 863-871.

Lynch, M. (1991). Analysis of population genetic structure by DNA fingerprinting. Pp. 113-126 i*n* DNA Fingerprinting Approaches and Applications, ed. by T. Burke, G. Dolf, A.J. Jeffreys and R. Wolf. Basel: Switzerland.

Lynch, M. and Milligan, B.G. (1994). Analysis of population genetic structure with RAPD markers. Molecular Ecology 3: 91-99.

Murray, M.G. and Thompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acid Research 8: 4321-4325.

Nei, M. and Li, W.H. (1985). Mathematical model for studying genetic variation in terms of restriction nucleases. Pp. 5269-5273 *in* Proceedings of the National Academy of Sciences: USA.

Ray, D.A. and Densmore, L.D. (2002). The crocodilian mitochondrial control region: General structure, conserved sequences and evolutionary implications. J. Exp. Zool. (Mol. Dev. Evol.) 294: 334–345.

Tourn, S, Imhof, A., Costa, A.L., von Finck, M.C. and Larriera, A. (1993). Colecta de sangre y procesamiento de muestras en *Caiman latirostris* (Informe de avance). Pp. 25-30 *in* Memorias del IV Workshop sobre Conservación y Manejo del yacaré overo (*Caiman latirostris*). Octubre 1993. Santa Fe: Argentina.

Verdade, L.M., Zucoloto, R.B. and Coutinho, L.L. (2002). Microgeographic variation in *Caiman latirostris*. J. Exp. Zool. 294(4): 387-396.

Wu, B-X., Wang, Y-Q., Zhou, K-Y., Zhu, W-Q., Nie, J-S., Wang, C-L. and Xie, W-S. (2002). Genetic variation in captive population of Chinese alligator, *Alligator sinensis*, revealed by random amplified polymorphic DNA (RAPD). Biological Conservation 106(3): 435-441.

Zucoloto, R.B., Verdade, L.M. and Coutinho, L.L. (2002). Microsatellite DNA library for *Caiman latirostris*. J. Exp. Zool. (Mol. Dev. Evol.) 294: 346–351.

Caiman latirostris and *Caiman yacare* Population Surveys in Formosa Province, Argentina

Pablo Siroski

Proyecto Yacaré (MAGIC-MUPCN), Aristóbulo del Valle 8700, CP: 3000, Santa Fe, Argentina (latirostris@arnet.com.ar)

Abstract

Caiman latirostris and *Caiman yacare* are two crocodilians species that inhabit Formosa Province, Argentina. These first two surveys are attempt to determine spatial distribution, relative abundance and population size of both species. These studies were carried out during spring of 2002 and 2003. Night counts showed that caiman's populations have important densities for *C. latirostris* and *C. yacare* in this province. These surveys have covered a big part of the available habitat and these indicate that populations of caimans in Formosa appear to be adequate to sustain management programs based on ranching.

Introduction

Broad-snouted caiman (*Caiman latirostris*) and yacare caiman (*Caiman yacare*) are the two crocodilians species that inhabit Argentina, and they can be easily differentiated based on cranial and nuchals structures, among others (Medem 1983). The two species are simpatric over a large part of their distribution in Argentina, but because of their more southern and more western dispersion, broad-snouted caiman appears in an allopatric way in a bigger area. Occasionally, when the two species are seen together, the broad-snouted caiman would stop occupying certain environments trying to find others (Micucci and Waller 1995); however, they are used to find themselves sharing the same bodies of water (Micucci and Waller 1995; Siroski 2003). This suggests that ecological preferences are not absolute but incidental.

Caiman latirostris prefers densely vegetated environments of difficult access and usually basks in floating vegetation, while *Caiman yacare* generally appears in environments free of floating vegetation and normally basks on beaches or coasts (Medem 1983). The presence of *Caiman latirostris* in the superior riverbanks of Iguazú River (Fabri 1998) is significant due to its the great flow.

The eastern limit for broad-snouted caiman distribution is the Atlantic Ocean, and the western limit is the Mountain range of the Andes (Medem 1983). In Argentina, broad-snouted caiman inhabits Jujuy, Salta, Formosa, Santiago del Estero, Entre Ríos, Santa Fe, Corrientes, Chaco and Misiones Provinces (Yanosky 1990); while *Caiman yacare* inhabits the same provinces except for Salta, Jujuy and Entre Ríos.

Although our country is the southern limit of dispersion for the two species. *C. latirostris* reaches a bigger latitude 32ºSL (Freiberg and Carvalho 1965) while *C. yacare* reaches 30-31ºSL (Medem 1983), many specimen have been seen in bigger latitudes due to extraordinary situations (Micucci and Waller 1995).

There is no record of commercial use of caimans in Argentina from the end of the 1980s to the beginning of the 1990s. There was a differential use between both species of caimans, due to the biggest ossification in the osteoderms that the *Caiman yacare* possesses, the most looked for one was the broad-snouted caiman because of its greater economic value.

The diversity and extension of habitats that caimans take up in this province makes it very difficult to estimate an amount that represents the whole of populations. In these cases, it is convenient to estimate the abundance or relative density, for what it is necessary to choose work places that are representative and recurring for the different areas.

The objective of this work was to evaluate the population status, distribution and structure of sizes of *Caiman* gender in the Province of Formosa, Argentina.

Biogeographical Characterization

The province of Formosa occupies a part the Chaco eco-region. This eco-region is characterized to have a vast semi-arid plain that covers around 1,200,000 km^2 of South America, of which about 800,000 km^2 belong to the Argentina and the rest is distributed among Bolivia and Paraguay. This province is characterized by the prevalence of subtropical thorny forests, humid and semi-arid savannah, being very rich in wildlife, mainly in the northwest portion where the anthropic intervention is scarce. In spite of their semi-arid conditions, the abundance of wetlands stands out with a diversity in their origins as well as in their physical and biological characteristics caused by the variability in rains year after year. It also presents a gradient for north-south temperature (the annual average varies between 19ºC and 24ºC, with maximum of up to 49ºC), and one east-west for the rain (450 to 1200 mm). The availability of habitats during the dry season varies significantly year after year, depending on the duration of the drought (Coutinho *et al*. 1995). This is of ecological importance since the aquatic habitats during the drought can affect the dynamics of wildlife population, particularly caiman populations.

The province of Formosa finds in the climatic factor a great advantage for the establishment of caiman populations. This is due chiefly to the short duration and intensity of cold months. Consecutively, *Caiman yacare* possesses a small capacity to adapt to the lowest temperatures, contrary to *Caiman latirostris*.

Methodology

A description of crocodilians population's abundance and distribution is generally the first step in this study, and it often establishes the basic information for conservation and management programs. (Bayliss 1987).

The crocodilians abundance estimate is based on density indexes, for example, the number of specimen seen per km or hectare. The importance of monitoring is to very well define the conditions with as many data as possible. Sampling places were characterized *in situ* regarding the diverse data, for example: physiognomy, dimensions, vegetable covering, type of banks and representativeness relating to other environments, inside the same bio-region, etc.

In each monitoring, different variables were registered, like: time of beginning and finalization, temperature of the air and water at the same time, cloud, wind and accessibility. Temperature was measured with a mercury bulb thermometer, which ranges from 0 to 50ºC and a 0.5ºC precision. For each studied place, indexes of relative abundance were calculated dividing the number of caimans observed by the total covered distance during the observation expressed in km.

Structure of sizes was estimated, in most of the cases, at 5 m maximum distance of each caiman. Classification of sizes was: Class I: <40 cm, Class II: 40-120 cm, Class III: 120-180 cm, and Class IV: >180 cm. In the cases where the distance was not enough to assure the classification, (EO) eyes only was reported.

Results

From 4 November to 10 November 2002 and from 1 November to 7 November 2003 reports on *Caiman latirostris* and *Caiman yacare* populations were carried out in different places of the province of Formosa. The work evaluated distribution, relative densities and structures of monitored populations. The results are shown in the table on the following page.

Discussion

During the last 20 years there has been a very important change in the relationship between conservation, exploitation and the trade. Initially, trade was seen as a problem in conservation, however, these days it seems more a solution to the conservation problem.

A pre-requirement to fulfill the successful management of wildlife is to know the relative densities or tendencies of populations (Rhodes and Wilkinson 1994). There is not much precedent about the population situation of the caiman's species in the Province of Formosa, except for the first exhaustive study carried out by Siroski (2003); that is why this type of work carried out annually constitutes a fundamental database for the handling of conservation and exploitation.

Locality	Distance Covered (km)	Species	Class I	Class II	Class III	Class IV	EO	Relative Abundance
Las Lomitas	1.8	*C. yacare*						
		C. latirostris	6	29	26	13	35	60.5
El Colorado	1.3	*C. yacare*						
		C. latirostris			3			
Pirané	3.5	*C. yacare*						
		C. latirostris			9	4	1	4
Bdo. La Estrella	2.5	*C. yacare*	2	11	13	22		*
		C. latirostris				1		
		Species?					22	
Cnia. Mte. Lindo	*2*	*C. yacare*	11	12	1	-		*
		C. latirostris						
		Species?					9	
Cnia. San Pedro	1.2	*C. yacare*						
		C. latirostris	2	22	15	11	16	55
Cnia. El Olvido	1	*C. yacare*		17	9			*
		C. latirostris						
		Species?					14	

* In the sampling places where the presence of both species was observed, relative density was not reported for each species, since animals considered as EO cannot be assigned to none of them. Relative density was not informed either in the sampling places where just one species was observed, even though eggs from the two species were afterwards harvested.

In places where *Caiman latirostris* is predominant, *Caiman yacare* was not observed, however, in other places both were observed but *Caiman yacare* was predominant. This observations show that this kind of environment is not the preferred one by this species, but it confirms the flexibility of *Caiman latirostris* to tolerate a bigger diversity of environments.

Conclusions

These are the first methodical studies of caiman populations in the province of Formosa, carried out with the standard methodology of the Crocodile Specialist Group of the World Union for Conservation (CSG/SSC/IUCN), which makes it repetible and demonstrable.

In Colonia Monte Lindo, animals of both species were observed but *C.latirostris* nests were not harvested. However, in Bañado La Estrella and Colonia El Olvido , both species were observed; and in the nesting season nests from both species were harvested. Bearing this in mind, it is clear that *Caiman latirostris* has not disappeared from none the areas that historically occupies in its distribution. Furthermore, the densities found in monitored places, thoroughly surmount the habitual average values for the species in this country. The places that broad-snouted caiman prefers to inhabit usually constitute a permanent obstacle for its study (Larriera 1992)

In environments where *Caiman latirostris* was observed, *Caiman yacaré* was not. where *C. yacare* prevailed, *C. latirostris* could be also observed. According to the data of the eggs of the 2003 and 2004 harvest, it can be established that they share nesting habitat in some places. Disturbance factors for yacare populations in the studied areas are related to habitat loss in favor of agricultural production and some isolated deaths, generally owing to hunting for survival or elimination by accident, unawareness, or fear. There is no record of commercial hunting with the purpose of using neither its skin nor its meat.

The present work was useful not only to update the knowledge of the species in the region, but also, to verify the striking interest of local residents in the utilization of the resource in a sustainable way.

In the Province of Formosa, populations of the gender *Caiman* are in a very good condition, what is shown, as well in population counts as in the increased number of nests during the 2003 and 2004 harvests.

Finally, the educational aspects of work mechanics in the face of a flawed reality in local economies make us suppose that future economic retribution for the detection of yacare nests, will constitute a significant stimulus for the appraisal of ecosystems starting from its sustainable use in the province of Formosa.

Acknowledgements

To Cesar Pérez and Oscar Ramón Candia, for their very good and constant willingness to team up. To the vet. Juan Carlos Orozco, Director of Fauna and Parks of the Province of Formosa for his open support. To "Caimanes de Formosa SRL" corporation and the Fac. de Cs. y Tec., UADER, for financing these studies and to the vet. Alejandro Larriera for his critical review of this document and to the whole Projecto Yacaré (MAGIC-MUPCN agreement).

Literature

Bayliss, P. (1987). Survey methods and monitoring within crocodile management programes. Pp. 157-175 *in* Wildlife Managment: Crocodiles and Alligators, ed. by G.J.W. Webb, S.C. Manolis and P.J. Whitehead. Surrey Beatty and Sons: Chipping Norton: Sydney.

Coutinho *et al*. (1995). Survey of broad snouted caiman C*aiman latirostris,* marsh deer *Blastocerus dichotomus* and capybara *Hidrochaerys hidrochaerys* in the area to be inundated by Porto Primavera Dam, Brazil. Biological Conservation 73: 27-31.

Fabri, S.F. and Herrera, J.R. (1998). Prospección de *Caiman latirostris* en la margen Argentina del Río Iguazú superior. Pp. 14-15 *in* Resúmenes de la XIII Reunión de Comunicaciones Herpetológicas de Argentina. Asociación Herpetológica: Argentina.

Freiberg, M.A. and Carvalho, A.L. (1965). El yacaré sudamericano *Caiman latiostris* (Daudin) Physis 25(70): 351-360.

Larriera, A., Del Barco, D., Imhof, A. and Von Fink, C. (1992). Environmental variables and its incidence on *Caiman latirostris* counts. Pp. 256-260 *in* Crocodiles. Proceedings of the 11th Working Meeting of the IUCN-SSC Crocodile Specialist Group, Vol. I. IUCN: Gland, Switzerland.

Medem, F. (1983). Los crocodylia de Sudamérica. Vol. II. Universidad nacional de Colombia. COLCIENCIAS: Bogotá, Colombia.

Micucci, P. and Waller, T. (1995). Los Yacares en Argentina: hacia un aprovechamiento sustentable. Pp. 81-112 *in* La Conservación y el Manejo de Caimanes y Cocodrilos de América Latina, ed. by A. Larriera and L.M. Verdade. Fundación Banco Bica: Santo Tomé.

Siroski, P. (2003). Relevamiento de las poblaciones de caimanes en la región centro y sureste de la provincia de Formosa 2002. 40 pp.

Rhodes, W. and Wilkinson, P. (1994). Alligator night-light surveys of impoundment habitat in coastal South Carolina - a preliminary validation. Pp. 66-73 *in* Crocodiles. Proceedings of the 12th Working Meeting of the IUCN-SSC Crocodile Specialist Group, Vol. 2. IUCN: Gland, Switzerland.

Yanosky, A.A. (1990). Histoire naturelle du Caiman á museau large (*Caiman latirostris*), un alligatoriné mal connu. Rev. Fr. Aquariol. Herpetol. 17(1): 19-31.

"Pancuronium Bromide" - an Immobilising Agent for Crocodiles

Laurelle Bates[1], Grahame Webb[2], Ken Richardson[1], Adam Britton[2], Jacob Bar-Lev[2] and Charlie Manolis[2]

[1]Division of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch, WA 6150, Australia; [2]Wildlife Management International Pty Limited, PO Box 530, Sanderson, NT 0813, Australia (corresponding author: gwebb@wmi.com.au)

Abstract

Flaxedil (gallamine triethiodide) was used extensively by crocodile farms and wildlife managers for the immobilisation of Saltwater Crocodiles (*Crocodylus porosus*). However, as it became increasingly difficult to obtain and the cost became prohibitive, alternative immobilising agents were sought. Preliminary testing suggested that Pavulon (pancuronium bromide) could be a potential replacement for Flaxedil. Extensive experimentation with juvenile *C. porosus* allowed dosage rates for Pavulon to be quantified. Dosage rates for immobilisation for 6-10 h, for large crocodiles (>2.9 m total length), were extrapolated from limited data, and continue to be tested. Nonetheless, Pavulon has proven to be a cheap, reliable immobilising agent for *C. porosus*.

Introduction

Crocodile management and research have benefited greatly from the use of injectable immobilising agents. Not only do these contribute to "safety" for handlers, they reduce stress, and risk of injury or death to the animals. A variety of drugs have been used for crocodilians, the effect of each drug on a particular species being somewhat unique in terms of dose rates, degree of immobilisation and recovery rates.

Flaxedil (gallamine triethiodide) has been the main immobilising agent used in Australia for Saltwater Crocodiles for over 30 years. It recently became unavailable in Australia, which motivated the search for an alternative agent. Pavulon (pancuronium bromide) is a closely-related drug widely used in human medicine, and preliminary testing by WMI indicated that it could be a suitable alternative. More extensive testing was then undertaken to derive dosage rates for Saltwater Crocodiles. A range of dosage rates was tested against a number of variables, including: size of crocodile, sex, bodyweight, body temperature, and degree of exercise. From this we determined induction time, duration of immobilisation, recovery time, and upper lethal dose. The physiological effects of the drug were examined, and we also tested the effectiveness of the antidote, neostigmine methylsulphate.

Both Flaxedil and Pavulon are non-depolarising neuromuscular blocking agents - they impair nerve impulses to the striated muscles, rending the animal helpless and unable to move until the drug has been metabolised. Paralysis tends to be specific to striated muscle, leaving vital functions such as ventilation and heart function relatively unaffected except at high dose rates.

Methods

Crocodiles in three size classes: juveniles (2.4-5.1 kg; <1.2 m TL), sub-adults (5.1-40 kg; 1.2-2.3 m TL) and adults (>40 kg; >2.3 m TL) were used in this study, although most testing was confined to juveniles. Dosage rates for larger animals were extrapolated from the results obtained for juveniles, and then tested on a limited sample of large crocodiles.

Pavulon's performance was evaluated through a number of experiments, including recording the time for specific responses to disappear and reappear as a measure of the level of immobilisation, assessing the influence of key variables (eg bodyweight, body temperature, exercise, sex) to understand the dynamics of immobilisation, physiological effects, and exploring upper lethal limits.

Results

Induction time: The time from injection to immobilisation (induction time) decreased with increasing dose (Fig. 1). Minimum effective dose was 0.019 mg/kg. Induction time does not decrease significantly at dose rates above 0.025 mg/kg due to limits on diffusion rate from muscle to bloodstream. The average induction time within the size range tested was 21.8 minutes.

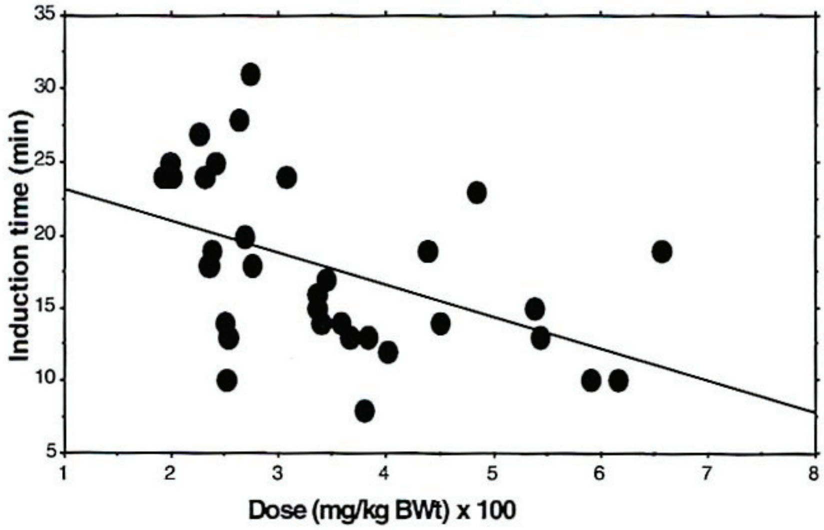

Figure 1. Relationship between dose rate and induction time for 34 *C. porosus* immobilised with Pavulon. Line shows significant linear regression (r^2= 0.23, P< 0.005.).

Duration of immobilisation: Duration of immobilisation increased at higher dose rates (Fig. 2), although both exercise and body temperature also influenced duration. Crocodiles remained immobilised for longer when exhausted prior to immobilisation, but recovered faster at higher body temperatures because the drug metabolises more rapidly.

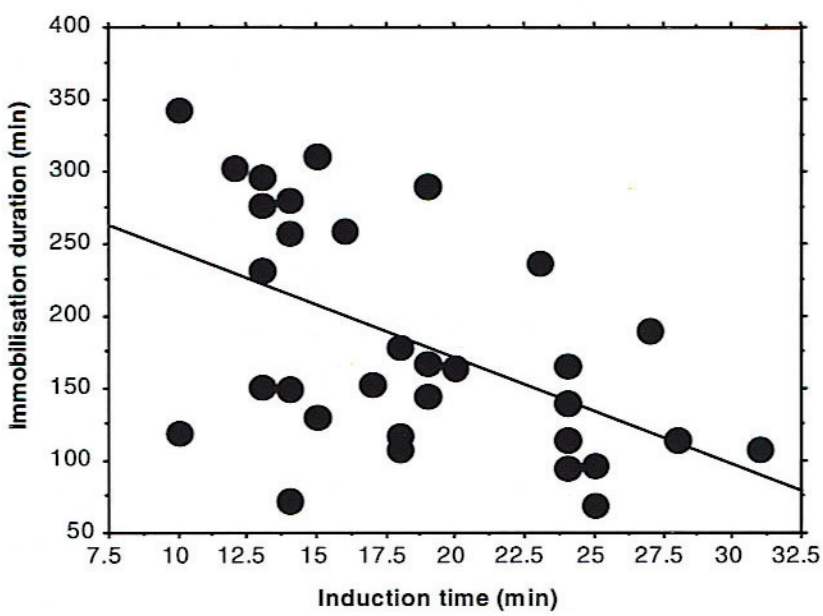

Figure 2. Relationship between dose rate and duration of immobilisation for 36 *C. porosus* immobilised with Pavulon. Closed circles = animals not exercised (N= 29); open circles = animals exercised (N= 7).

Duration of immobilisation can be predicted from induction time (Fig. 3) - crocodiles that take longer to become immobilised tend to recover more rapidly. However, once recovery had started (ie some reflexes were regained), the time taken for full recovery of all reflexes was not influenced by dose rate, sex, body weight or induction time.

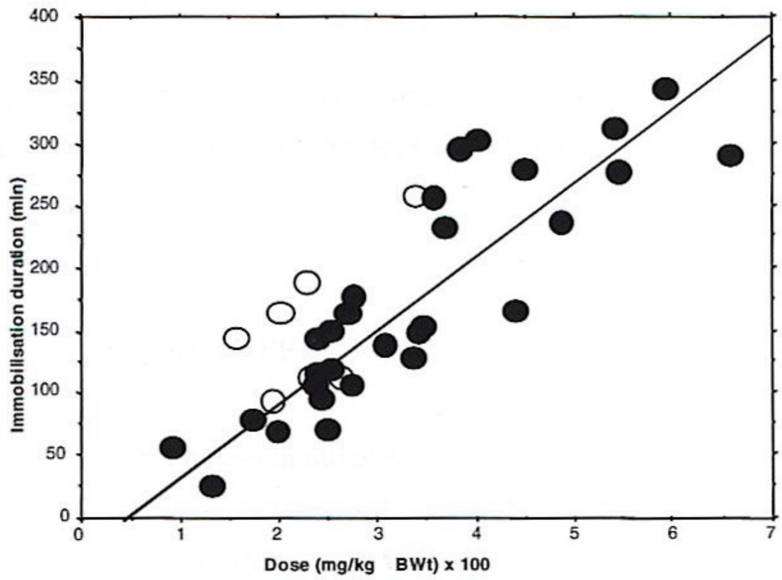

Figure 3. Relationship between dose rate and induction time for 36 *C. porosus* immobilised with Pavulon.

Antidote*:* Neostigmine methylsulphate was found to be highly effective at reversing the effects of Pavulon. Recovery time was significantly reduced (<5 minutes) in all animals following injection of >0.02 mg/kg neostigmine (Fig. 4).

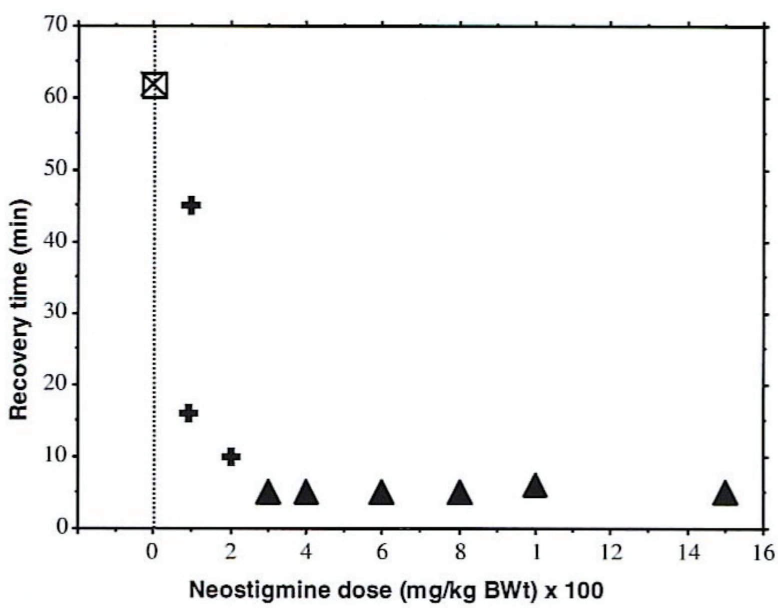

Figure 4. Relationship between neostigmine dose rate and recovery time for 10 *C. porosus* previous immobilised with 0.034 mg/kg Pavulon. Solid triangles = neostigmine dose >0.02 mg/kg (N= 6); crosses = neostigmine dose £0.02 mg/kg (N= 3); crossed box = no neostigmine.

Lethal dosage rates*:* Dosage rates greater than 0.4 mg/kg (>20 times minimum effective dose rate) proved lethal in non-exercised crocs. Dosage rates of 0.2 mg/kg were lethal in crocodiles exercised to exhaustion and in crocodiles with higher body temperatures. Even in non-exercised crocodiles, dosage rates greater than 0.2 mg/kg (>10 times minimum effective dose) could lead to respiratory distress and death unless administered with the antidote. Neostigmine can prevent mortality if administered early, but is ineffective at restoring muscular movements at high dosage rates (>0.14 mg/kg). Very rapid induction time (<10 minutes) indicates a potentially lethal dose.

Physiological effects*:* The physiological effects appear to be a 10% increase in heart rate (tachycardia; Fig. 5) and a 66% decrease in respiration rate (hypoventilation; Fig. 6) between injection and complete immobilisation. Tachycardia was not observed using Flaxedil, and appeared to be independent of the stress response to handling. Hypoventilation was greater than that observed using Flaxedil (66% instead of 50%).

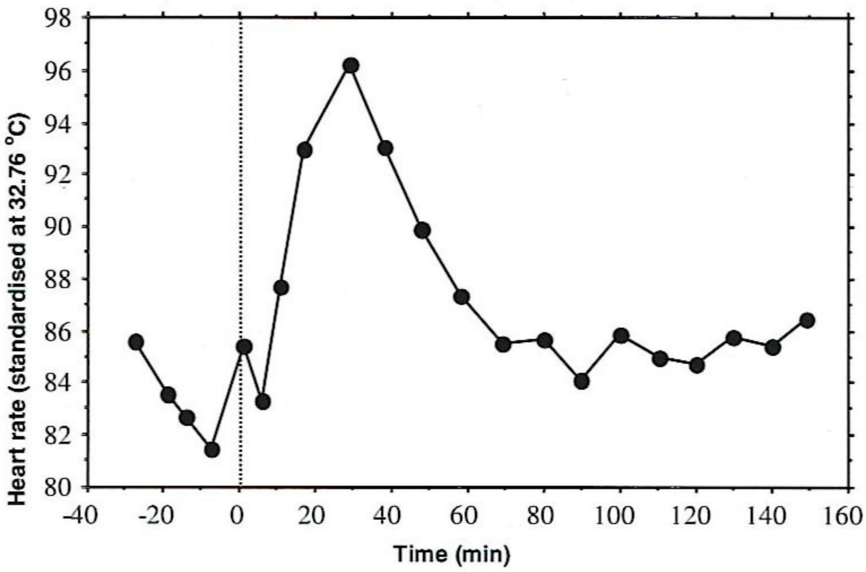

Figure 5. Apparent physiological effect of 0.034 mg/kg Pavulon on heart rate of juvenile *C. porosus* with body temperature standardised at 32.8ºC.

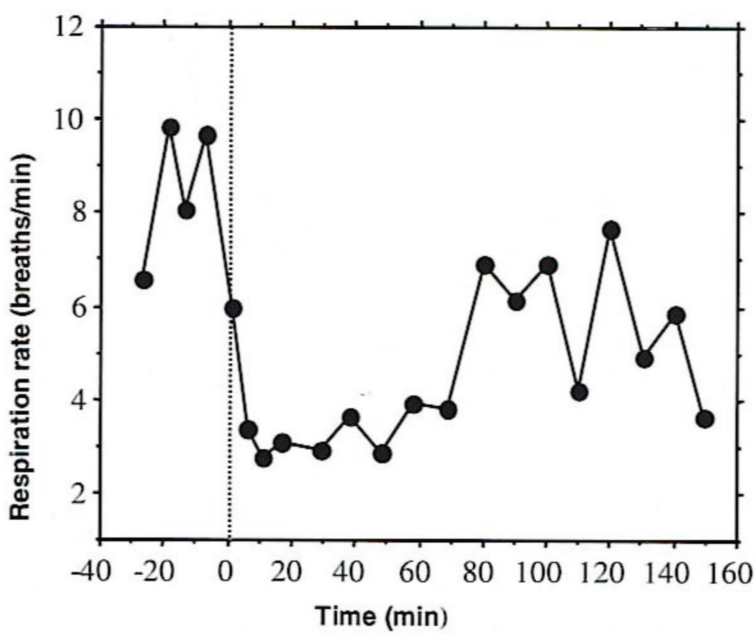

Figure 6. Apparent physiological effect of 0.034 mg/kg Pavulon on respiration rate of juvenile *C. porosus.*

Larger crocodiles*:* To test dose rates extrapolated for larger crocodiles, the predicted effects of a normal dose were compared with actual effects in 10 large *C. porosus* (12.1-240.5 kg BWt). There was a significant difference between the predicted and actual effects - induction time, immobilisation duration and recovery times were all higher than predicted from smaller crocodiles. Minimum effective dose was also predicted to be lower than 0.02 mg/kg. These effects are consistent with crocodiles of higher bodyweight taking longer to metabolise the drug.

Conclusions

These results indicate that Pavulon is an effective immobilisation agent, and a suitable replacement for Flaxedil. The effective dose rate is relatively low, and there is a considerable safety margin before lethal levels are reached. There are relatively clear indicators if dose rates are excessively high, and unless extreme there is a possibility for administering neostigmine - an effective antidote, particularly at normal dose rates. There appeared to be a change in dynamics of immobilisation between smaller and larger crocodiles, the latter's higher bodyweight and lower metabolism of the drug meaning that smaller dose rates relative to body size are required. Based on the results, provisional dosage rates of Pavulon for wild Saltwater Crocodiles were derived (Table 1). However, caution should be exercised with large crocodiles, as the dosage rates were derived from limited data.

Table 1. Provisional dosage rates of Pavulon (4 mg/2 ml) for wild *Crocodylus porosus*. Dosage rates in shaded area are extrapolations, and should be used with caution. Line beneath "total length of 2.9 m" denotes a change in dose rate. Concentration of neostigmine methylsulphate is 2.5 mg/ml.

TL (m)	BWt (kg)	Partial Immobilisation (ml)	Immobilisation 3-6 h (ml)	Immobilisation 6-10 h (ml)	Neostigmine (ml)
2.0	25	0.1-0.2	0.3	0.6	0.2
2.1	30	0.1-0.2	0.3	0.7	0.2
2.2	35	0.2-0.3	0.4	0.8	0.3
2.3	40	0.2-0.3	0.4	0.9	0.3
2.4	45	0.2-0.3	0.5	1.0	0.3
2.5	52	0.3-0.4	0.6	1.1	0.4
2.6	59	0.3-0.4	0.7	1.3	0.4
2.7	67	0.3-0.5	0.7	1.5	0.4
2.8	76	0.4-0.6	0.8	1.7	0.5
2.9	85	0.4-0.6	0.9	1.9	0.5
3.0	95	0.3-0.4	0.5-0.7	0.8-0.9	0.5-1.0
3.1	106	0.3-0.4	0.5-0.7	0.8-1.0	0.5-1.0
3.2	118	0.4-0.5	0.6-0.8	0.9-1.1	0.5-1.0
3.3	131	0.4-0.5	0.7-0.9	1.0-1.2	0.5-1.0
3.4	144	0.4-0.6	0.7-1.0	1.2-1.3	0.5-1.0
3.5	159	0.5-0.6	0.8-1.1	1.3-1.4	1.0
3.6	175	0.5-0.7	0.9-1.2	1.4-1.6	1.0
3.7	188	0.6-0.8	0.9-1.3	1.5-1.7	1.0
3.8	205	0.6-0.8	1.0-1.4	1.6-1.8	1.0
3.9	224	0.7-0.9	1.1-1.6	1.8-2.0	1.0
4.0	243	0.7-1.0	1.2-1.7	1.9-2.2	1.0
4.1	264	0.8-1.1	1.3-1.8	2.1-2.4	1.0
4.2	286	0.9-1.1	1.4-2.0	2.3-2.6	1.0
4.3	305	0.9-1.2	1.5-2.1	2.4-2.7	1.0
4.4	329	1.0-1.3	1.6-2.3	2.6-3.0	1.0
4.5	355	1.1-1.4	1.8-2.5	2.8-3.2	1.0
4.6	382	1.1-1.5	1.9-2.7	3.1-3.4	1.0
4.7	410	1.2-1.6	2.1-2.9	3.3-3.7	1.0
4.8	440	1.3-1.8	2.2-3.1	3.5-4.0	1.0
4.9	471	1.4-1.9	2.4-3.3	3.8-4.2	1.0
5.0	504	1.5-2.0	2.5-3.5	4.0-4.5	1.0
5.1	538	1.6-2.2	2.7-3.8	4.3-4.8	1.0
5.2	574	1.7-2.3	2.9-4.0	4.6-5.2	1.0
5.3	612	1.8-2.4	3.1-4.3	4.9-5.5	1.0

Study and Conservation of Black Caiman (*Melanosuchus niger*) in French Guiana: a 4-years Record

Benoit de Thoisy

Kwata NGO, Cayenne, French Guiana (thoisy@nplus.gf)

Introduction

Formerly considered as "endangered", the status of the Black Caiman (*Melanosuchus niger*) has been reevaluated recently on the basis of ecological monitoring (eg da Silveira and Thorbjarnarson 1999), and the species is now considered as "vulnerable". Nevertheless, this status remains closely dependant to conservation program at the populations levels (Hilton-Taylor 2000). The monitoring of the French Guianan population was initiated in the late 1999 (Blanc and de Thoisy 2001; de Thoisy *et al*. 2001, 2002). Since the population was formely supposed to be restricted to the Kaw Swamps area (but see de Thoisy and Auffret 2003), primary aims of the program funded by the Kaw Swamps Nature Reserve and the French Ministry of Ecology, were:

- to assess the status of the population, using both field surveys and molecular markers;
- to identify areas of major importance for the species;
- to gather new data on ecology and biology; and,
- to propose recommentations for a better management of habitat and species conservation.

Area of Study and Methods

The Kaw Swamp Nature Reserve (04°25-04°50 N, 52°00-52°15 W) comprises several habitats for caimans, including swamps, flooded forests, and mangroves. These areas are facing different levels of anthropic pressure.

The core area of the reserve: the Angélique Swamp

Due to its inaccessibility, this 80 sq. km flooded herbaceous swamp remained in a rather pristine state. Five surveys were realized in several permanent ponds.

The Kaw River

This 70 km-long river crosses the reserve. Its upper part is dominated by a herbaceous habitat, the lower part crosses a galery forest, and, closer to the sea, a mangrove. A small village is located on the edges of the river. Caimans were extensively hunted during decades, until the complete protection of the species by Ministerial Decree in 1986. To date poaching still occurs, although at a low scale. The area is exploited by unmanaged tourism, cattle ranching, and fishing. Count surveys were undertaken monthly since 4 years; as far as possible animals were marked for capture/recapture (C/R) purposes, as well as for the genetic study.

Approuague Estuary

This mangrove habitat has been identified as a important nesting site. The area was chosen for study of the growth and dispersal of youngs using C/R method. Also, a genetic approach was developed, using 2 distinct molecular markers: nuclear microsatellite DNA, and mitochondrial cytochrome-b in collaboration with Izeni Farias from the University of Manaus.

Results

Abundances on the Kaw River

Three species are present: the Dwarf Caiman *Paleosuchus palpebrosus*, the Black Caiman *Melanosuchus niger*, and the spectacled caiman *Caiman crocodilus*. A fourth species, the smooth-fronted caiman *P. trigonatus*, is recorded in forest tributaries, but not in the main river course.

The abundance of the Dwarf caiman ranged from 2.8 to 5.6 individuals per 10 km. Whatever the season and the water level, the Dwarf caiman was more present in the gallery forest area, where 67 to 100% of monthly sightings were recorded.

The kilometric index of both Black and Spectacled caimans are shown on Figure 1. The decrease of the abundance of the Black caiman is significant since 4 years, and the increase of the Spectacled caiman could be an ecological response to this feature (Herron 1991, 1994). Aditionnally to very low KIs, Black caimans on the river were only young animals; alternatively the size structure recorded in the Angélique Swamps reflects a healthy population. Also, hatchlings and nesting activities were not recorded on the Kaw River. Nevertheless, large animals, although still poorly represented, were recorded at a higher occurrence during the last year of survey, and may be considered as an encouraging feature (Fig. 2). The Black caiman marks a significant preference for forest habitats (40% of records located in the gallery forest area, 18% in the mangrove area, vs. 42% in savannas); the Spectacled caiman used preferably the savanna area (94% of sightings).

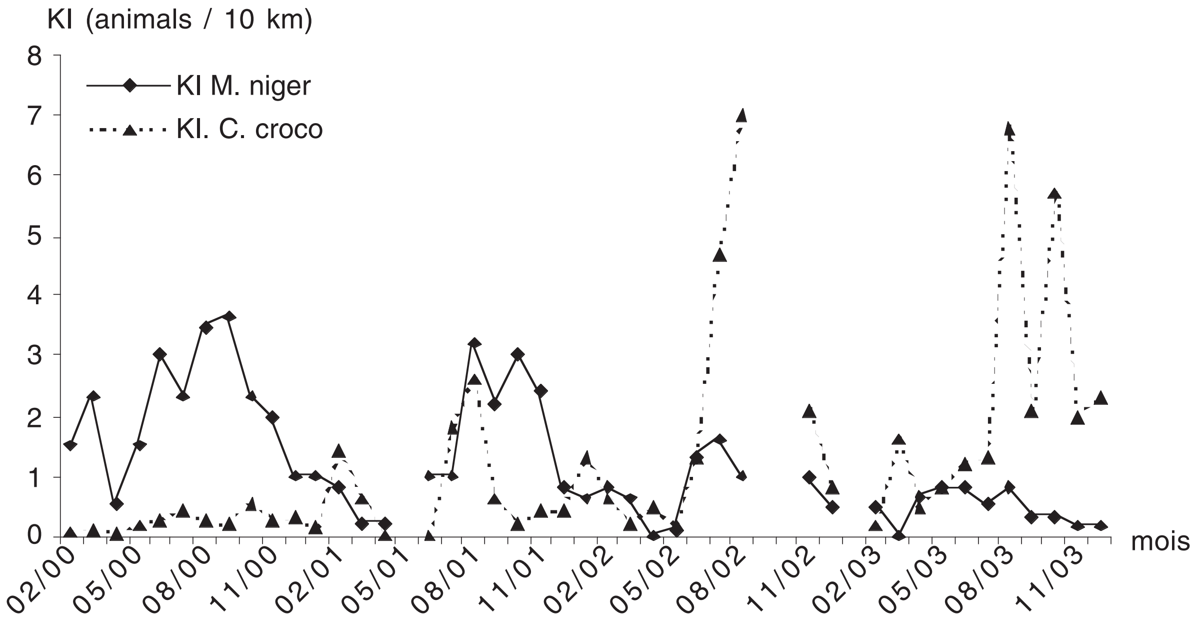

Figure 1. Evolution of monthly kilometric index (KI) of Black caiman *Melanosuchus niger* and Spectacled caiman *Caiman crocodilus* on the Kaw River, Kaw Swamps Nature Reserve, French Guiana. Period 2000-2003.

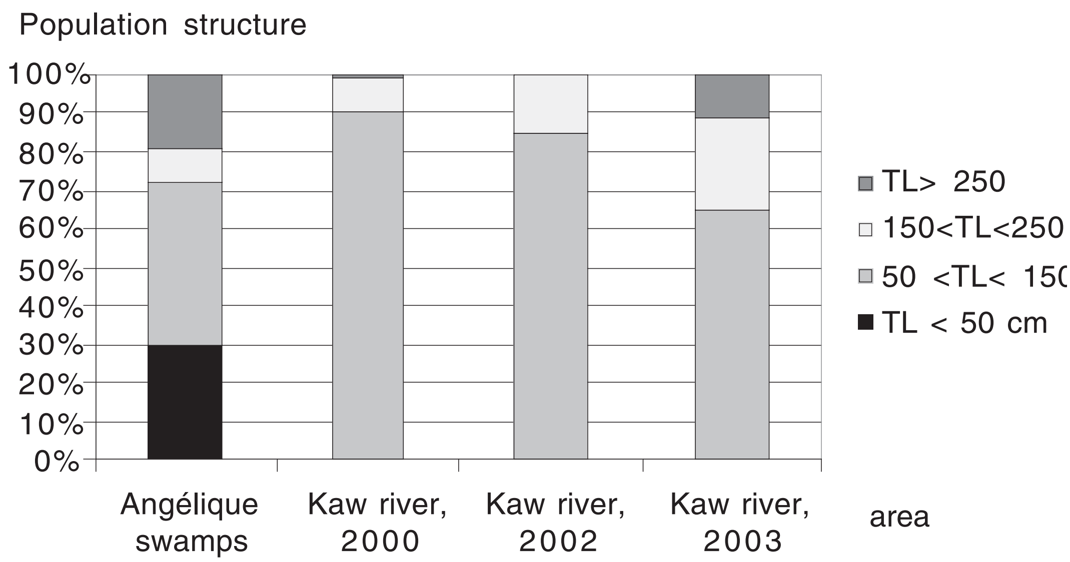

Figure 2. Sizes structure of Black caimans on the Kaw Nature Reserve, French Guiana. Period 2000-2003.

Growth

Data are very preliminar; only 10 C/R events were recorded, the difference of total lenghts between the 2 captures, for each C/R (with 1 to 11 months between the 2 events), are shown on Figure 3. Mean increase ranges from 1 to 3.5 cm/month, and is significantly higher in youg animals. To date, no animal marked in one of the three areas: Angélique Swamp, Kaw River, and Approuague, were recaptured in another.

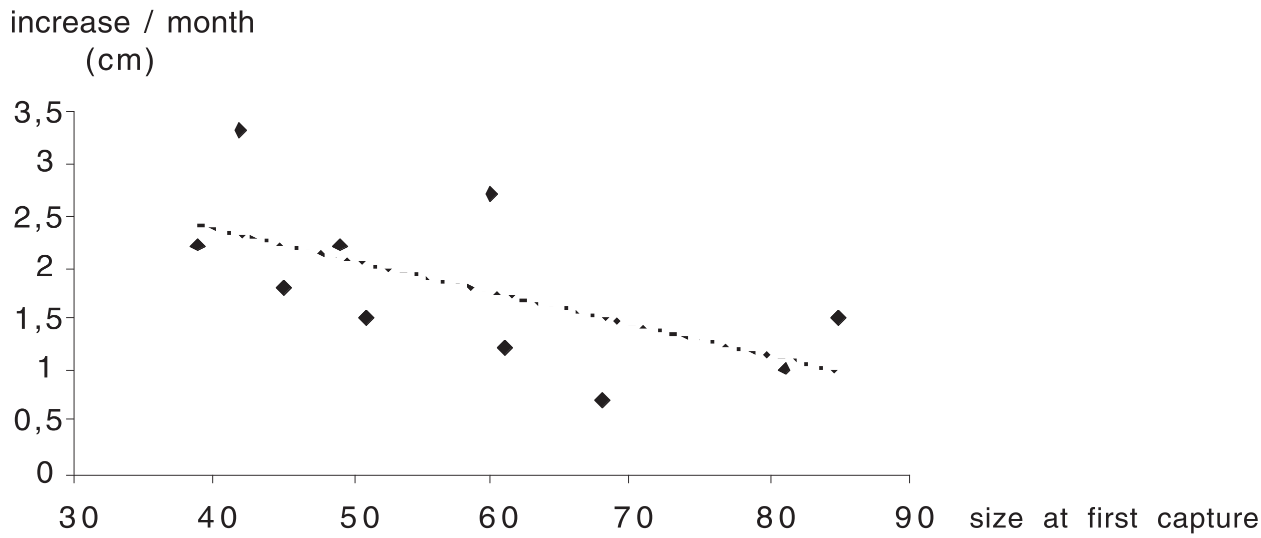

Figure 3. Growth rate of *Melanosuchus niger* in French Guiana.

Genetic diversity

Analysis of both nuclear and mitochondrial DNA suggested a high genetic diversity and a significant recovering potential (Farias *et al*. 2004; de Thoisy and Lavergne 2004). Nuclear markers suggested that gene flows are important between Angélique and Kaw River. On the other hand, animals from the Approuague would be related to breeders from Pointe Béhague, another large swamp inacessible area located in the East of the river, close to the brazilian boundary (de Thoisy and Lavergne 2004).

Conclusions

A large population of Black caiman is still present in French Guiana, due to the remoteness of large swamps. Although the population is severley depleted in bordering areas, a recovering potential is expected, but a strict management of the area is fully necessary, and is not yet sufficient. Considering together surveys and genetic data, we can suggest that:

(i) at the population scale, the high diversity and the absence of significant probability of consaguinity means an overall satisfactory status, with the evidence of gene flows between pristine areas (ie the Angélique Swamp), that may act as sources, and depleted areas; and,

(ii) on the Kaw River, the continuous decrease has to worry the reserve managers, and may be explained, at least in part, by unsufficient management: overfrequentation, inadequate behaviors of visitors and tourism operators, and maintenance of a silent poaching pressure.

Forthcoming actions will include VHF telemetry and extension of the genetic survey towards animals located outside the reserve, and as far as possible with the Brazilian population of Cabo Orange.

Literature

Blanc M. and de Thoisy B. (2001). Black caimans (*Melanosuchus niger*) in the Kaw swamps natural reserve, French Guiana: a first year survey. Crocodile Specialist Group Newsletter 20: 30-31.

da Silveira R. and Thorbjarnarson, J.B. (1999). Conservation implications of commercial hunting of black and spectacled caimans in the Mamiraua sustainable development reserve, Brazil. Biological Conservation 88: 103-109.

Farias I.P., Da Silveira, R., de Thoisy, B., Monjelo M.A., Thorbjarnarson, J., and Hrbek T. (2004). Genetic diversity and population stucture of Amazonian crocodilians. Animal Conservation (in press).

Herron J.C. (1991). Growth rates of black caiman *Melanosuchus niger*, and spectacled caiman *Caiman crocodilus* and the recruitment of breeders in hunted caimans populations. Biological Conservation 55: 103-113.

Herron J.C. (1994). Body size, spatial distribution, and microhabitat use in the caimans, *Melanosuchus niger* and *Caiman crocodilus*, in a Peruvian lake. J. Herp. 28: 508-513.

Hilton-Taylor, C. (2000). 2000 IUCN Red List of Threatened Species. IUCN: Gland.

de Thoisy, B., Blanc, M. and Joly, A. (2001). Distribution, abundance, and habitat use of black caiman in the Kaw swamps, French Guiana: a preliminary report. V° Congreso sobre el Manejo de la fauna silvestre en Latin America y Amazonia, Cartagena, Colombia.

de Thoisy, B., Lavergne, A., Blanc, M. and Joly, A. (2002). Status of the French Guianan black caiman (*Melanosuchus niger*) population. *In* Crocodiles. Proceedings of the 16th Working Meeting of the IUCN-SSC Crocodile Specialist Group.

de Thoisy, B. and Auffret, E. (2003). Possible extension of the distributional area of Black caiman in French Guiana. Crocodile Specialist Group Newsletter 27: 17-18.

de Thoisy, B. and Lavergne, A. (2004). Population status, structure and dynamics in Black caimans (*Melanosuchus niger*) in French Guiana. Submitted.

Blood Parasites of *Crocodylus siamensis* in Thailand

Jindawan Siruntawineti[1], Win Chaeychomsri[1], Boongeua Vajarasathira[1], Anussara Ritwichai[1], Tawatchai Ong-aat[2] and Yosapong Temsiripong[2,3]

[1]Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand (fscijws@ku.ac.th);
[2]R & D Laboratory, Sriracha Crocodile Farm Co., Ltd., Chonburi 20110, Thailand
[3]Crocodile Management Association of Thailand (CMAT) (yosapong@crocodileuniverse.com)

Abstract

Blood samples of 383 captive freshwater crocodiles, *Crocodylus siamensis,* from three different parts of Thailand, central-eastern (n= 231), northeastern (n= 38) and southern parts (n= 114) were examined. Blood smears, whole blood, white cells and red cells, were prepared, stained with Wright stain, and microscopically examined. To evaluate the relation between hematological values and blood parasite infection, 128 whole blood samples were identified for the pack red cell volume (PCV), differential of white blood cell (WBC) count and thrombocyte count per 100 WBC. Standardized questionnaires, concerning the diets and management practices, were collected and evaluated for their association with parasitological prevalence. The results show no parasite in crocodile blood (prevalence rate= 0%). The hematological values (mean ± SD) were as follows: PCV 28.90 ± 2.90%, heterophil 77.52 ± 3.70%, eosinophil 1.30 ± 0.90%, basophil 1.09 ± 0.78%, lymphocyte 18.55 ± 3.39%, monocyte 1.46 ± 1.03%, and 117.25 ± 19.76 thrombocytes per 100 WBC. These values were not significantly different between sexes. However, some hematological values of crocodiles from farms that changed water once a week were significantly ($P< 0.05$) lower than those from farms that changed water once a month. These results should prove safety in crocodile blood consumption.

Introduction

Freshwater crocodiles, *Crocodylus siamensis*, are endemic in Thailand. At present, they are commercially important. Thus they are more frequently found in captivity than in the wild. Recently, crocodile production is increased because of the progress in the farming technology. Crocodile skin is the principal product for annually exporting in the exotic leather trading. Besides, crocodile by-products such as meat, fat, bile, and penises are increasing important in adding values of captive crocodiles. The crocodile byproducts are influential in some farming areas and are conveniently classified into two categories, curios/novelty items and ingredients for medicines and other products (Hutton and Webb 1993; Huchzermeyer 2003). Crocodile blood, one of the by-products, is traditionally consumed for preventing and curing allergy and asthma.

Crocodiles may be infected by a number of blood-inhabiting protozoa and nematode worms transmitted by haematophagous arthropods. Protozoan parasites included haemosporidia in the genera *Hepatozoon,* and *Progarnia* (Siddall 1995; Smith 1996; Lainson 1995), and haemoflagellates in the genus *Trypanosoma* (Hoare 1931; Lainson 1977). Nematode worms in the family Filariidae occur in many crocodile species (Huchzermeyer 2003). Blood-inhabiting parasites of crocodiles are of particular interest because a high incidence of parasitic infections lowers productivity leading to important economic losses. Some blood parasites are zoonoses, transmitted between humans and animals.

The prevalence of blood parasites and parasitic species of zoonotic importance in *C. siamensis* has never been reported in Thailand. To ensure safety in crocodile blood consumption, the prevalence of the parasites in crocodile blood was observed. The relationships between hematological values and parasite infections were also investigated.

Materials and Methods

<u>Sample size and study area</u>: The sample size was calculated following the formula described by Thrusfield (1995). The total samples (n= 383) consisted of crocodiles from three parts of Thailand, central-eastern (n= 231 from 7 Provinces, 33 samples/Province), northeastern (n= 38 from 2 Provinces, 19 samples/Province) and southern parts (n= 114 from 6 Provinces, 19 samples/Province).

Designed questionnaires and collection: The questionnaires were designed concerning the diets and management practices. The data in each questionnaire were collected by personally communicating with crocodile farm owners or via mail. They were analyzed and evaluated for their association with parasitological prevalence.

Subjects and sample collection: Crocodiles, 2 to 3-year-old, were caught from the captive farms and transferred for slaughtering at Sriracha Crocodile Farm, Chonburi, during April 2003 and March 2004. Their weight, thorax length, and total length, from head to tail, were measured. Blood samples (5 ml) were collected from the post-occipital venous sinuses with sterile-technique. The samples were kept in tubes containing ethylene diamine tetraacetic acid (EDTA). The pack red cell volume (PCV) was prepared and determined according to the standard microcentrifugation method. The blood samples were used to produce three types of blood smear namely, whole blood, white cell and red cell smear. To make the whole blood smear, approximately 5 μl of blood was smeared on a microscope slide. The remaining blood was centrifuged at 3000 rpm for 10 min. Afterwards, blood was divided into two layers, the upper white blood cell (WBC) with thrombocyte and the lower red blood cell (RBC). Each portion was then used to prepare the white and red cell smears. All blood smeared slides were individually labeled, air-dried, fixed in 100% methanol for 15 sec, and stained with Wright stain (Dip-Quick®, Clinical Diagnoties, Ltd., Part, Bangkok, Thailand).

Smear examination: To identify parasite species, all blood smears were examined at 1000x magnification under oil immersion. The crocodiles were classified as negative for the absence of parasites and as positive for the presence of various parasite types. The prevalence of infection was defined as the percentage of infected individuals in a sample. In addition, the whole blood smears were checked for the differential WBC counts and the number of thrombocyte per 100 WBC at 1000x magnification.

Statistical analyses: The prevalence of blood parasites in freshwater crocodiles was calculated as a percentage of crocodile infected. Group differences were assessed by ANOVA.

Results

The sizes of crocodiles collected from different parts of Thailand are summarized in Table 1, together with the prevalence of blood parasites. The captive-bred crocodiles, 2-3 years-old, were 189.54 ± 10.48 cm in length and weighed 28.23 ± 5.25 kg. Their thorax length averaged 56.01 ± 4.62 cm. However, parasites were not detected in all types of blood smears, 383 specimens.

Table 1. Prevalence of blood parasites in freshwater crocodiles (*Crocodylus siamensis*) from three parts of Thailand.

Part/Province	Crocodile size (Mean ± SD)			No.	Prevalence of parasites in blood smears (%)		
	Length (cm)	Weight (kg)	Thorax length (cm)		WhB*	WBC*	RBC*
Central-Eastern							
Bangkok	194.21±8.42*	30.98±3.97	58.58±3.32	33	0	0	0
Ayuthaya	190.81±15.62	26.94±7.18	55.30±5.98	33	0	0	0
Samut Prakan	195.67±17.61	32.30±10.86	57.95±8.82	33	0	0	0
Nakhon Pathom	185.61±10.67	26.42±4.99	56.27±4.96	33	0	0	0
Trat	197.30±7.92	34.90±2.91	59.42±3.95	33	0	0	0
Rayong	193.25±11.93	28.75±5.28	56.58±4.72	33	0	0	0
Chanthaburi	185.76±6.78	27.85±3.65	56.12±3.82	33	0	0	0
Northeastern							
Loei	183.89±7.87	24.33±4.07	53.63±4.27	19	0	0	0
Nakhon Phanom	175.21±11.06	22.01±4.69	53.79±3.95	19	0	0	0
Southern							
Petchaburi	197.21±9.37	27.34±4.75	55.84±4.09	19	0	0	0
Chumporn	200.20±8.44	32.69±5.34	58.05±4.84	19	0	0	0
Ranong	197.68±14.20	33.28±8.23	58.32±5.39	19	0	0	0
Surat Thani	176.50±10.02	23.40±4.53	52.74±4.16	19	0	0	0
Songkhla	173.95±7.08	21.82±2.89	51.21±3.44	19	0	0	0
Phatthalung	195.89±10.18	30.45±5.38	56.35±3.98	19	0	0	0
Total	189.54±10.48	28.23±5.25	56.01±4.62	383	0	0	0

*WhB: whole blood; RBC: red blood cell; WBC: white blood cell

The hematological values in relation to parasitic infection could not be evaluated because there were no parasites in the crocodile blood. However, the hematological values, PCV, differential WBC count and the number of thrombocytes per 100 WBC, of 128 samples from 6 Provinces were analyzed.

The hematological values of male and female crocodiles from different parts of Thailand are presented in Tables 2 and 3, respectively. These values were not significantly different between sexes ($P > 0.05$). The type of water changes significantly affected male hematological values, PCV, %heterophil, %eosinophil and %lymphocyte (Table 4). Similar results were observed in females (Table 5). The frequency of water changes significantly influenced %monocyte in female. On the contrary, the regularity of water changes did not affected %monocyte in male.

Table 2. Hematological values of male freshwater crocodiles (*Crocodylus siamensis*) from three parts of Thailand.

Hematological tests	Hematological values (Mean ± SD)		
	Central-Eastern (n = 25)	Northeastern (n = 22)	Southern (n = 35)
PCV (%)	28.20 ± 3.06	30.68 ± 2.56	28.27 ± 3.35
Differential count (%)			
Heterophil	75.52 ± 3.33	80.23 ± 3.12	74.40 ± 5.22
Eosinophil	1.72 ± 1.12	0.77 ± 0.52	1.31 ± 1.13
Basophil	0.68 ± 0.48	1.32 ± 0.78	1.96 ± 1.41
Lymphocyte	20.78 ± 3.00	15.27 ± 3.16	21.83 ± 5.24
Monocyte	1.30 ± 0.91	1.48 ± 1.04	1.29 ± 0.82
Thrombocytes/ 100 WBC	112.52 ± 26.02	121.02 ± 12.81	105.39 ± 26.22

n: number of analyzed samples, PCV: pack red cell volume, WBC: white blood cell.

Table 3. Hematological values of female freshwater crocodiles (*Crocodylus siamensis*) from three parts of Thailand.

Hematological tests	Hematological values (Mean ± SD)		
	Central-Eastern (n = 8)	Northeastern (n = 16)	Southern (n = 22)
PCV (%)	27.38 ± 2.10	28.53 ± 3.87	30.36 ± 2.61
Differential count (%)			
Heterophil	75.00 ± 3.76	81.22 ± 2.73	78.77 ± 4.03
Eosinophil	1.69 ± 1.07	0.72 ± 0.52	1.61 ± 1.11
Basophil	0.56 ± 0.32	1.19 ± 0.81	0.82 ± 0.89
Lymphocyte	21.25 ± 2.82	15.03 ± 2.85	17.14 ± 3.24
Monocyte	1.50 ± 1.22	1.66 ± 1.06	1.55 ± 1.13
Thrombocytes/ 100 WBC	107.81 ± 7.54	122.91 ± 21.44	133.84 ± 24.53

n: number of analyzed samples, PCV: pack red cell volume, WBC: white blood cell.

Table 4. Effect of the types of water change on hematological values of male freshwater crocodiles (*Crocodylus siamensis*).

Hematological tests	Hematological values (Mean ± SD)	
	Types of water change	
	Once a week	Once a month
PCV (%)	27.69 ± 2.91[a]	30.98 ± 2.64[b]
Differential count (%)		
Heterophil	73.72 ± 3.63[a]	80.78 ± 2.96[b]
Eosinophil	1.52 ± 1.15[a]	0.90 ± 0.69[b]
Basophil	1.49 ± 1.33[a]	1.23 ± 0.78[a]
Lymphocyte	22.46 ± 3.70[a]	15.05 ± 2.83[b]
Monocyte	1.23 ± 0.87[a]	1.53 ± 0.95[a]
Thrombocytes/ 100 WBC	106.40 ± 27.18[a]	121.03 ± 12.63[b]

Values with different superscripts in the same column, are different (ANOVA; $P \leq 0.05$), PCV: pack red cell volume, WBC: white blood cell.

Table 5. Effect of the types of water change on hematological values of female freshwater crocodiles (*Crocodylus siamensis*).

Hematological tests	Hematological values (Mean ± SD)	
	Types of water change	
	Once a week	Once a month
PCV (%)	28.00 ± 1.94[a]	30.06 ± 3.65[b]
Differential count (%)		
Heterophil	75.76 ± 3.86[a]	81.22 ± 2.47[b]
Eosinophil	1.89 ± 1.22[a]	0.91 ± 0.59[b]
Basophil	1.03 ± 0.84[a]	0.81 ± 0.80[a]
Lymphocyte	20.29 ± 2.80[a]	14.89 ± 2.30[b]
Monocyte	1.00 ± 0.93[a]	1.98 ± 1.04[b]
Thrombocytes/ 100 WBC	121.53 ± 26.13[a]	128.33 ± 20.82[a]

Values with different superscripts in the same column, are different (ANOVA; $P \leq 0.05$), PCV: pack red cell volume, WBC: white blood cell.

The questionnaire data revealed that crocodiles were usually individually maintained in a single pen with smooth concrete ground. They were fed with chicken skeleton (94%), fish (38%) and pork remains (6%) every other day (87%). Sources of water supply were from artesian well (56%), piped water (25%), pond (19%) and other natural water sources (6%). The water was regularly changed once a week (63%) rather than once a month (19%).

Discussion

Our study examined captive or farmed crocodiles from 15 Provinces in three parts of Thailand. Most crocodile farms (60%) were concentrated in the central-eastern areas. The others were scattered in the southern (30%) and northeastern (10%) parts of Thailand. The crocodiles from these farms were delivered to Sriracha Crocodile Farm located in Chonburi Province, in the eastern part of Thailand. This farm is a center for crocodile trading and slaughtering. Moreover, Sriracha Farm owner advises the smaller operations in transfer technology of crocodile husbandry

According to the questionnaires the crocodiles were kept singly in a pen with smooth concrete ground. They were fed mainly with chicken skeleton every other day. The source of water was mostly from artesian well water (56%). The water was changed predominantly once a week (63%).

In general, parasitic infections in crocodiles are acquired by eating or drinking contaminated food or water. The crocodiles may also be infected through direct contact with soil or water containing parasitic eggs or larvae, or by being bitten by infectious insects. Temperature, food and pen design were the main factors influencing the occurrence of many diseases in farmed crocodiles (Buenviaje *et al.* 1994).

Our results showed no parasites in the crocodile blood. This may due to good farming condition and valid management. The infection rate also depended upon the crocodile physiological status. Some hematological values of crocodiles were significantly influenced by the regularity of water change indicating the association between the management practice and the crocodile physiological status.

The crocodile blood is one of interesting by-products. It is traditionally consumed for medicinal purpose. However, little information is available on its safety for human consumer. The blood is usually processed by freeze drying or lyophilizing. This process does not harmfully affect active substances in blood but it cannot destroy many pathogens. These viable pathogens may pass to consumer and cause serious health problems. For example food contaminated with common parasites, *Giardia* and *Strongyloides* species can cause zoonosis. Thus food safety is gaining increase attention.

The results in this study, the prevalence of blood parasites in captive or farmed crocodiles, serve as mark information that is important in improving husbandry management and assuring safety in consuming crocodile blood.

Literature

Buenviaje, G.N., Ladds, P.W., Melville, L. and Manolis, S.C. (1994). Disease- husbandry associations in farmed crocodiles in Queensland and Northern Territory. Aust. Vet. J. 71: 165-173.

Hoare, C.A. (1931). Studies on *Trypanosoma grayi*. III. Life-cycle in the tsetse-fly and in the crocodile. Parasitology 23: 449-484.

Huchzermeyer, F.W. (2003). Crocodiles: biology, husbandry and diseases. CABI Publishing: Oxon.

Hutton, J.M. and Webb, G.J.W. (1993). The principles of farming crocodiles. *In* Proceeding of the 2nd Regional (Eastern Asia, Oceania, Australasia) meeting of the Crocodile Specialist Group. Darwin, Australia, 12-19 March 1993. IUCN: Gland.

Lainson, R. (1995). *Progarnia archosauriae* nov. gen., nov. sp. (Haemosporina: Garniidae), a blood parasite of *Caiman crocodilus crocodilus* (Archosauria: Crocodilia), and comment on the evolution of reptilian and avian haemosporines. Parasitology 110: 513-519.

Lainson, R.J. (1977). *Trypanosoma cecili* n. sp., a parasite of the South American cayman, *Caiman crocodilus crocodilus* (Linnaeus, 1785) (Crocodilia: Alligatoridae). Protozoology 3: 87-93.

Siddall, M.E. (1995). Phylogeny of adeleid blood parasites with a partial systematic revision of the haemogregarine complex. J. Eukaryot. Microbiol. 42: 116-125.

Smith, T.G. (1996). The genus *Hepatozoon* (Apicomplexa: Adeleina). J. Parasitol. 82: 565-585.

Thrusfield, M. (1995). Veterinary Epidemiology. Blackwell Science: London.

Intestinal Parasites of Freshwater Crocodiles, *Crocodylus siamensis*, in Thailand

Win Chaeychomsri[1], Jindawan Siruntawineti[1], Boongeua Vajarasathira[1], Komsun Komoljindakul[1], Tawatchai Ong-aat[2] and Yosapong Temsiripong[2,3]

[1]Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand (fsciwcc@ku.ac.th); [2]R & D Laboratory, Sriracha Crocodile Farm Co., Ltd., Chonburi 20110, Thailand; [3]Crocodile Management Association of Thailand (CMAT) (yosapong@crocodileuniverse.com)

Abstract

The prevalence of intestinal parasites in farmed juvenile crocodiles, *Crocodylus siamensis,* was compared with that of adults in breeding pens. Faecal samples of 383 juveniles were gathered from three parts of Thailand, central-eastern, northeastern and southern parts. Thirty faecal specimens of adult crocodiles were also taken from Sriracha Crocodile Farm. They were examined for parasitic infection by direct smear and Modified Kato thick technique. Standardized questionnaires, dealing with the diets and management practices, were collected and evaluated for their association with parasitological prevalence. The overall prevalence of coccidian parasites in juveniles was 3.13% (12/383). The highest prevalence of coccidian infection, 5.26% (2/383), was observed in north-northeastern part of Thailand. The prevalent rates of 3.51 (4/114) and 2.60% (6/231) were observed in southern and in central-eastern part of Thailand, respectively. In contrast, 23.33% (7/30) coccidian infection was found in adult crocodiles. The difference in coccidian prevalence between juveniles and adults, relative to diets and management practices, was discussed.

Introduction

Freshwater crocodiles, *Crocodylus siamensis*, are promising economic animals in Thailand. The crocodile farming business is progressively growing. At present, there are over 500 crocodile farmers in Thailand. Approximately 30,000 juvenile crocodiles are available for marketing as classical crocodile leather and other by-products. Parasitic infection is one of the most important diseases affecting a wide range of animals including crocodiles. In livestock, both clinical and sub-clinical infections cause mortality, decrease growth rate and productivity and increase treatment cost leading to economic loss. Thus far there is no information on the nature and prevalence of intestinal parasitic infections in Thai crocodiles. Our study was therefore aimed to determine the prevalence of intestinal parasites in captive breeding crocodiles.

Materials and Methods

<u>Sampling frame</u>*:* Sample size was calculated using the formula $n= 1.96^2 P (1-P)/d^2$ according to Thrusfield (1995), with *P*= 0.5 and d= 0.05. As the prevalence of the parasites was unknown, each was assumed to be 50% where the maximum sample size is calculated. To meet a desired absolute precision of 5% and a 95% level of confidence, a sample size of at least 383 crocodiles was required. Samples were taken from both juvenile and adult crocodiles. The juveniles, 383, were from three parts of Thailand and the adults, 30, were from breeding pens, Sriracha Farm.

<u>Sample preparation and examination</u>*:* The direct smear method was used to detect trophozoites of amoebae and flagellates. Faecal specimens were placed in a small container and a drop of physiological saline was added for emulsification. Subsequently, two drops of the emulsified stool was placed on a slide and observed under the microscope. Iodine was used to stain parasitic eggs and cysts. The total area of the cover slip was scanned using 10x and 40x objectives. Trophozoite mobility and the presence of ingested red blood cells (RBCs) were observed to differentiate between *Entamoeba histolytica* and other amoebae. *Eimeria* species were identified based on the morphology of oocysts (shape, colour, form index, presence or absence of micropyle and its cap, presence or absence of residual, polar and stieda bodies) and time of sporulation (Coudert 1992; Eckert *et al.* 1995).

The Modified Kato thick smear technique was also used to estimate worm egg number in the fecal samples, especially of Ascaris, Opisthorchis, Trichuris, Hookworm, Schistosomes (Martin and Beaver 1968). Forty micrograms of faecal material was passed through a template onto a slide and covered with a cellophane cover slip soaked in 0.3% malachite green. The slide was inverted against a smooth surface and pressed down gently until the sample spread uniformly over the whole cellophane cover slip. The slide was left 20-30 min before microscopic examination. Parasite ova were identified according to the species characteristics described in Soulsby (1982), Campbell (1988), Anderson (1992), Khalil *et al*. (1994), and Calnek (1997).

Designed questionnaires and collection*:* The questionnaires were designed according to the diets and management practices. The questionnaire data were collected by personally contacting with crocodile farm owner or via postage. The data were analyzed and evaluated for their association with parasitological prevalence.

Calculation of overall prevalence*:* To determine the overall prevalence of intestinal parasites in the sample population the number of positive faecal samples was divided by the total number of faecal samples collected.

Results

The general data of crocodiles was summarized in Table 1. The crocodiles were individually maintained in a single cage with smooth concrete floor. They were fed with chicken skeleton (94%), fish (38%) and pig remains (6%). The feeding interval was mainly every two days (87%). Water supply for caging was from artesian well (56%), piped water (25%), pond (19%) and other natural water sources (6%). Frequency of water change was once a week (63%) rather than once a month (19%).

Table 1. General data of crocodile farms. * *N* = number from returned questionnaires.

	N*	%
Number of crocodiles		
Mean number of captive crocodiles per farm (Range 30-1600 crocodiles)	15/15	100
Mean number of ranched crocodiles per farm (Ranges 10-40 crocodiles)	2/15	13.33
Type of crocodile feed		
Chicken skeleton	15/16	93.75
Fish	1/16	6.25
Pig remains	6/16	37.50

The overall prevalence of crocodile infection with intestinal parasites was lower in juveniles (3.13%) than in adult crocodiles (23.33%). Only coccidian parasite was detected in faecal samples of both adult and juvenile crocodiles (Table 2). By using direct faecal smear technique, 3.13% (12/383) juvenile crocodiles were found positive for coccidian parasite. The highest prevalence of coccidian, 2/38 (5.26%), was in the Northeastern part of Thailand. The lower prevalent rates, 4/114 (3.51%) and 6/231 (2.6%), were in the southern and the central-eastern parts of Thailand, respectively. By using similar method, 23.33% (7/30) adult crocodiles were positive for coccidian parasite (Table 2). In contrast, protozoa and other intestinal parasites were not found by Modified Kato thick smear technique (Table 3).

Table 2. Prevalence of intestinal parasites observed by direct faecal smear technique.

Faecal exam.	Adult Crocodile			+ Juvenile crocodiles: Central-Eastern			Southern			Northeastern			Total		
	No.	+	%	No.	+	%	No.	+	%	No.	+	%	No.	+	%
Coccidia	30	7	23.33	231	6	2.60	114	4	3.51	38	2	5.26	383	12	3.13
Other	30	0	0	231	0	0	114	0	0	38	0	0	114	0	0

Table 3. Prevalence of intestinal parasites observed by direct faecal smear technique and Modified Kato Thick smear technique.

	Juvenile crocodile											
Faecal exam.	Central-Eastern			Southern			Northeastern			Total		
	No.	+	%	No.	+	%	No.	+	%	No.	+	%
Direct smear	132	0	0	57	0	0	38	0	0	227	0	0
Kato's thick	132	0	0	57	0	0	38	0	0	227	0	0

Discussion

The parasitic prevalence in captive breeding crocodiles was very low. This may be the results of good farm management and hygienic measures, regular deworming and improved caging system. Usually, parasites are likely to buildup when animals are held in confinement without proper hygiene. The incidence of protozoan infections in farmed crocodiles indicated a need for prophylactic anti-protozoan therapy as a basic farm management. Such procedure will minimize the likelihood of in-house parasite buildup and improve safety in the colony staff, because these parasites are capable of infecting and causing disease in crocodiles.

Coccidiosis is the disease caused by coccidia when the host ingests many oocysts of a pathogenic strain. It is a frequent problem when raising large numbers of domesticated animals in a confined space (Fayer and Reid 1982). Poultry men, cattlemen, rabbit breeders, pigeon raisers, etc., are familiar with the mortality and morbidity effects of coccidiosis. Coccidiosis is probably not a problem in wild populations because the hosts are widely distributed. They are not confined to limited areas where the oocysts concentration can increase in the environment to the point that the hosts would ingest large numbers of oocysts. However, from the parasite's point of view, distribution would be more difficult in the natural setting. This study suggests that adult crocodiles that spend much of their time in a flock are more likely to be infected with coccidia than juvenile crocodiles that spend their time in a single cage.

Clustering behavior fosters oocyst transmission because of the close proximity of infected crocodiles passing oocysts to potential new hosts. Oocysts, develop endogenously, are passed in the feces, and sporulate in the environment. Crocodiles become infected when they ingest the sporulated oocysts while feeding. Transmission of the parasite is facilitated by close contact with infected hosts who are actively passing oocysts in their feces. The results from questionnaires indicate that crocodiles were fed chicken skeleton (94%), fish (38%) and pig remains (6%). Their feeding habit was controlled by management program. They were fed every two days in the cage. The water supply was changed either once a week or once a month depending on the farm management. Thus the chance for the farmed crocodiles exposing to pathogenic agents or infective stage of parasites was very low. Although the transmission of coccidian parasites is well known, there are few reports of the prevalence of coccidian parasites in wild animal populations and no reports of the prevalence of coccidian in the farmed crocodiles in Thailand.

Habitat also influences the prevalence of coccidian in crocodile populations. Adult crocodiles, that were ground feeders, had higher prevalence of coccidian than juvenile crocodiles that were fed in the single concrete floor cages. Oocyst viability is dependent on moisture and oxygen (Brotherston 1948; Farr and Wehr 1949; Marquardt *et al*. 1960). Oocysts are very susceptible to desiccation and oxygen is required for sporulation and development to the infective stage. The soil in dens would be moist and conducive to oocysts survival and development. Wild crocodiles and the forest canopy would have more sunlight and higher temperatures that would enhance desiccation and reduce oocyst viability (Long 1959; Farr and Wehr 1949).

In conclusions, both adult and juvenile crocodiles harbored similar species of coccidian parasites. However, the prevalence of protozoan infections was higher in the adults than in the juveniles. These findings could not be easily explained based on conventional epidemiological criteria. Colony management, such as housing, sanitation and veterinary interventions such as a regular chemoprophylaxis against protozoa, was expected to be responsible for the lower level of coccidian infection in the juveniles. The lack of chemoprophylaxis against protozoa could have been responsible for the relatively higher prevalence in the captive breeding adults.

Literature

Anderson, R.C. (1992). Nematode parasites of vertebrates: their development and transmission. CAB International: Wallingford, UK.

Brotherson, J.G. (1948). The effect of relative dryness on the oocytes of *E. tenella* and *E. bovsis.* Transactions of the Royal Society of Tropical Medicine and Hygine 42: 10-11.

Calnek, B.W. (1997). Nematodes and acanthocephalans. Pp. 815-849 *in* Diseases of Poultry, 10th Edition. Iowa State University Press: Ames, IA.

Campbell, T.W. (1988). Avian hematology and cytology, PhD Thesis, Iowa State University Press: Ames, IA.

Coudert, P. (1992). *Eimeria* species from the goat. *In* Proceedings of the Fourth Conference COST-89. Tours, INRA, October 1992.

Eckert, J., Taylor, M., Catchpole, J., Licois, D., Coudert, P. and Bucklar, H. (1995). Identification of *Eimeria* species and strains. Pp. 108-109 *in* Guidelines on Techniques in Coccidiosis Research, COST 89/820. Biotechnology. European Commission, Directorate-General XII, Science Research and Development Agriculture Biotechnology L-2920, Luxemburg.

Farr, M.M. and Wehr, E.E. (1949). Survival of *Eimeria acervulina*, *E. tenella* and *E. maxima* oocyts on soil under various conditions. Annuals of the New York Academy of Science 52: 468-472.

Fayer, R. and Reid, W.M. (1982). Control of coccidiosis. Pp. 453-487 *in* The Biology of the Coccidia, ed. by P.L. Long. University Park Press: Baltimore.

Khalil, L.F., Jones, A. and Bray, R.A. (1994). Keys to the Cestode Parasites of Vertebrates. CAB International: Wallingford, UK.

Long, P.L. (1959). A study of *Eimeria maxima*, a coccidium of the fowl (*Gallus gallus*) Annual of Tropical Medicine and Parasitology 35: 325-333.

Marquardt, W.C., Senger, C.M. and Seghetti, L. (1960). The effect of physical and chemical agents on the oocysts of *Eimeria zuernii* (Protozoa, Coccidia). Journal of Protozoology 7: 186-189.

Martin, L.K. and Beaver, P.C. (1968). Evaluation of Kato thick smear technique for quantitative diagnosis of helminth infection. Am. J. Trop. Med. 17: 382-391.

Soulsby, F.J.L. (1982). Pp. 767 *in* Helminths, Arthropods and Protozoa of Domesticated Animals. Bailliere Tindal: London.

Thrusfield, M. (1995). Veterinary Epidemiology. Blackwell Science: London.

Efficacy of Siamese Crocodile (*Crocodylus siamensis)* Serum on Bacterial Growth Inhibition

Jindawan Siruntawineti [1] Win Chaeychomsri [1] Boongeua Vajarasathira[1], Tawatchai Ong-aat[2] and Yosapong Temsiripong[2,3]

[1]Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand (fscijws@ku.ac.th);[2]R & D Laboratory, Sriracha Crocodile Farm Co., Ltd., Chonburi 20110; [3]Crocodile Management Association of Thailand (CMAT)(yosapong@crocodileuniverse.com)

Abstract

Bactericidal efficacy of Thai freshwater crocodile (*Crocodylus siamensis*) serum was tested. Various serum concentrations, 100, 75, 50 and 25%, were mixed with *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*, incubated at 37°C for 12 h. The samples, 100 ml, were collected at 0, 1, 3, 6, 9, and 12 h intervals. The crocodile serum, inoculated with *E. coli* and *P. aeruginosa*, showed bactericidal activity depending on incubation time and serum concentration. However, the serum, inoculated with *B. subtilis* and *S. aureus*, exhibited a time- and concentration-independent inhibition of bacterial growth. The bactericidal activity of crocodile serum was completely inhibited by preincubating the serum at 56°C for 30 min. The mechanism of antimicrobial activity may due to complement system and/or antimicrobial peptides presented in the crocodile serum.

Introduction

Crocodiles infrequently exhibit impairment health conditions due to infection. They often sustain serious injuries by rapidly healing and always without infection. Crocodilians have been known to live with opportunistic pathogenic bacteria with no physiological effects (Madsen *et al*. 1998). Their immune systems consist of two types of acquired and innate immune systems. The first line of defense of them against pathogens and parasites is of physical nature via their hard skin. However, once this barrier is passed, a complex interaction of innate humoral and cellular immune reactions is induced in both tissues and body fluid in circulatory system, which results in a fast elimination of microorganisms. High avidity binding of proteins to foreign material is a requisite of most humoral immunological recognition events (Dodds and Alex Law 1998).

Blood is a liquid tissue that has three major functions; as a transportation, for regulation, and protection. It holds specialized cells and chemicals that defend the body against diseases. As in other higher vertebrate, crocodilian also has chemicals involving in host defense mechanisms against infection. Discerning the nature of crocodile immunity, Siamese freshwater crocodile, *C. siamensis*, serum was evaluated for the efficacy of antimicrobials, and validated antimicrobial activity.

Materials and Methods

Crocodile samples**:** Samples were conducted on portions of Sriracha Crocodile Farm, Chonburi, Thailand. Crocodiles in 3 years age, at least 25 kg weight and 1.5-2.0 m length, were used. A blood sample, 5-10 ml, was collected from the anterior dorsal sinus, centrifuged and separated for serum. The serum was kept at -20°C until used.

Bacterial samples and cultures**:** Lyophilized bacterial strains were purchased from National Institute of Health (NIH, Thailand). The following ATCC-registered strains were used: *Escherichia coli* (25922), *Pseudomonas aeruginosa* (27853), *Staphylococcus aureus* (5638), and *Bacillus subtilis* (6633). Bacteria were preserved on nutrient agar slants at 4°C. The bacteria were inoculated in 4 ml of nutrient broth culture and incubated at 37°C overnight to raise log phase growth. Serial dilutions of the log-phase cultures were performed and plated on nutrient agar in 100 x 15 mm petri dishes. The dishes were incubated at 37°C for 18 h. Then, the cultures were determined for the colony-forming unit (CFU).

Optimized antibacterial assays**:** Each bacterial culture, *E. coli, P. aerugenosa, B. subtilis* and *S. aureus,* in log phase growth was prepared in 10-fold serial dilutions using sterile physiological saline. The samples were measured for optical densities using a spectrophotometer (Spectronic 20 Genesys, Spectronic Instruments, Inc., USA) at 600 nm. Fresh crocodile serum samples, 450 ml, were treated with 50 ml of bacterial cultures or a dilution containing different amounts of bacteria. The samples were incubated at 37°C for 18 h. One hundred ml of a dilution of each sample was spread onto the surface of nutrient agar plates to determine the CFU. Samples were plated at three different dilutions to obtain plates with a quantity of colonies such to provide a reasonable estimate of bacterial density (30-300 CFU/plate).

Antibacterial assays**:** Fresh crocodile serum (F-CS), was diluted to 25, 50, 75 and 100% with physiological saline for variation of time and concentration dependent. Nine hundred ml samples containing various concentrations of crocodiles sera were inoculated with 100 ml of log phase culture of bacteria, approximately 1 x 10^7 cell/ml. The samples were incubated at 37°C for 0, 1, 3, 6, 9, and 12 h. One hundred ml of each sample was spread onto the surface of nutrient agar plates to determine the CFU.

Inhibition of antibacterial activity**:** The inhibition of antibacterial activity in crocodile serum was performed by serum incubation at 56°C 30 min. Then, the inactivated sera (I-CS) were diluted to 25, 50, 75 and 100% with physiological saline for variation of time and concentration in dependent. Nine hundred ml of samples containing various concentrations of crocodile sera were inoculated with 100 ml of optimum concentration that previously performed. The samples were incubated at 37°C for 0, 1, 3, 6, 9, and 12 h. One hundred ml of each sample was spread onto the surface of nutrient agar plates to determine the bacterial CFU.

Statistical analysis**:** To obtain valid statistical evaluation of the results, all experiments were performed in triplicate. The percentage of relative growth for each bacterial strain was calculated.

Results

The dilution of bacteria in log phase growth provided optimal colony-number for manually counting exhibited 0.2 of optical density at 600 nm. In this study, we used 10^{-7} dilution of bacteria that showed 30-300 of viable cells on nutrient agar plate. The results showed that concentration, 50-100% of fresh crocodile serum (F-CS) particularly inhibited *E. coli* growth in 3 h of incubation (Fig. 1a). However, these gram-negative bacteria were incompletely inhibited when treated with in 25% F-CS. *Pseudomonas aeruginosa* growth was totally suppressed by all concentrations of F-CS approximately in 1 h of incubation (Fig. 1c). Concentration-dependent inhibition of the bacterial growth by F-CS was observed. Moreover, all concentrations of F-CS incompletely inhibited *S. aureus* and *B. subtilis* growth (Figs. 2a and 2c). These bacteria remarkably exhibited their colonies less than 150 after all period of incubations. In contrast, heat-inactivated crocodile serum at 56°C, 30 min (I-CS) fully obliterated the antimicrobial activities (Figs. 1b, 1d, 2b and 2d).

Discussion

The crocodile serum exhibited antimicrobial activity including in gram-negative and gram-positive bacteria.In this study, high efficacy in bactericidal activity was observed in gram-negative bacteria, *E. coli* and *P. aeruginosa* (Figs. 1a and 1c). The results revealed that the antibacterial capacity of F-CS in *E. coli* was higher than in *P. aeruginosa*. Both bacteria, as shown in Figures 1b and 1d, were inhibited depend on F-CS concentration and incubation time. The strengths of antibacterial activities against bacteria are probably related to bacterial membrane structure. In present study, gram-positive bacteria, *S. aureus* and *B. subtilis*, cultured with crocodile serum exhibited fluctuating relative growth (Figs. 2a and 2c), however, their relative growth were not over than 150%. Overall results implying that in the crocodile serum may have antimicrobial factors responsible for defense microbial invaders. Firstly, we hypothesized that the antimicrobial factors in serum were including proteins in complement system and antimicrobial peptides.

During the past few years, studies on the components of an immediate immune response against infectious microorganism or innate immune system have established the contribution of antimicrobial peptides (AMPs). AMPs are polypeptides of fewer than 100 amino acids, found in host defense settings, and exhibiting antimicrobial activity at physiologic ambient conditions and peptide concentrations. They are expressed in many tissues, polymorphonuclear leukocytes, macrophages and mucosal epithelial cells (Brogden *et al*. 2003). AMPs neutralizes bacteria by interacting specifically with their cell membranes (Lehrer and Ganz 1999). Previously, saltwater crocodile, *Crocodylus niloticus*, has been exhibited antibacterial activities of lung and adrenal tissues (Shaharabany *et al.* 1999).

Complement is a necessity part of the innate immune system and contains about 35 soluble and membrane-bound proteins. The complement system performs a major role in killing and neutralizing microorganisms. These events are mediated by activation of the lytic pathway and by opsonization. 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*.

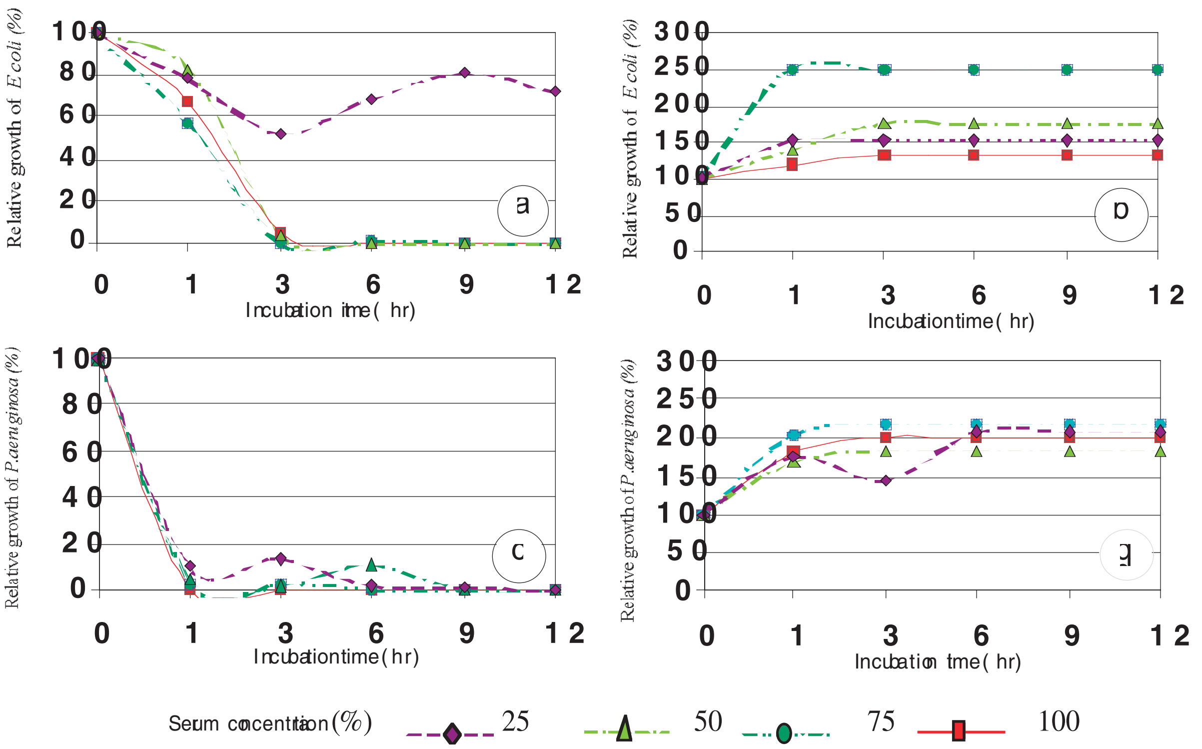

Figure 1. Relative growth of gram-negative bacteria (*E. coli* and *P. aeruginosa*) after treated with fresh and heat-inactivated *C. siamensis* sera.

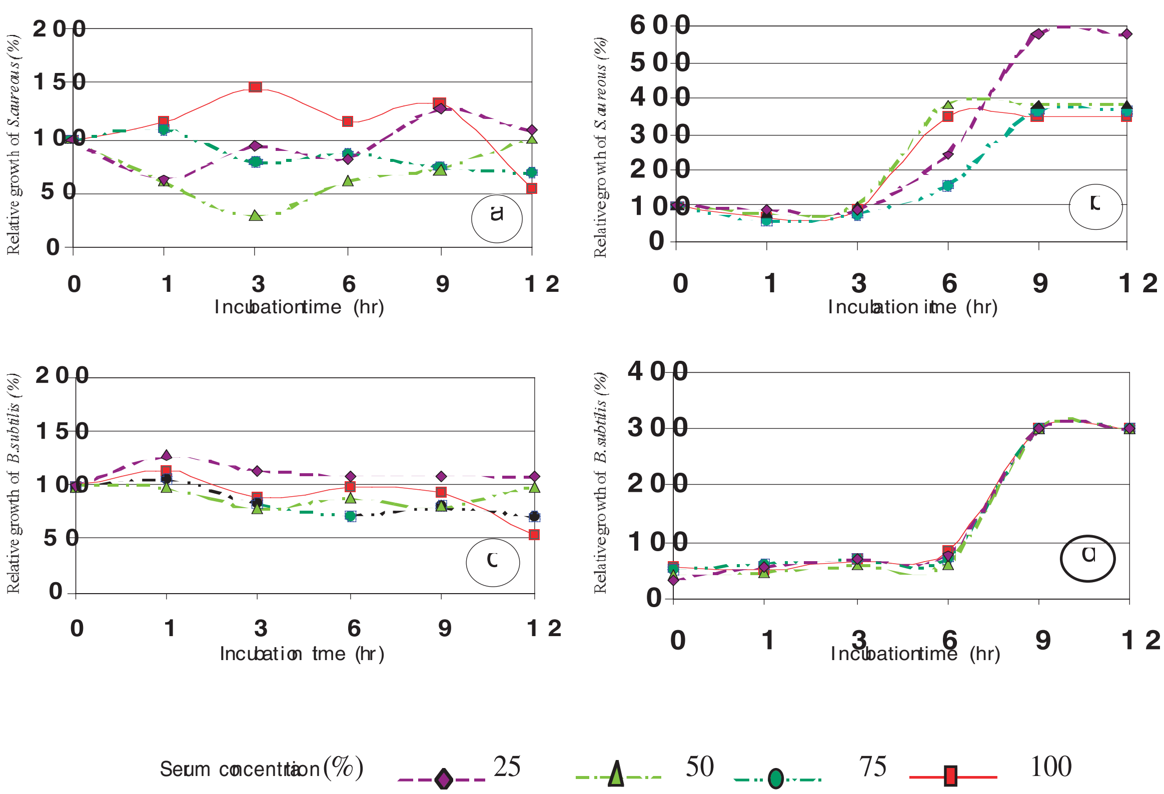

Figure 2. Relative growth of gram-positive (*S. aureus* and *B. subtilis)* after treated with fresh and heat-inactivated *C. siamensis* sera.

1988). The complement cascade can be initiated by three distinct mechanisms, an antibody-dependent classical pathway, an antibody-independent alternative pathway and lectin pathway (Fujita 2002). Several investigators have reported the existence of an active serum complement system in a variety of reptilian species (Koppenheffer 1987). For example, Koppenheffer (1986) has evidenced the presence of both classical and alternative pathways in turtle serum. In reptiles, the complement system has been studied almost exclusively in the cobra (Sunyer and Lambris 1998). Moreover, Kuo and coworkers (2000) characterized the complement-mediated killing of the Lyme disease spirochete (*Borrelia burgdorferi*) in the western fence lizard.

Using classical human serum complement inactivation conditions (incubation at 56ºC, 30 min), we found antibacterial activities obliteration in the treated crocodile serum (Figs. 1 and 2). With evidences that the major sources of AMPs are in various tissues, as described above, and concerning our data, bacterial growth inhibition of the crocodile blood serum should correspond to serum complement system. Recently, Merchant *et al*. (2003) have presented antibacterial properties of serum from the American alligator (*Alligator mississippiensis*) and suggested an active serum complement system. We have done a preliminary study of a sheep red blood cell hemolytic assay, CH50, for the function of crocodile complement system. Its results also supported the finding in this study. However, to our knowledge, a detailed functional description of Thai freshwater crocodile complement has not been published to date. Further studies will have to be performed to find more detail in crocodile complements and AMPs.

Literature

Brogden, K.A., Ackermann, M., McCray, P.B. and Tack, B.F. (2003). Antimicrobial peptides in animals and their role in host defences. Int. J. Antimicrob. Agent 22: 465-478.

Campbell, R.D., Law, S.K.A., Reid, K.B.M. and Sim, R.B. (1988). Structure, organization, and regulation of the serum complement genes. Ann. Rev. Immunol. 6: 161.

Dodds, A.W. and Alex Law, S.K. (1998). The phylogeny and evolution of the thioester bond-containing proteins C3, C4 and a_2-macroglobulin. Immunol. Rev. 166: 15-26.

Fujita, T. (2002). Evaluation of the lectin-complement pathway and it's role in innate immunity. Nature Rev. Immunol. 2: 346-353.

Koppenheffer, T.L. (1986). Activation of the alternative pathway by both high and low molecular weight turtle antibodies. Am. Zool. 26: 86A.

Koppenheffer, T.L. (1987). Serum complement systems of ectothermic vertebrates. Dev. Comp. Immunol. 11: 279-286.

Kuo, M.M., Lane, R.S., and Giolas, P.C. (2000). A comparative study of mammalian and reptilian alternative pathway of complement-mediated killing of Lyme disease spirochete (*Borrelia burgdorferi*). J. Parasitol. 86: 1223-1228.

Lehrer, R.I. and Ganz, T. (1999). Antimicrobial peptides in mammalian and insect host defence. Curr. Op. Immunol. 11: 23-27.

Madsen, M., Hangartner, P., West, K. and Kelly, P. (1998). Recovery rates, serotypes, and antimicrobial susceptibility patterns of salmonellae isolated from cloacal swabs of wild Nile crocodiles (*Crocodylus niloticus*) in Zimbabwe. J. Zoo Wildl. Med. 29: 31-34.

Merchant, M., Roche, C., Elsey, R. and Prudhomme, J. (2003). Antibacterial properties of serum from the American alligator (*Alligator mississippiensis*). Comp. Biochem. Physiol. B, 136: 505-513.

Shaharabany, M., Gollop, N., Ravin, S., Golomb, E., DeMarco, L., Ferreira, P.C., Boson, W.L. and Friedman, E. (1999). Naturally occurring antibacterial activities of avian and crocodile tissues. J. Antimicrob. Chemother. 44: 416-418.

Sunyer, J.O. and Lambris, J.D. (1998). Evolution and diversity of the complement system of poikilothermic vertebrate. Immunol Rev. 166: 39-57.

Amoebacidal Effects of Serum from the American Alligator (*Alligator mississippiensis*)

Mark Merchant[1], Ruth M. Elsey[2] and Phillip Trosclair III[2]

[1]McNeese State University, Department of Chemistry, PO Box 90455, Lake Charles, Louisiana 70609, USA (mmerchan@mcneese.edu); [2]Louisiana Department of Wildlife and Fisheries, Rockefeller Wildlife Refuge, 5476 Grand Chenier Hwy, Grand Chenier, Louisiana 70643, USA (Elsey_RM@wlf.state.la.us)

Treatment of axenic *Naegleria gruberi* cultures with alligator serum resulted in time-dependent amoebacidal activity, with measurable activity at 5 min and maximal activity occurring at 20 min. The amoebacidal activity was concentration-dependent, with measurable activity at 25% serum, while treatment of amoebas with undiluted serum resulted in only 16% survival. The efficiency was dependent on the concentration of amoebas, with higher survival rates at high amoeba densities and lower survival at low amoeba densities. The amoeba-killing effects of alligator serum were observed to be broad in spectrum, as the serum was effective against three strains of *Naegleria* species tested. In addition, alligator serum was effective against four *Acanthamoeba* strains, which have been reported to be resistant to human serum complement-mediated lysis. The amoebacidal effects of alligator serum were shown to be temperature-dependent, with optimal activity at 15-30°C and a decrease in activity below 15°C and above 30°C. The amoebacidal activity of alligator serum was heat labile and protease sensitive, indicating the proteinaceous nature of the activity, and was also inhibited by EDTA, which indicated a requirement for divalent metal ions. These characteristics strongly suggest that the amoebacidal properties of alligator serum are due to complement activity.

GIS-based Habitat Suitability Analysis of Saltwater Crocodiles (*Crocodylus porosus*) in the Northern Territory

Yusuke Fukuda[1], G. Boggs[1] and P. Whitehead[2]

[1]RS/GIS Group, Charles Darwin University, NT 0909, Australia (s108037labmail.cdu.edu.au); [2]Key Centre for Tropical Wildlife Management, Charles Darwin University, NT 0909, Australia (peter.whitehead@cdu.edu.au)

Abstract

A habitat suitability model for saltwater crocodiles (*Crocodylus porosus*) in north-west coastal regions of the Northern Territory was constructed using GIS techniques. The requirements of saltwater crocodiles for nesting, feeding and access to open water, as defined by a review of the literature, were linked to surrogates mapped at 1:250,000 in coverages assembled in a Geographic Information System (GIS). GIS layers were assigned a weighting based on assessments of the relative importance of each layer for defining suitable habitat. Intersections of the layers were used to assign a total "habitat suitability" score to all cells in the study area, derived from the sum of the weighted scores across all layers. The predicted habitat suitability map showed distinct areas with high habitat suitability, particularly in the lower floodplains of the region. The results of the habitat suitability were summarised on a catchment basis. The Mary River Catchment had the greatest proportion of highly suitable habitat (12%) followed by the Finniss/Reynolds River Catchment (8%) and the Adelaide River Catchment (6%) whilst the Darwin/Blackmore River catchment had the lowest proportion of suitable habitat (0%). A simple validation of the model (using data from the Parks and Wildlife Service of the Northern Territory) showed a relatively high correspondence between the habitat suitability prediction and the actual population data, with an overall accuracy of approximately 35%. This model was based on readily available but relatively broadly defined attributes mapped at coarse scales. We argue that, with refinement, GIS analyses may help inform management of crocodiles and their habitat in the Northern Territory and elsewhere.

Introduction

Geographical Information Systems (GIS) are much used in natural resource management as a powerful tool for managing spatial and temporal information (eg Bridgewater 1993; Stow 1993; Berry 2000). Applications to wildlife management have taken advantage of the opportunities to link GIS with environment models to investigate and model dynamic species-environment relationships (Ackakaya 1994; Koeln *et al*. 1996). One of the most common GIS applications is habitat suitability analysis and mapping. A habitat suitability map is defined as a map displaying the suitability of land or water as a habitat for specific wildlife species (Leeuw *et al*. 2002). Production of a habitat suitability map requires a predictive model with a set of environmental variables that define the resources required by the species. GIS combines spatial representations of these variables to create the predicted habitat suitability maps.

We applied GIS techniques to predict suitable habitat on catchment basis for Saltwater Crocodiles (*Crocodylus porosus*) in the Northern Territory of Australia. The objectives of the analysis were to: 1) model habitat suitability based on environmental attributes thought to influence crocodile distribution, 2) generate a habitat suitability map of crocodiles based on the habitat suitability derived from the model, and 3) examine the validity of the predicted habitat suitability by comparing it to actual crocodile density data.

Methods

Study Area

The study area is the north west coastal regions of the Northern Territory, covering the major river systems and floodplains between the Moyle River in the west and the Glyde River (Arafura Swamp) in the northeast coast of the Northern Territory. The total area of the study site is approximately 109,500 km^2.

Input Data

Five different input datasets were used in this study:

- GEODATA TOPO 250K SERIES 2 - Hydrography Theme
- GEODATA Australian Surface Water Management 2000 (SWMA 2000)
- GEODATA 9-Second Grid Digital Elevation Model (9 Second DEM)
- Wetland Vegetation Map in the NT
- Crocodile Population Data in the NT

All the GEODATA datasets were obtained from Geoscience Australia. The Wetland Vegetation Map in the NT and Crocodile Population Data in the NT were provided by the Parks and Wildlife Service of the NT and Wildlife Management International through the Key Centre for Tropical Wildlife Management.

Habitat Modelling

Requirements for suitable habitat were determined by a literature review and from expert opinion. To reflect various aspects of crocodile biology, the habitat criteria were defined in terms of accessibility to a waterbody, availability of food, and influence on successful breeding and nesting. Based on these criteria for rating habitat favourability, environmental variables to be incorporated in the model were derived from the available data (Table 1).

Each variable was divided into 3 classes based on the statistical distribution of the variable. These classes referred to Low, Medium and High values and were assigned suitability values of 1, 2 and 3, respectively. The variables were further multiplied by a weighting value based on their impact on habitat suitability. The weightings were calculated based on the variable's relevance to the habitat criteria and their importance as determined by the literature review (eg suitability values of any variable related to permanent water were doubled) (Table 1).

After assigning the weighted habitat suitability value to each variable, the GIS layers were overlayed and the individual suitability values summed to calculate an overall habitat suitability score in the study area. The final habitat suitability scores were divided into three equal classes to represent low, medium and highly suitable areas.

Data validation of the results was performed to assess the accuracy of the model. The habitat suitability map was compared to actual crocodile population data in the NT provided by the PWCNT. An error matrix was constructed to see the fitness of the predictive model to the observed population data.

Results and Discussion

Overall habitat suitability

The output showed some distinct areas with high habitat suitability, particularly in the lower floodplains of the region (Figs. 1 and 2). In terms of habitat quality, the Mary River Catchment had the greatest proportion of high suitability habitat (12%), followed by the Finniss/Reynolds River Catchment (8%) and the Adelaide River Catchment (6%). The Darwin/Blackmore River catchment had the lowest proportion of high suitability (0%). Interestingly, the East Alligator River had the greatest proportion of habitat rated above zero suitability (59%) although the proportion of high suitability habitat was very low (0.3%).

Model validation

At the relatively crude level of our validation, the error matrix showed an overall accuracy of approximately 35% (Table 2). It should, however, be noted that, since most animal species are mobile and use different habitat types for different purposes at different times, a single assignment of habitat suitability represents a gross oversimplification (Leeuw *et al.* 2002). We conclude that the match between predicted habitat suitability and observation of variation in densities is sufficient to show the potential to rank habitat suitability at large spatial scales by application of GIS to readily available mapped information.

Limitations

It is important that the application of such approaches be matched to the management objectives. We regard this

Table 1. The variables, including different waterbody types and other environmental factors, used in the habitat suitability model, their relevance to different habitat requirements of *C. porosus* (indicated by !) and their final weighting value.

Type	Variables	Water	Food	Nest	Weight
Stream	Permanent – individual area	!			2
	Permanent – total area per catchment	!			2
	Permanent – distance to individual stream	!			2
	Permanent fringed by mangrove – individual area		!		2
	Permanent fringed by freshwater floodplain – individual area			!	2
	Temporary – individual area	!			1
	Temporary – total area per catchment	!			1
	Temporary – distance to individual stream	!			1
	Temporary fringed by mangrove – individual area		!		1
	Temporary fringed by freshwater floodplain – individual area			!	1
Swamp	Swamp – individual area	!		!	4
	Swamp – total area per catchment	!			2
	Swamp – distance to individual swamp	!			2
Lake	Permanent – individual area	!			2
	Permanent – total area per catchment	!			2
	Permanent – distance to individual permanent lake	!			2
	Temporary – individual area	!			1
	Temporary – total area per catchment	!			1
	Temporary – distance to individual temporary lake	!			1
Tidal area	Tidal area – distance to permanent freshwater stream	!			2
Freshwater floodplain	Freshwater – coastal or inland	!	!		2
	Freshwater – individual area	!	!	!	3
	Freshwater – total area per catchment	!			1
	Freshwater – ratio to length of permanent freshwater stream	!			2
	Freshwater – ratio to length of temporary freshwater stream	!			1
	Freshwater – ratio to number of permanent lake	!			2
	Freshwater – ratio to number of temporary lake	!			1
	Freshwater – ratio to number of swamp	!			2
	Freshwater – ratio to area of permanent lake	!			2
	Freshwater – ratio to area of temporary lake	!			1
	Freshwater – ratio to area of swamp	!			2
Saline floodplain	Saline – individual area	!	!		2
	Saline – total area per catchment	!			1
	Saline – ratio to area of permanent freshwater stream	!			2
	Saline – ratio to number of permanent lake/billabong	!			2
	Saline – ratio to number of swamp	!			2
	Saline – ratio to area of permanent lake	!			2
	Saline – ratio to area of swamp	!			2
Mangrove	Mangrove – individual area		!		1
	Mangrove – distance to permanent stream		!		2
Vegetation	Vegetation – vegetated by melaleuca			!	1
Elevation	Elevation – < 15m			!	1

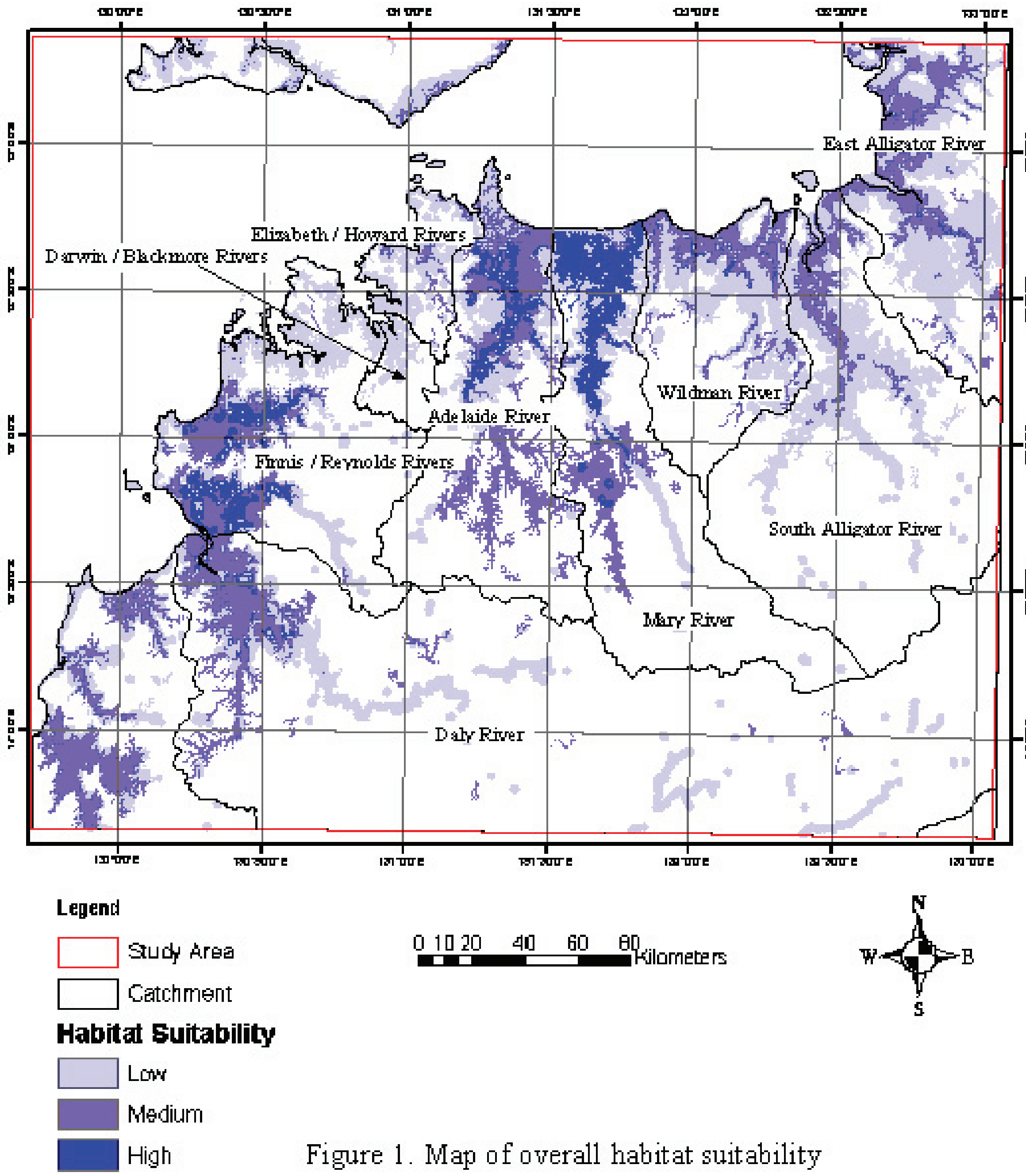

Figure 1. Map of overall habitat suitability

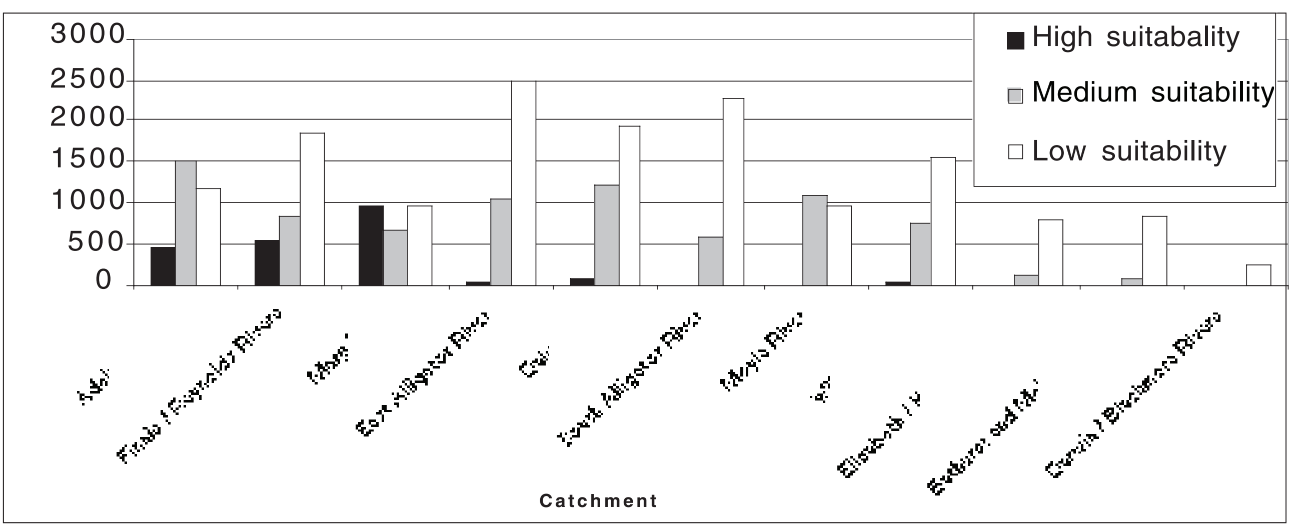

Figure 2. Area of each suitability class in each catchment.

Table 2. Error matrix of the predicted habitat suitability classes compared to the observed density classes. The values are shown as a percentage of the predicted recordings.

		Observation				
		High	Medium	Low	N/A	Total
Prediction	High	23	44	23	10	100
	Medium	18	48	28	6	100
	Low	16	33	34	16	100
	N/A	6	20	38	36	100

study as no more than a "proof of concept" in its present form. Enhancement to permit predictions relevant to management (eg sites warranting special protection) will require identification of additional data sources, perhaps at finer spatial scales, relevant to habitat needs of estuarine crocodiles. Exploration of options and refinement of methods are presently under investigation.

Acknowledgements

We would like to thank Professor Grahame Webb and Charlie Manolis who gave us the opportunity to present this work at the 17th Working Meeting of the CSG. Yusuke Fukuda also appreciates the support of Asako Kobayashi.

Literature

Akcakaya, H.R. (1994). GIS Enhance Endangered Species Conservation Efforts. GIS World 7: 36-40.

Berry, J.K. (2000). GIS Technology in Environmental Management: a Brief History, Trends and Probable Future. Pp. 49-76 *in* Global Environment Policy and Administration, ed. by D.L. Soden and B.L. Steel. Marcel Dekker: New York.

Bridgewater, P.B. (1993). Landscape ecology, geographic information systems and nature conservation. Pp. 23-36 *in* Landscape Ecology and Geographical Information Systems, ed. by R. Haines-Young, D.R. Green and S.H. Cousins. Taylor and Francis: London.

Leeuw, J.E., Ottichilo, W.K., Toxopeus, A.G. and Prins, H.T. (2002). Application of remote sensing and geographic information systems in wildlife mapping and modelling. Pp. 121-145 *in* Environmental Modelling with GIS and Remote Sensing, ed. by A. Skidmore. Taylor and Francis: London.

Stow, D.A. (1993). The role of geographic information systems for landscape ecological studies. Pp. 365-378 *in* Landscape Ecology and Geographical Information Systems, ed. by R. Haines-Young, D.R. Green and S.H. Cousins. Taylor and Francis: London.

Workplace Health and Safety Queensland - Guidelines for the Exhibition and Farming of Crocodiles

Ryan Anderson

(Ryan.Anderson@dir.qld.gov.au)

Workplace Health and Safety Queensland, a Division of the Queensland Department of Industrial Relations, is currently rewriting its guidelines for the crocodile industry. The guidelines relate to the exhibition and farming of crocodiles only and not to activities such as meat processing.

Workplace Health and Safety Queensland invites stakeholders to comment on a draft copy of the guidelines available at the poster stand. Through working with industry practical guidance material can be developed that will make the exhibition and farming of crocodiles a healthier and safer activity.

Officers from Workplace Health and Safety Queensland will be attending the stand. Please feel free to make enquiries regarding the guidelines, the process for review or any general workplace health and safety issues.

Temperature-sex Determination in the Broad-snouted Caiman (*Caiman latirostris*)

Luciano M. Verdade[1,2], Carlos I. Piña[2] and Maristela P. Miranda-Vilela[1]

[1] Animal Ecology Lab/ESALQ, University of São Paulo, PO Box 09, Piracicaba, SP 13418-900, Brazil (Tel./Fax: 55 (19) 3433-1922; lmv@esalq.usp.br);
[2] C.I.C. y T.T.P. - CONICET, Fac. de Cs. y Tec., UadER, Dr. Matteri y España, 3105 Diamante, Entre Ríos, Argentina (cidcarlos@infoaire.com.ar)
[CIP = Author for Correspondence]

The present study was carried out with the captive colony of Broad-snouted caiman (*Caiman latirostris*) at the Laboratório de Ecologia Animal/ESALQ/USP, in the summer of 2001. We collected eggs from two clutches within the first 24 hours after egg-laying and incubated them at temperatures of 28, 30, 32 and 34ºC. The eggs were placed on plastic trays and covered with vermiculite, which was kept humid. Digital Thermolab® 6030 thermometers were attached to each incubator near the egg trays, and the temperature was registered every morning. The mean hatching success at 30°C was 70%, at 32°C was 79% and at 34°C was 65%, and at 28°C was nil. At temperatures of 30°C the sex ratio produced was 10% male and 90% female; at 32°C it was 46% male and 54% female; and, at 34°C the gender produced in both nests was 100% male.

Diet and Condition of American Alligators (*Alligator mississippiensis*) in Three Central Florida Lakes

Amanda N. Rice

Everglades National Park SFNRC, 40001 SR 9336, Homestead, FL 33034, USA (amanda_rice@usgs.gov, anrzoo@aol.com)

Understanding the diet of crocodilians is important because diet affects condition, behavior, growth, and reproduction. In this study, I examined the diet and condition of adult American alligators (*Alligator mississippiensis*) in three central Florida lakes, Griffin, Apopka, and Woodruff. Alligators ate a variety of vertebrates and invertebrates, but vertebrates were more abundant and fish dominated alligator diets in the lakes. Species composition of fish varied among the lakes. The majority alligator diets from Lakes Apopka and Woodruff was fish, 90% and 84% respectively. Lake Apopka alligators consumed a significantly ($P = 0.006$) higher proportion of fish. Fish were 54% of Lake Griffin alligator diets and the infrequent occurrence of reptiles, mammals, birds, and amphibians often resulted in a large biomass.

Alligator condition (Fulton's Condition Factor, K) was significantly ($P < 0.001$) different among the lakes. Alligators from Lake Apopka had the highest condition, followed by those from Lake Griffin, and alligators from Lake Woodruff had the lowest condition. Composition of fish along with diversity and equitability of fish in alligator diets may have contributed to differences in condition among lakes. The observed diet and condition differences probably reflect both habitat differences and prey availability in these three lakes.

Cranial Morphometric Study of Chinese Alligators in Different Age Groups

Xue Hui[1] Wu Xiaobing[1], Jiang Hongxin[2], Ruan Xiangdong[2] and Nie Jishan[3]

[1]College of Life Sciences, Anhui Normal University, Key Lab for Conservation and Utilization of Biological Resources in Anhui, Wuhu 241000; [2]Department of Wildlife Conservation, SFA, Beijing 100091; [3]Anhui Researching Center for Chinese Alligator Reproduction, Xuancheng 242000, China

Abstract

Morphology study is the most basic part of biology, while cranial morphology study is especially important for crocodilians. In this paper, 98 samples were taken from different age groups of Chinese alligator according to the measurements adapted from Iordansky (1973), 14 variables were achieved from each specimens. Meanwhile, 10 ratio variables were also introduced because the cranial morphology traits depend on some of these ratios. The ratio variables characterized the short and broad trait of head, as well as indicated that the orbital is rather large and broad. Most of the ratios of different age groups have statistical difference except relative length of mandibular symphysis (RLSS; p= 0.369), which indicated that there were distinctions between different phases ontogenetically. This study suggested that ratio variables vary regularly most of the time. It's obviously useful to grasp those ratios and their variations among different age groups for alligator's cranium and fossil identification, age-estimation, and wild investigation.

Introduction

Cranial morphology traits depend on some optimal ratio variables in crocodylidae and cranial morphology research has been done on many species, such as *Alligator mississippiensis*, *Crocodylus novaeguineae*, and *Caiman latirostris* (Allstead *et al*. 1995; Hall *et al*. 1994; Verdade *et al*. 1998). Beside the visual observation, the pertinent datum is much more necessary for qualitative analysis of cranial shape, containing the traits of length, width and height, and comparison between interspecies, different geographical populations, and different age groups. Iordansky (1973) defined 14 measurement variables and 10 ratio variables to depict the configuration and appearance of the whole cranium. These variables were received by most of the researchers (Verdade *et al*. 1998; Cong *et al*. 1998). Measurement record about Chinese alligator (*Alligator sinensis*) had been seen in several papers (Zhu *et al*. 1957; Huang *et al*.; Cong *et al*. 1998). But small sample size and uncoincident measurement standards didn't suit for comparison and analysis of cranial morphology.

Mook (1921) and Iordansky (1973) found that the cranial shape varied with the animals growing, and snout changes from acute to blunt, from narrow to broad, and from short to long; orbital shape and size vary at the same time; the cranial sculpturing become more and more. Chen (1985) did some primary research on the difference of cranium between the young and the old, while the few samples can't draw a conclusion for them. In this paper, the cranial shape of different age groups was compared to explore the rule in the varying course.

Materials and Methods

The samples were taken from Anhui Researching Center for Chinese Alligator Reproduction (ARCCAR). There are five age group animals in the center, including eight-month-old, one-year-old (about 15 months), two-year-old (about 27 months), three-year-old (about 39 months) and four-year-old (more than 51 months, containing few five-year-old individuals, as the four-year-old group and the five-year-old group were bred together and unable to be identified in ARCCAR, the four-year-old is considered as matured). Fourteen head variables were taken for each alligator, in addition to total length (TL) and snout-vent length (SVL) (Table 1). Instruments needed are simple: a steel electronic digital caliper (0.01 mm precision, third decimal unconsidered) and a tape. The study began at the onset of the animals' hibernation period to reduce the infection on the animals.

Before analyzing the data, normality and homogeneity were tested by Kolmogorov-Simirnov, Shapiro-wilk and

Table 1. Measurements (adapted from Iordansky 1973).

Abbreviation	Explanation	Unit
DCL	Dorsal cranial length: anterior tip of snout to posterior surface of occipital condyle	mm
CW	Cranial width: distance between the lateral surface of the mandibuar condyles of the quadrates	mm
SL	Snout length: anterior tip of snout to anterior orbital border	mm
SW	Basal snout width: width across anterior orbital borders	mm
OL	Maximal orbital length	mm
OW	Maximal orbital width	mm
IOW	Minimal interorbital width	mm
LCR	Length of the postorbital cranial roof: orbital border to the posterolateral margin of the squamosal	mm
WN	Maximal width of external nares	mm
PXS	Length of palatal premaxilary symphysis	mm
ML	Mandible length	mm
LMS	Length of the mandibular symphysis	mm
WSR	Surangular width	mm
LM	Length of lower ramus	mm

Levene-test on them. After tested, some data was transformed to fit for the need of the analysis later. One-way ANOVA and Tukey' HSD were used to test the difference significance and compare the correspondent data.

Results

Descriptive statistics for measurements of the head of each age group were shown in Table 2. Ten ratio variables were introduced (see Table 3) and the ratio were tested by One-way ANOVA and Tukey' HSD to find the difference between age groups (see Table 4). The result indicates that most of the ratio variables exist difference between each group.

Analysis and Discussion

<u>Ratios about cranium</u>

In other species of Crocodylidae, there is a difference between two measurements - dorsal cranial length and axial length of cranium and axial length cranial (between the tip of the snout and the posterior margin of the occipital condyle) is longer usually, but the two measurements are very similar in Chinese alligator. So the dorsal cranial length (DCL) is regarded as cranial length approximately. Cranial length of 8-month old Chinese alligator is about (28.35 ± 0.19) of SVL, (13.53 ± 0.10) of TL, while the length of the 4-year old is about (22.75 ± 0.41) of SVL, (11.34 ± 0.07) of TL. The proportion in SVL becomes less and less gradually from 8-month to 4-year-old ($F_{4, 93}$= 33.062, $p<0.001$). Thus it can be seen, the relative length of cranium takes on reverse ratio function relationship with age (see Fig. 1).

Usually cranial width index was used to express relative cranial width, which is the major factor to decide the cranial type, as well as directly reflect whether the cranium is broad or not. The high value of cranial width index embodies the short broad trait of cranium of Chinese alligator obviously. Cong *et al.* (1998) compared the Chinese alligator with other species, and found that the index of Chinese alligator was greater than most of other crocodilians. When testing RCW of 5 age phases of Chinese alligators, we found that the value of one-year and four-year individuals is relatively high; that of 8-month and three-year individuals was not high; and it is relatively low in the two-year individuals (see Fig. 1, RCW). The cranial width index of different age varies irregularity and the RCW has no linear correlation with age.

<u>Ratios about snout</u>

Snout also can be called rostrum, whose relative length determines the stretch of itself and effects on the cranial

Table 2. Descriptive statistics for head-size in Chinese Alligator. N= sample size; M= mean; CV= coefficient of variation; O.R.= observed value range.

	8 month (N= 18)			One year (N= 20)			Two year (N= 20)			Three year (N= 20)			Four year (N= 20)		
	M	CV	O.R.	M	CV	O.R.	M	CV	O.R.	M	CV	O.R.	M	CV	O.R.
DCL	39.74	0.033	37.96-43.60	52.21	0.054	47.32-58.49	68.99	0.051	62.21-75.61	95.67	0.051	86.49-105.14	132.42	0.050	117.18-142.46
CW	24.51	0.054	23.08-25.88	32.67	0.074	28.80-39.32	41.14	0.045	37.82-44.77	58.01	0.053	52.48-63.61	82.85	0.068	70.76-90.74
SL	16.72	0.086	11.86-19.00	24.37	0.069	21.36-28.44	35.01	0.082	30.08-39.81	48.66	0.062	42.73-54.86	69.49	0.060	62.19-76.73
SW	18.88	0.093	13.60-21.80	25.09	0.073	20.83-28.44	33.96	0.049	30.72-36.69	48.90	0.067	40.76-54.37	69.46	0.065	58.79-79.01
OL	12.85	0.038	12.16-13.62	15.49	0.054	13.98-17.39	20.11	0.058	17.91-22.41	23.42	0.054	20.76-25.54	31.08	0.087	27.03-37.54
OW	10.99	0.043	10.20-12.00	12.65	0.062	11.61-14.34	15.89	0.083	13.63-18.36	18.30	0.088	15.23-22.70	22.41	0.070	19.11-26.57
IOW	2.68	0.132	1.94-3.34	4.48	0.124	3.38-5.53	5.32	0.124	3.87-6.44	6.78	0.128	5.21-8.46	8.44	0.170	5.27-12.27
LCR	13.73	0.062	12.24-16.48	16.24	0.072	14.43-19.13	20.17	0.061	18.03-22.24	25.60	0.072	22.55-28.82	31.76	0.069	28.02-36.15
WN	6.70	0.029	6.28-7.00	8.48	0.080	6.98-9.73	10.99	0.069	9.39-12.09	16.86	0.064	14.92-19.43	24.36	0.052	21.27-26.25
PXS	8.07	0.081	6.56-8.84	11.02	0.074	9.70-12.38	14.58	0.086	12.24-16.64	20.77	0.083	17.06-24.12	28.94	0.118	24.55-38.85
ML	39.32	0.031	37.52-41.68	55.39	0.068	44.44-62.95	74.29	0.052	66.99-79.75	103.26	0.080	75.22-114.93	145.88	0.051	128.70-156.58
LMS	6.84	0.056	6.12-7.70	13.09	0.097	7.62-10.84	11.53	0.085	10.26-13.87	16.43	0.067	14.52-18.96	22.66	0.090	17.83-27.20
WSR	23.53	0.045	22.00-26.36	30.42	0.073	26.05-33.90	40.33	0.062	35.17-45.01	58.27	0.056	52.01-65.35	85.49	0.065	72.85-95.42
LM	22.70	0.060	20.54-25.62	39.22	0.070	32.99-45.49	54.48	0.069	48.01-61.57	74.58	0.066	66.50-81.97	101.59	0.058	90.42-115.56

Table 3. Head-size ratios (introduced from Iordansky 1973). Usually use the ratio as index form, meaning 100 times of the relative length.

Abbreviation	Explanation
RCW	Relative cranial width: CW/DCL
RLST	Relative length of snout: SL/DCL
RWST	Relative width of snout: SW/SL
ROL	Relative orbital length: OL/DCL
ROW	Relative orbital width: OW/OL
RWI	Relative interorbiatl width: IOW/OL
RWN	Relative width of external nares: WN/(DCL-SL)
RPXS	Relative length of premaxillary symphysis: PXS/DCL
RLSS	Relative length of mandibular symphysis: L MS/ML
RWM	Relative width of mandible: WSR/ML

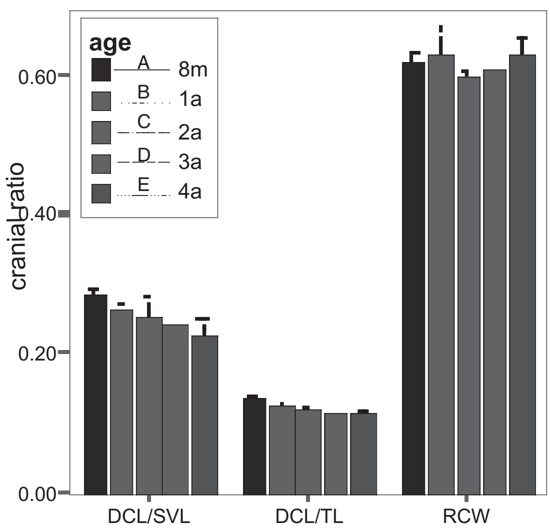

Figure 1. The bars show the mean ratios about cranium of different age groups, Y scale axis refer to the ratio value.

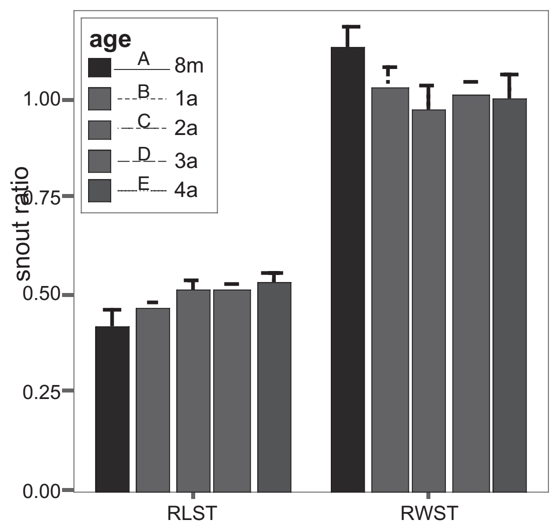

Figure 2. The bars show the mean ratios about snout of different age groups, Y scale axis refer to the ratio value.

length and shape of whole cranium. Figure 2 indicates that RLST become greater gradually and the values of different ages were documented to have significant difference by test. Obviously, the extension of snout increases step by step, at the same time the orbital location move backward relatively.

The width index of snout (100 times of RWST) is used to judge the extent of long or narrow, short or broad: °‹40 refers to elongate, °›70 refers to broad, and >40, <70 refers to intermediate. Mean width indexes of snout of the young and the old are all about 100, even more than 100. The snout width index listed in this paper is greater than that with 88-83 (Cong, L.Y, 1998), which may be caused by the measurement difference between the living and the skeleton or other reasons. However, the Chinese alligator belong to broad type without any question. In the same type, index of Broaded-snouted Caiman (*Caiman latirostris*) is especially great, and even reaches near to 130 in some large individuals (Verdade 1998). RWST of 8-month-old individuals is the greatest among all objects measured, one-year secondly, the three- and four-year old have no significant difference with the two aforementioned, the two-year individuals is lowest. Without considering the two-year old, snout width index take on decreasing trend with the animals growing up.

<u>Ratios about the orbita</u>

The length of orbita is always greater than the width of orbita in crocodilians, the smaller the individual, the bigger the proportion of orbita in cranium, which presents as ROL and ROW decreases with the animals growing up in the

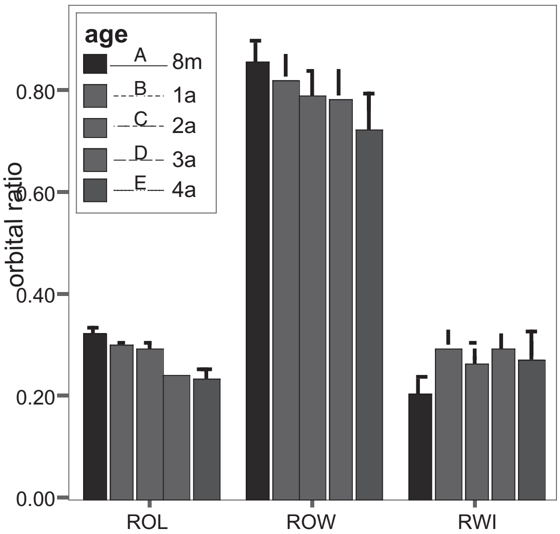

Figure 3. The bars show the mean ratios about orbita of different age groups, Y scale axis refer to the ratio value.

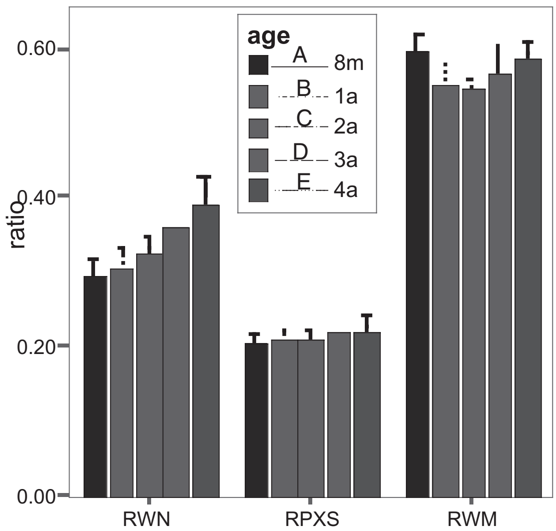

Figure 4. The bars show the mean ratios of other variables of different age groups, Y scale axis refer to the ratio value.

measurements, whereas RWI takes on increasing trend. As Figure 3 shows, RLST and RWST reduce gradually with growing. This indicates the proportion of orbita in cranium shows descending trend and the shape becomes narrow relatively. RWI is relatively low in the 8-month old; other age stages don't differ significantly. Through comparison, values of RWST and RLST are greater than that of other species, nevertheless the RWI are lower than other ratios. These differences make up of the large and broad trait of orbita.

Other ratios

The relative length of premaxillary symphsis (RLSS) increases with the age increasing, that is to say, the proportion of premaxillary symphsis in cranium appears increasing trend and they grow relatively fast in contrast to whole cranium. The relative width of mandibular of 8-month individuals is the greatest, four-year-old individuals in the next place, one-year, three-year-old thirdly, the two-year-old is the lowest, whole trend take on "V" shape (see Table 4): the 8-month value is the greatest, then the ratio descends with growth, later rise again. No age differences were recognized in RLSS. RWN of different age groups differs significantly and takes on rapidly increasing tendency.

Conclusions

In taxonomy, Chinese alligator belongs to genus *Alligator*, Family Alligatoridae. High RCW and RWST values decide the outstanding morphology traits of them - broad cranium and broad snout. So, this species is classified as short-snouted type. The value of ROW is high relatively, and the orbita is relatively broad, when compared with other species. Some ratio variables (RLST, RPXS, RWN) take on increasing trend, some (ROL, RWST) take on descending trend, and several (RWM) takes on descending then increasing trend. Thus it can be seen, the whole ontogenetic trends of cranium in Chinese alligator take on: With age increasing, the trend of relative cranial width was not evident, probably the hatchlings and adults have great RCW value, while the middle ages have low value; The snout extend fast relatively, which make the snout turn narrow relatively; Both ROL and ROW reduce with individuals growing up, which reflect the relatively lessening of orbita against the age. In addition, both the length of premaxillary symphsis and width of nares increase rather rapidly, and the relative width of mandibular vary in V-shaped.

It is obviously useful to grasp those ratios and their variations of different age groups for alligator's cranium and fossil identification. It also offered a feasible way to estimate the age of Chinese alligators. When identifying the cranium and fossil, the pertinent head-size variables and ratio variables can be used as standard to compare with the values of cranium and fossil being identified. Meantime, the regression equations between head-size variables and snout-vent length, the head-size variables, head ratio variables and age can predict the body length and age of the alligator met in wild.

Table 4. Head-ratio variables' mean value and comparison between different age groups of Chinese alligator. One-way ANOVA to test the difference between different age groups (significance to 0.05 level), the result indicates except the relative length of mandibular symphysis (RLSS) all of other variables have significant difference. Means with different superscripts differ significantly _ a>b>c>d.

Age	RCW (M±SE)	RLST (M±SE)	RWST (M±SE)	ROL (M±SE)	ROW (M±SE)	RWI (M±SE)	RWN (M±SE)	RPXS (M±SE)	RLSS (M±SE)	RWM (M±SE)
8 m	0.6168±0.0031^{ab}	0.4214±0.0095^{c}	1.1301±0.0131^{a}	0.3234±0.0027^{a}	0.8562±0.0089^{a}	0.2085±0.0058^{b}	0.2932±0.0056^{d}	0.2030±0.0034^{b}	0.1739±0.0022	0.5985±0.0052^{a}
	0.5826-0.6356	0.2720-0.4664	1.0356-1.2862	0.3060-0.3436	0.7944-0.9155	0.1588-0.2504	0.2073-0.3188	0.1702-0.2184	0.1592-0.1940	0.5366-0.6324
1a	0.6262±0.0092^{a}	0.4666±0.0037^{b}	1.0304±0.0121^{b}	0.2969±0.0024^{b}	0.8175±0.0113^{bc}	0.2895±0.0080^{a}	0.3053±0.0061^{cd}	0.2111±0.0025^{ab}	0.2363±0.0731	0.5497±0.0062^{cd}
	0.5834-0.7915	0.4431-0.4943	0.9559-1.1468	0.2764-0.3236	0.7066-0.8875	0.2120-0.3563	0.2497-0.3539	0.1894-0.2288	0.1399-1.6239	0.4819-0.6085
2a	0.5965±0.5965^{b}	0.5072±0.0067^{a}	0.9737±0.0133^{c}	0.2917±0.0031^{b}	0.7901±0.0110^{b}	0.2654±0.0078^{a}	0.3241±0.0051^{c}	0.2111±0.0025^{ab}	0.1551±0.0021	0.5428±0.0031^{d}
	0.5749-0.6245	0.4639-0.6008	0.8247-1.0888	0.2654-0.3163	0.7088-0.8707	0.1861-0.3267	0.2880-0.3880	0.1922-0.2299	0.1355-0.1739	0.5222-0.5728
3a	0.6065±0.0040^{ab}	0.5086±0.0040^{a}	1.0053±0.0080^{bc}	0.2453±0.0036^{c}	0.7834±0.0175^{b}	0.2905±0.0092^{a}	0.3591±0.0043^{b}	0.2170±0.0029^{a}	0.1600±0.0035	0.5665±0.0085^{bc}
	0.5825-0.6696	0.4796-0.5465	0.9490-1.0819	0.2153-0.2828	0.6876-0.9627	0.2130-0.3732	0.3252-0.4030	0.1953-0.2602	0.1384-0.2123	0.5371-0.7148
4a	0.6255±0.0057^{a}	0.5252±0.0060^{a}	1.0010±0.0139^{bc}	0.2347±0.0035^{c}	0.7249±0.0153^{c}	0.2733±0.0118^{a}	0.3895±0.0081^{a}	0.2185±0.0051^{a}	0.1553±0.0023	0.5859±0.0046^{ab}
	0.5576-0.6625	0.4709-0.5956	0.8920-0.1422	0.2013-0.2671	0.6135-0.8810	0.1771-0.4301	0.3261-0.4904	0.1897-0.2847	0.1360-0.1739	0.5494-0.6342
F	$F_{4, 93}$= 5.387 P= 0.001	$F_{4, 93}$= 44.311 P<0.001	$F_{4, 93}$= 23.119 P<0.001	$F_{4, 93}$= 140.544 P<0.001	$F_{4, 93}$= 12.861 P<0.001	$F_{4, 93}$= 13.627 P<0.001	$F_{4, 93}$= 43.651 P<0.001	$F_{4, 93}$= 3.074 P_0.020	$F_{4, 93}$= 1.085 P_0.369	$F_{4, 93}$= 15.856 P<0.001

Acknowledgements

We thank the Anhui Research Center for Chinese Alligator Reproduction for their gracious assistance in the data collection. This study was sponsored by National Natural Science Foundation of China (NSFC, No. 30270213), Natural Science Foundation of Anhui Province (No. 01043501), special funds for Anhui Young Leaders Fellowship Fund (04043049), the grants from National Forestry Administration and Conservation and Utilization of Important Biological Resources of Anhui Key Laboratories.

Literature

Allstead, J. and Lang, J.W. (1995). Sexual dimorphism in genital morphology of alligators, *Alligator mississippiensis.* Herpetologica 51(3): 314-325.

Booktein, F.L. (1989). Size and shape; comment on semantics. Systematic Zoology 38(2): 173-180.

Chen B.H and Hua, Z.H. (1985). Chinese alligator. Science and Technology Press, Hefei 2: 19-28.

Cong L.Y. and Hou, L.H. (1998) The cross anatomy of *Alligator sinensis* Fauvel. Science Press, Beijing 2: 82-107.

Cynthia, L.G. (2003). A first look at estimating body size in dentally conservative marsupials. Journal of Mammalian Evolution 10(1/2): June 2003.

Hall, P.M. and Portier, K.M. (1994). Cranial morphometry of New Guinea crocodiles (*Crocodylus novaeguineae*): ontogenetic variation in relative growth of the skull and an assessment of its utility as a predictor of the sex and size of individuals. Herpetological Monographs 8: 203-225.

Herbert, J.D. and Coulson, T.D. (2002). Growth rate of Chinese and American alligators. Comp. Biochem. Physiol. Part A 131: 909-916.

Iordansky, N.N. (1973). The skull of the Crocodilia. Pp. 201-262 *in* Biology of the Reptilia. Vol. 4. Morphology D, ed. by C. Gans and T.S. Parsons. Academic Press: London.

Magnusson, W.E. (1982). Techniques of surveying for crocodiles. Pp. 389-403 *in* Crocodiles. Proceedings of the 5th Working Meeting of the IUCN-SSC Crocodile Specialist of Group. Florida State Museum, Gainesville, Florida, USA.

Mook, C.C. (1921). Individual and age variation in the skulls of Recent Crocodilia. Bull. Amer. Mus. Nat. Hist. 44 (7): 51-56.

Verdade, L.M. (1990). Regression equations between body and head measurements in the Broad-snouted Caiman. Rev. Brasil. Biol. 60(3): 469-482

Verdade, L.M. (1997). Morphometric Analysis of the Broad-snouted Caiman (*Caiman latirostris*): An Assessment of Individual's Clutch, Body Size, Sex, Age, and Area of Origin**.** PhD Dissertation, University of Florida, Gainesville, Florida, USA, 174 pp.

Webb, G.J.W. and Messel, H. (1977). Crocodile capture techniques. J. Wildl. Manage. 41: 572-575.

Wermuth, H. (1953. Systematik der rezenten Krokidile. Mitt. Zool. Mus. Berlin 29(2): 375-514.

Wermuth, H. and Mertens, R. (1961). Schildkröten. Krokodile Brückeneschsen. Veb Gustav Fisher Verlag: Jena, Germany.

Wild Harvest of Crocodile Eggs: The Economic Benefits to Resource Owners and its Effect on Habitat Conservation.

David H Wilken and Eric Langelet

Mainland Holdings Ltd, PO Box 196, Lae 411, Papua New Guinea
(crocfarm@mainland.com.pg)

Introduction - Background

Papua New Guinea's two species of crocodiles, the Saltwater crocodile (*Crocodylus porosus*) and the New Guinea Freshwater crocodile (*Crocodylus n. novaguineae*) are not only an integral part of the traditional culture for the Sepik River people but, since the 1950s have also become a commercially valuable natural resource.

Because both species were extensively exploited for their skin and to a lesser extend their meat, the PNG Government in 1966 enacted the *Crocodile Trade (Protection) Act* to regulate the crocodile industry. Monitoring mechanisms to ensure their long term survival and, consideration for the welfare of the rural population have also been introduced.

In the early 1980s, the introduction of ranching and farming schemes added even more incentives for village people to continue benefiting from their natural resources while also encouraging them to actively protect the environment.

To further enhance the impact of its crocodile management program, the Department of Environment and Conservation in 1985 introduced a selective harvest of wild crocodile eggs in the surveyed area of the Middle Sepik River. This activity is run conjointly with the aerial survey and is closely monitored by DEC wildlife rangers. Mainland Holdings Ltd (MH), the largest commercial farm in Papua New Guinea, is an integral part of this national conservation policy. The company's ranching program largely contributes to the economic success of the wild harvest of juvenile crocodiles and crocodile eggs.

Eggs Harvest - 2002 and 2003

Over the years, the combination of regular awareness campaigns, the effective enforcement of the *Crocodile Trade (Protection) Act* and various private commercial incentives have encouraged the steady recovery of both crocodile species in the Sepik.

Since 2000, the Sepik Wetlands Management Initiative, a local non-Government organization based in Ambunti, has also actively campaigned to strengthen the concept of "sustainable use of the wildlife" amongst the villagers. A Participatory Rural Appraisal has concluded that crocodiles still constituted a major source of incomes for the Middle Sepik communities but, also raised concerns about the long term sustainability of the nesting habitat.

Consequently, in March 2002, the DEC agreed to further increase the harvest program for the wild Saltwater crocodile eggs in the Middle Sepik, in return for habitat conservation. A total of 3542 fertile eggs from 62 nests were purchased in this first motorised canoe harvest.

Again, in February 2003, a team of two Mainland employees accompanied by a DEC wildlife ranger and local guides departed Ambunti in two groups to harvest saltwater crocodile nests. One team visited the areas from Kubkain to the mouth of the May River, while the other team concentrated its search from Kubkain down to Wagu Lakes.

During this time 138 nests and 8346 eggs were harvested. Later, some more nests were brought in by the landowners during the annual aerial nests survey conducted by the DEC. At third trip was organized to make cash payment for the eggs harvested. Unfortunately by that time most of the remaining nests had already hatched or were flooded.

In 2003, it was generally observed that most of the habitats had well regenerated since the widespread burning of the 1997 "El Niño" drought and, only one hook / trap was found. The landowners were found to be very enthusiastic with the concept and keen to protect the nesting areas while at the same time reaping a better financial benefit as compared to hunting juvenile crocodiles.

Financial perspective of eggs harvesting for the landowners

Average price paid by MH for a 2-year-old live crocodile (71-75 cm TL) **= K50.00**

Average price paid by a local crocodile buyer to the hunter (landowner) for the same crocodile = **+/- K25.00**

Net benefit earned by the resource owner

Price paid per crocodile	K25.00
Hunting costs (petrol, batteries, …), freight, mortality losses	-K15.00
Net profit per crocodile =	K10.00

It is estimated by MH suppliers that the mortality of small crocodiles so caught could be as high as 50%. That is, 100 crocodiles supplied to MH = 200 wild crocodiles taken from the wild.

On the other hand, MH pays K7.00 per fertile egg. In 2003, the incubator hatchability reached 80%. That is, 100 hatchlings = 125 fertile eggs taken from the wild**.**

If we also take into account that only 4.1% of the eggs laid (with a 25% hatchability) in the wild will survive as juveniles (Webb and Manolis 1989), the ecological cost of small crocodile hunting versus egg harvesting is:

100 live crocs = 200 wild animals = 1242 fertile eggs
100 hatchlings = 125 fertile eggs.

These 1242 fertile eggs translate into no more than K3750 (200 animals removed from the wild - 25% mortality = 150 crocs sold to local buyer x K25.00 each) for the landowner, or a value of K3.02 per fertile egg, compared to K7.00 per fertile egg paid by MH! There is also a 10x "ecological" saving factor in harvesting fertile eggs versus live crocodiles.

It is therefore our belief that by giving the resource owners a much greater return from the assets, they will continue supporting the initiative of eggs harvesting and refrain from burning the nesting habitats.

Eggs Harvest - 2004

In February 2004, another Saltwater crocodile harvest was undertaken, at an earlier date to avoid the flooding of the river and to precede the peak hatchling period, again with two teams.

It was decided not to harvest eggs in areas where the nesting habitats were still threatened or where villagers did not yet show much interest in preserving it. The villagers were informed of the harvesting dates and more actively searched for the nests. As a result, this year's harvest yielded 12,756 eggs from 215 nests.

As in 2003, the accurate position of all the accessible nests by motorized and paddle canoes were recorded on a "Garmin etrex" handheld GPS.

Conclusions

It is believed that the economic incentive to protect the breeding stock and the habitat is having a very positive effect on the recovery of the crocodile population. Most of the swamps and scrolls visited showed healthy vegetation suitable as nesting habitat and quite a few nests were built by young females. The teams noticed that less nests had been "raided" for egg consumption.

It appears that the recent initiative by SWMI, in collaboration with DEC and MH, to increase the annual saltwater crocodile egg harvest in the Middle Sepik has been positively supported by the villagers. This year's overwhelming participation by communities even exceeded our predictions.

From a conservation point of view, it must be emphasized that only the accessible nests, built on the fringes of the swamps were harvested. Those situated deep in the herbaceous swamps or a long distance from the villages were spared. Many landowners also chose to save some nests to hatch naturally. Because of the nature of the nesting habitat and the harvesting technique, it was virtually impossible to quantify the proportion of nests left. Only a systematic aerial survey would enable an observer to scientifically determine the harvesting index.

Literature

Cox, J. (2002). Participation Rural Appraisal Techniques for Conservation of Crocodile Nesting Habitat in the Middle Sepik. Unpublished Technical Report No. 1, Port Moresby.

Genolagani. J.M.G. (1994). Conservation, management and farming of crocodiles in Papua New Guinea. In Crocodiles. Proceedings of the 2nd Regional (Eastern Asia, Oceania, Australasia) Meeting of the Crocodile Specialist Group, IUCN: Gland.

McKie, R. and McKie, G. (1993). Reflections of the Sepik. Crawford House Press.

Solmu, G.C. (2002) Participatory Rural Appraisal. A report produced in participation with the Sepik Wetlands Management Initiative. DEC: Port Moresby.

Webb, G.J.W and Manolis, S.C. (1989). Crocodiles of Australia. Reed Books: Australia.

Whitaker, R. and Mitchell, G. (1989). Comparison of farms. Pp. 214-215 *in* Crocodiles and Alligators, ed. by C.A. Ross and S. Garnett. Golden Press: Silverwater.

Observations of the Effect of Toxic Blue Green Algae on Crocodiles

Vicki R. Lowing

6 Minnipa Drive, Hallett Cove 5158, SA, Australia (vicki@txc.net.au)

Introduction

An 8-year-old Australian Freshwater Crocodile (*Crocodylus johnstoni*), raised from a hatchling in a domestic environment near the township of Deniliquin, located in the Murray-Darling Basin Region, southwestern New South Wales, Australia, was observed to develop abnormal various signs and symptoms following exposure to water containing high blue green algal cell counts.

This area is predominately rice production, requiring high levels of irrigation with increasing usage of herbicides, pesticides and fertilizers (nutrient pollutants, particularly phosphates). Eutrophication is recognized as a critical factor in creating an algal bloom; as well as low flow of water (irrigation) and high summer temperatures. The environmental impact is one of increasing mixed strains of blue green algal cells in the local water supply.

Findings

During the period from December 2002 to April 2003, environmental daytime temperatures ranged from approximately

Murray Irrigation Limited- *Algal Alert*
Friday 31st January 2003

443 Charlotte St (P.O. Box 528) Deniliquin NSW 2710
Phone (03) 5881 9300 Fax (03) 5881 9317

Murray Irrigation Limited

High Algae Alert for Mulwala Canal

High levels of blue green algae in the Murray River system have moved into the Mulwala Canal.

Murray Irrigation has announced a high alert for the Mulwala Canal between Lake Mulwala and Lawson's Syphons after preliminary testing this week.

If high levels of algae continue in the Murray River system it is likely the contamination will spread to the remainder of the channel system.

The discovery of blue green algae in the Mulwala Canal follows a high alert issued for the Hume Dam late last week. Murray irrigation is closely monitoring the situation and will continue to inform shareholders about the occurrence of algae.

High levels of blue-green algae make water unsuitable for drinking or cooking. **Boiling does not destroy algae toxins.** Any contact with the affected water should be minimised. Blue Green algae is potentially toxic and may cause gastroenteritis and liver damage in humans if consumed. It is also known to cause stock illness or even death. Dogs are particularly sensitive.

Landholders who draw a domestic supply from the channel system should make contingency arrangements for alternative water supplies. Activated carbon is the only effective filtration system to remove toxins.

Landholders using water for stock from the channel system should regularly check water supplies for bright green colouration and odours. They should check stock for scours, disorientation or other illness and consider contingencies and possible alternative water supplies.

Irrigation of pastures may pose some risk to stock who could take up toxins while grazing. However, it is unknown how much toxin the pasture would take up, or how long the pasture would be affected. To minimise any risk, leave irrigated pastures for as long as possible before grazing. Rainfall or irrigating with algae free water will help wash any potential toxins from the pasture.

Landholders are asked to be watchful for earthy or musty smells, tastes or surface scums of green, yellow or blue-green. Only examination of a water sample under the microscope will confirm the presence of blue-green algae.

Further information on blue green algae can be found at www.murraybluegreenalgae.com or by ringing Department of Land and Water Conservation's toll free hotline 1800 088510.

Deputy General Manager
Warren Elsbury

Figure 1. High algae alert.

Murray Irrigation Limited- *Algal Alert*
Thursday 6th February 2003

443 Charlotte St (P.O. Box 528) Deniliquin NSW 2710
Phone (03) 5881 9300 Fax (03) 5881 9317

Murray Irrigation Limited
ACN 067 197 933

Medium Algae Alert for Mulwala Canal

Alert levels for blue green algae in the Mulwala Canal have been downgraded from a high alert to a medium alert level, co-inciding with a fall in algae levels in the Murray River.

Murray Irrigation has reduced its alert level after extensive monitoring of all its main irrigation supply channels. Algae levels in the Mulwala Canal and main lateral channels were at a medium alert level when samples were taken on Monday and Tuesday this week.

Murray Irrigation's environment manager Alex Marshall said "although algae levels appeared to have fallen, landholders should not use channel water for domestic supplies and should make contingency arrangements for alternative water supplies."

"Blue green algae is potentially toxic and dangerous to human and animal health," Mr Marshall said. "Water affected by blue green algae is not suitable for drinking or cooking and skin contact should be avoided. **Boiling affected water will not make it safe to use. Drinking affected water may cause** gastroenteritis and liver damage in humans. It is also known to cause stock illness or even death and dogs are particularly sensitive."

Murray irrigation is closely monitoring the situation and will continue to inform shareholders about the occurrence of algae.

Landholders with stock are asked to be watchful for signs of blue green algae in their water supplies. Scums can form, varying in colour from pale green/brown to bright fluorescent green, and may appear paint-like or granular in texture. Under windy conditions, the scum can accumulate along leeward banks. Blooms of blue green algae often give off a characteristic musty odour. Only examination of a water sample under the microscope will confirm the presence of blue-green algae.

Symptoms of blue green algae affected stock include scours, disorientation or other illness.

Further information on blue green algae detection and treatment can be found by calling the DLWC hotline 1800 088 510 or at the following web site: www.murraybluegreenalgae.com.

Ends

For further information please contact:
Mr Warren Elsbury, Deputy General Manager 03 5881 9352, mobile 0429 819 351
Mr Alex Marshall, Environment Manager 03 5881 9331, mobile 0428 819 331

Figure 2. Medium algae alert.

30°C to 45°C, resulting in Medium to High Blue Green Algae Alert Warnings for the local water supply in this area (Figs. 1 and 2).

However, these alert warnings were only advertised in a limited way, thus exposing the crocodile to water heavily contaminated with blue green algal cells over long periods of time throughout the summer months. High counts of toxic blue green algal cells were also identified from water samples taken from the local water supply (Fig. 3 and 4).

The crocodile (a healthy and alert subject prior to exposure or submersion in water contaminated with high counts of toxic blue green algal cells), was observed to show the following signs and symptoms immediately afterwards:

- Change in skin colour (head and jaw) from a pale creamy colour to a dark grey, almost black in colour. On occasions, a distinctive, bright yellowish tinge colour was also observed;
- Dilated pupils and depressed respirations;

Vicki Lowing

From: "Penny Sloane" <pennys@murrayirrigation.com.au>
To: <vlowing@mcmedia.com.au>
Cc: "Chris Shaw" <chriss@murrayirrigation.com.au>
Sent: Wednesday, 4 February 2004 12:59 PM
Subject: BGA

Vicki

First last years results:

Sample date: 10 Feb 03
Description: Q722B dam
Results: anabaena 2370 cells/ml
coelosphaerium present in large numbers (not counted)
(Counted at MIL lab.)

Sample date: 29 Jan 03
Description: Mundiwa 1 @ Q721
Results: coelosphaerium 26768 cells/ml
(Counted at Water Environment Lab. Sydney)

Coelosphaerium is a species found in Australia of the same genus as those known to be toxic elsewhere. These were the only two samples in the entire MIL area to have coelosphaerium identified as present.

This years samples:

Sample date: 30 Jan 04
Description: Q722B dam water
Results: anabaena (mixed species) 12423 cells/ml
Green algae and diatoms present
(Counted at MIL lab.)

Figure 3. Results of water sample testing.

- Mouth ulcers;
- Increasing difficulty in mobilization including sliding on belly and dragging rear limbs, with loss of power in these limbs for a period of time; and,
- Circling, postural abnormalities, and disorientation for short periods of time (approximately 10 to 20 minutes), were also observed on many occasions.

Following observations of the above signs and symptoms, a recovery period lasting four to five days was then observed. During this period the crocodile remained out of water for the majority of the time, displaying acute lethargy and appearing very dark in colour. These signs and symptoms were also complicated by those of dehydration. Following this recovery period, the crocodile would once again appear healthy and active, until further exposure to water contaminated with high counts of toxic blue green algal cells.

Serum pathology was also attended on the crocodile following several exposures to the contaminated water and the results received indicated the following:- Decreasing blood glucose levels; elevated se.calcium levels; anaemia; increased white cell count; basophilia, and heterophilia (interpreted as: “ may reflect sub-acute inflammation”).

Conclusions

These observations were made over a four year period on the crocodile, on a one to one basis. A journal was also maintained over this period reflecting the health changes observed. It is considered that both the chronic and the acute exposure to the toxic blue green algal cells were responsible for these health changes. The health of the crocodile has appeared to have improved markedly since a recent move to another State and subsequent removal from further exposure to water contaminated with high counts of blue green algal cells.

LAND & WATER CONSERVATION

Report Number 3606
Report Date 26-Mar-03

Water Environmental Laboratory
Analytical Report

		ARN-03-03-280/001	ARN-03-03-280/003
		Whitham - Dam	Whitham - Canal
		18-Mar-03	18-Mar-03
		16:00	16:00
**Blue Green Algae (Potentially Toxic)*	Anabaena (other species)	**503**	
Blue Green Algae	Aphanocapsa	**68408**	**201**
	Coelosphaerium	**5634**	**4024**
	Oscillatoria		**604**
	Planktolyngbya		**5030**
Total Counts	Total Blue Green Algal Count	**74545**	**9859**

All Results Reported in Cells/mL

Page 2 of 2

4 Guess Ave, Arncliffe, NSW 2205 Ph : 02 9597 4444 Fax : 02 9597 4808

Figure 4. Results of water sample testing.

Note : The above Blue Green Algae Alert Warnings and Results from water samples were not received until March 2003 and February 2004 respectively).

Relationship between Telomere Length and Body Length in *Alligator mississippiensis*

Nicole Scott[1], M. Haussmann[1], C. Vleck[1], Phillip Trosclair[2] and Ruth Elsey[2]

[1]Dept of EEOBiology, Iowa State University, Ames, IA USA; [2]Louisiana Dept. of Wildlife and Fisheries, Grand Chenier, LA, USA

Free-living American alligators grow about 30 cm per year until they reach about 2 m. Then growth slows, so size is not an accurate estimator of age. Our goal was to determine whether telomere lengths in blood cells shorten with growth in a predictable manner. Telomeres are DNA sequences at the end of each chromosome that do not fully replicate at cell division in most animals; thus telomeres are usually shorter in older animals. We sampled erythrocyte DNA from wild-caught animals of different body lengths and from captive-reared yearlings. Telomere length was determined by separation of restriction-digested DNA by electrophoresis, followed by hybridization to P^{32}-labeled oligonucleotides specific for the telomere. Mean telomere length for each animal was determined by densitometry. We found no significant difference in telomere lengths over the range of animals sampled (~30-300 cm), although males tended to have longer telomeres than females. The mechanisms preserving telomere length in these long-lived animals are not known.

Thyroid Hormone Biology of Embryonic Saltwater Crocodiles (*Crocodylus porosus*)

Caroline A. Shepherdley[1], Samantha J. Richardson[1], Julie A. Monk[1], Barbara K. Evans[2], Christopher B. Daniels[3], Sandra Orgeig[3], Eduard R. Kühn[4] and Veerle M. Darras[4]

[1]Department of Biochemistry and Molecular Biology, The University of Melbourne, VIC 3010, Australia; [2]Department of Zoology, The University of Melbourne, VIC 3010, Australia; [3]School of Earth and Environmental Sciences, University of Adelaide, SA 5005, Australia; [4]Laboratory of Comparative Endocrinology, Zoological Institute, Catholic University Leuven, B-3000 Leuven, Belgium

Thyroid hormones (THs) are essential for vertebrate development. This study investigated three aspects of thyroid hormone biology during the development of embryonic saltwater crocodile. Free thyroxine (T_4) and free triiodothyronine (T_3) levels in blood gradually rose during the late stages of incubation (days 60, 68 and 75), and were maximal at hatching (day 80). These changes could have been brought about by an increase in T_4 release from the thyroid gland, and changes in the metabolism of THs by iodothyronine deiodinases. Specifically, we showed a decrease in hepatic T_3 to diiodothyronine (T_2) conversion by a low Km inner ring deiodinase, and an increase in T_4 to T_3 conversion by a high Km outer ring deiodinase. Thyroid hormone distribution proteins (THDPs) are required for distribution of THs. Albumin was present as a THDP in serum at all ages examined. A second THDP was identified as transthyretin and was detected in serum at days 60, 68, 75 of incubation, and at day-1 post-hatch, but not in serum from 6 month-old or adult crocodiles. Changes in TH serum levels, TH metabolism and the presence of a second THDP demonstrate the importance of THs during the late stages of embryonic development.

Pit Holes in Nile Crocodile Skins

Fritz W. Huchzermeyer[1] and J F Putterill[2]

[1]PO Box 12499, Onderstepoort, 0110, South Africa; [2]Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort 0110, South Africa

Abstract

Deep holes in skins of three-year-old farmed Nile crocodiles were dubbed "pit holes". In histopathological sections they appeared to be branched and were lined by an intact epidermis. Earlier lesions from two-year-old animals were in similar form but the surrounding tissue contained remnants of inflammatory cells. The skins of one-year-old animals contained large cysts of a diameter equal to the thickness of the dermis. These cysts were surrounded by an intense inflammatory reaction and only partially lined by epidermis. Investigations into the cause of the pit holes continue.

Introduction

The skin diseases of crocodiles were reviewed by Huchzermeyer (2003). Small holes of unknown causes in American alligator skins were described by Haire (1997). These were dubbed "pix" and later found to be caused by erupting dermal granulomata from which the fungus *Hortaea werneckii* was isolated (Dickson *et al*. 2002).

A South African crocodile farmer had a number of skins downgraded because of small holes, "pit holes", found prevalently in the ventrolateral region of affected Nile crocodile skins. At close examination these holes were visible also in the skin of live crocodiles before slaughter. This paper describes the preliminary findings of an investigation into the cause of the pit holes.

Materials and Methods

Samples were taken from the skins of slaughtered crocodiles as well as from selected younger age groups. These were fixed in 10% buffered formalin and after fixation processed for routine histological sectioning, stained with haematoxylin-eosin stain and examined using light microscopy.

Results

The histopathological examination of the skins from slaughtered crocodiles (three years old) showed deep holes lined with a healed epidermis and without any inflammatory reaction. Two-year-old crocodiles had similarly healed lesions but with some inflammatory and pigment cells in the dermis surrounding the lesion. The one-year-old crocodiles had active lesions consisting of deep cysts penetrating the whole dermis, empty or filled with debris and surrounded by an intense inflammatory reaction.

Discussion

The canals seen in the histopathological specimens from 2- and 3-year-old crocodiles are believed to be collapsed cysts seen in skin preparations of the youngest animals. Preliminary investigations appear to indicate that the pit holes are caused by an infectious agent and that the incidence of this condition is linked to poor hygiene, although bacterial and fungal cultures so far gave negative results. The incidence appears not to be limited to one farm only. It also appears that the condition has been seen before but veterinarians were not consulted. Investigations continue.

Literature

Dickson, H., Cardeilhac, P. and Ashley, J.D. (2002). Pix skin disease in the American alligator (*Alligator mississippiensis*). Pp. 187 *in* Crocodiles. Proceedings of the 16th Working Meeting of the IUCN-SSC Crocodile Specialist Group. IUCN: Gland.

Haire, D. (1997). Concern over American alligator skin pitting. Crocodile Specialist Group Newsletter 16(1): 22-23.

Huchzermeyer, F.W. (2003). Crocodiles: Biology, Husbandry and Diseases. CABI Publishing: Wallingford, UK.

Economic Valuation of the Tarcoles River *C. acutus* Population Using the Travel Costs Method

Gerardo Abadia

Programa Regional en Manejo de Vida Silvestre para Mesoamérica y el Caribe, Universidad Nacional de Costa Rica, Apartado Postal 1350-3000 Heredia, Costa Rica (gabadia@una.ac.cr)

The value of crocodiles as an incentive for ecotourism at the Río Grande de Tárcoles in Costa Rica was measured by using the travel cost method. By analyzing travel behavior, the study reveals that foreigners are willing to pay considerably for the experience of visiting the site to see crocodiles. The travel cost method takes into account the total expenses undergone by visitors and assumes that costs increase with the distance to the site and therefore the number of visitors decrease with increasing costs. The economic value of a natural resource is estimated according to people's willingness to assume the cost of visiting a natural site. The present value of the Río Grande de Tárcoles' crocodile population, based on tourism, is found to be 19.5 times in magnitude greater than the purchase price currently paid for the hides. Ecoturism then proves to be an important conservation strategy because it focuses on the sustainable use of natural resources by providing an income to local communities.

The Ecological Distribution of Crocodiles in South West Cameroon: Threats and Conservation Potentials

Gonwouo Nono Legrand

University of Yaounde, Cameroon

The ecological distribution of crocodiles from the rain forest of South West Cameroon was carried out between 2002 and 2003. Investigations indicate that three species of the African crocodile, *Crocodylus niloticus, Crocodylus cataphractus* and *Osteolaemus tetraspis,* still exist in southwest Cameroon. Evidence reveals the later has a scattered distribution all over the region in tributaries of larger rivers, swamps and water ponds. It appears to be abundant but threatened by bush meat trade. Five smoked specimens where observed from an individual in a bush meat market. Larger rivers harbour considerable populations of the Nile crocodile with seven sites identified. It is hunted for its flesh, skin and eggs (an individual found with 53 eggs) sold at high prices. *C. cataphractus* is the rarest in the region and no direct evidence was recorded. These observations suggest areas where efforts of conservation and management should be implemented.

Research on Habitat Selection by Wild Chinese Alligator (*Alligator sinensis*)

Wu Lu-Sheng[1], Wu Xiao-Bing[1], Jiang Hong-Xing[2,3], Ruan Xiang-Dong[3] and Gu Chang-Ming[4]

[1]College of Life Sciences, Anhui Normal University, Wuhu 241000, China; [2]Research Institute of Forestry Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing 100091, P.R. China; [3]National Wildlife Research and Development Center, State Forestry Administration, Beijing 100091, P.R. China; [4]Anhui Natural Conservation Management, Hefei 230001, P.R. China

Research on the habitat selection of wild Chinese Alligator was conducted from September 2002 to July 2003 at Natural Conservation Regions of Chinese Alligator in southern Anhui Province. We sampled 22 specimens in order to research habitat selection by Chinese alligator. Eight ecological factors in correlation to subsistence of alligator such as shelter condition of bank, island of water area, stabilization of water, pH value of water, snail abundance, soil texture, density of bamboo and vegetation type were selected according to data of investigation on the spot. We adopted the methods of resource selection functions and principal components to analyze habitat selection. The results show that the model of first and second Principal Component: Z_1 the first Principal Component, $Z_1 = 1.312X_1 - 0.253X_2 - 0.269X_3 + 0.035X_4 - 0.149X_5 + 0.160X_6 - 0.070X_7 - 0.174X_8$; Z_2 the second Principal Component, $Z_2 = -0.127X_1 - 0.047X_2 - 0.092X_3 - 0.054X_4 + 1.138X_5 + 0.123X_6 - 0.145X_7 + 0.095X_8$. The Logistic regression model is logist $P = -2.451 + 2.612Z_1 + 1.434Z_2 + 1.564Z_3 + 0.941Z_4 - 1.044Z_5 + 1.408Z_6 + 1.423Z_7 - 0.039Z_8$. The results suggested that shelter condition of bank influenced habitat selection significantly and the subordinate ecology factor was pH value of water, while the ecological factors (Island of water area and Vegetation type) influenced habitat selection slightly.

The Population Status of Rescued Chinese Alligators From Wild in Anhui Research Center for Chinese Alligator Reproduction

Nie Jishan[1], Wang Chaolin[1], Gu Changming[2] and Xie Wanshuo[1]

[1]Anhui Research Center for Chinese Alligator Reproduction, Xuanzhou, 2320034, P.R. China; Anhui Natural Conservation Management, Hefei 230001, P.R. China

We analysed 212 rescued Chinese alligators and 21 nests in the wild from 1980 to 1988. The rescued alligators were mainly distributed in Xintian, Yishan and Yangling (24.5, 14.6 and 12.7% respectively), and included hatchlings (8.5%), subadults (18.5%) and adults (73%). These alligators laid a mean of 3 nests with mean of 13 eggs per nest every year. After breeding for several years, 14 individuals (6 males, 8 females) produced by the rescued alligators were released into the wild. The rescued alligators were also bred as stock populations of present breeding populations in Anhui Research Center for Chinese Alligator Reproduction.

Spotlight Surveys of New Guinea Freshwater Crocodile (*Crocodylus novaeguineae*) in Mid-Zone Memberamo River (Memberamo and Rouffaer River Systems), Papua Province, Indonesia

Hellen Kurniati[1] and Charlie Manolis[2]

[1]Research Centre for Biology, Indonesian Institute of Sciences (LIPI), Widyasatwaloka Building-LIPI, Jalan Raya Cibinong Km 46, Cibinong 16911, West Java, Indonesia, PO Box 2 (*hkurniati@yahoo.com);*
[2]Wildlife Management International Pty. Limited, PO BOX 530, Sanderson, NT 0812, Australia (cmanolis@wmi.com.au)

Abstract

All available spotlight survey data for the New Guinea Freshwater Crocodile (*Crocodylus novaeguineae*) in mid-zone Memberamo River, Papua Province, Indonesia, were analysed. Survey areas consist of four rivers (Memberamo Mati, Jaro, Baso and Soi) and 10 lakes (Sobaki, Kamika, Waropen, Kweri Satu, Kweri Dua, Apuse, Bernekam Satu, Bernekam Dua, Cabang Tiga Satu and Cabang Tiga Dua), surveyed at different times between 1987 and 2002.

Results from the Jaro River (1989-2002) indicated a significant increase in non-hatchling density over time. All other areas surveyed indicated stable non-hatchling densities over the periods of survey. The latest surveys (2001-2002) were affected by high water levels (flooding), which are likely to have reduced the sightability of crocodiles.

Trends in live crocodile and skin harvests appear to be driven by market forces and a moratorium (early 1990s), rather than any limitations of the crocodile resource. In general, the population of *C. novaeguineae* in Mid-zone Memberamo River has been relatively stable, despite extensive harvesting.

Introduction

The people of Indonesia have historically used crocodiles for a variety of non-commercial purposes. In the past ten years, commercial collection from the wild has threatened the population status of Indonesian crocodiles. As pressure from the commercial hunting is continuous, the successful conservation of Indonesian crocodiles will depend on the constant monitoring of their populations. Papua is one of several provinces in Indonesia which historically accommodate unknown numbers of commercial crocodiles (Webb and Jenkins 1991).

Monitoring of the crocodile population in the Mid-zone Memberamo River (see Appendix 1 for the region) was conducted from 2000 to 2002, with spotlight surveys in Kamika Lake (Kaureh District) and Waropen, Kweri Satu, Kweri Dua, Apuse, Bernekam Satu, Bernekam Dua, Cabang Tiga Satu and Cabang Tiga Dua Lakes (Memberamo Hulu District). Additional surveys were undertaken in 2001-2002 in the Memberamo Mati, Jaro, Baso and Soi Rivers, and Sobaki Lake (Memberamo Hulu District), all of which had been surveyed previously by the FAO-PHPA Project. All of these areas are within the Memberamo and Rouffaer River systems. Interviews with local people and harvest data indicate that the dominant species is *Crocodylus novaeguineae*.

This paper presents the most recent spotlight survey data (Table 1), and summarizes all spotlight data and population trends (total numbers and non-hatchlings) for *C. novaeguineae* in Papua Province.

Methodology

1. Timing of Surveys

   Surveys in 2000-2002 were undertaken in September-December each year (Table 1).

Table 1. Dates of *C. novaeguineae* spotlight surveys in mid-zone Memberamo and Rouffaer River systems, 2000-2002.

Year	Duration	Number of sites	Total length of survey	Conducted by	Reference
2000	September 27-October 2	9 lakes	29.9 km	KSDA	KSDA (2000)
2001	November 26-December 5	10 lakes, 4 rivers	162.5 km	LIPI-KSDA	Kurniati *et al.* (2001)
2002	December 13-23	10 lakes, 4 rivers	143 km	KSDA	KSDA (2002)

2. Spotlight Survey Technique

Crocodile densities (number of individuals per kilometre of river or lake) were assessed by spotlight surveys using the method described by Messel *et al*. (1981). Spotlight surveys do not calculate absolute abundance, but provided an index or relative density which allows changes in population size and structure to be quantified over time (Baylis 1987; Messel *et al.* 1981). A halogen torch, powered by 6 DD batteries (7.2V, 0.85 amp) was used. When an eyeshine was detected, an attempt was made to approach the crocodile in order to estimate total length (TL; see 3. below).

Surveys typically started downstream and proceeded upstream in rivers or to the mouth of lakes, except in the Soi River. All distances were determined using 1:250,000 maps (between 2°00'00"S and 4°00'00"S to 138°00'00"E and 139°30'00"E) and a GPS. The speed of the boat and canoe were recorded by GPS. Prevailing weather conditions at the time of survey were also recorded.

3. Species

The species of crocodile surveyed, *C. novaeguineae,* is known locally as Buaya Air Tawar or Buaya Bob. Crocodiles were categorized as hatchling (H, <50 cm TL); juvenile (J, 50-150 cm TL), adult (A, >150 cm TL) and Eyes Only (EO).

4. Locality

Areas surveyed were within the Memberamo and Rouffaer River systems. Specifically, they comprised Memberamo Mati, Jaro, Baso and Soi Rivers, Sobaki, Waropen, Kweri Satu, Kweri Dua, Apuse, Bernekam Satu, Bernekam Dua, Cabang Tiga Satu, Cabang Tiga Dua Lakes (Memberamo Hulu District), and Kamika Lake (Kaureh District).

5. Vessels

Survey areas varied in length, so the type of boat used varied accordingly. In long distance areas (Memberamo Mati, Jaro, Baso and Soi Rivers, and Kamika, Bernekam Satu, Sobaki, Kweri Satu and Kweri Dua Lakes) a long boat powered by a 40 HP motor was used. In other areas (Cabang Tiga Satu, Cabang Tiga Dua, Apuse, Waropen and Bernekam Dua Lakes) a canoe was used.

6. Interviews with local people

Interviews with local people were conducted to confirm the species of crocodile being harvested in each river and lake surveyed.

7. Data analysis

Linear regression analysis was used to describe population trends (SPSS statistic analysis, version 9.0). Significant level of statistic analysis is $p<0.05$.

8. Harvest data

Harvest data for *C. novaeguineae* were provided by one company (CV Bintang Mas), for the period 1995 to 2002.

Results

Generally higher water levels were encountered during surveys in 2001 and 2002, which affected the extent of riverbank that could be seen clearly (Table 2). Spotlight survey results for all years are in Table 3. This includes previous survey results for Memberamo Mati River, Jaro River, Baso River, Soi River and Sobaki Lake, collected by the FAO-PHPA Project.

Population Trends

Population trends of each area were determined using linear regression analysis. As hatchling numbers can vary greatly from year to year, and mortality rates for this size/age class can be high, analyses were carried using all data (ie H, A, J), and using only non hatchlings (Figs. 1-14).

Table 2. Weather conditions recorded during spotlight surveys of *C. novaeguineae* in the Memberamo and Rouffaer River systems, 1987-2002. Information for surveys carried out in 2000-2002 is more detailed. Surveys were carried out by FAO-PHPA, Division of Natural Resources and Conservation Papua I (KSDA) and the Indonesian Institute of Sciences (LIPI).

No	River/Lake	Weather description	Personnel team	Reference
1	**Memberamo Mati River**			
	1/10/87	Low water level, dark moon, clear sky.	FAO-PHPA	KSDA (2000)
	20/2/88	High water level (flood), dark moon, clear sky.	FAO-PHPA	KSDA (2000)
	3/10/89	Low water level, dark moon, heavy rain.	FAO-PHPA	KSDA (2000)
	6/4/90	Low water level, dark moon, clear sky.	FAO-PHPA	KSDA (2000)
	1/10/90	Low water level, dark moon, cloudy sky.	FAO-PHPA	KSDA (2000)
	12/10/92	Low water level, full moon, cloudy sky.	KSDA	KSDA (2000)
	26/11/01	High water level, but more than half of survey distance the river bank could be seen clearly, full moon, clear sky. Survey started 2105 h, finished 2215 h.	LIPI-KSDA	Kurniati *et al* (2001)
	20/12/02	High water level, but all of survey distance the river bank could be seen clearly, no moon, cloudy and dark sky. Survey started 1905 h, finished 2240 h.	KSDA	KSDA (2002)

No	River/Lake	Weather description	Personnel team	Reference
2	**Jaro River**			
	24/9/89	Low water level, dark moon, clear sky.	FAO-PHPA	KSDA (2000)
	30/7/91	Low water level, dark moon, clear sky.	FAO-PHPA	KSDA (2000)
	29/11/01	High water level (flood), but more than half of survey distance the river bank could be seen clearly, 3/4 full moon, cloudy sky and shower. Survey started 2105 h, finished 2205 h.	LIPI-KSDA	Kurniati *et al* (2001)
	19/12/02	High water level, flooding, full moon, clear sky. Survey started 1915 h, finished 2250 h.	KSDA	KSDA (2002)
3	**Baso River**			
	30/9/89	Low water level, dark moon, heavy cloudy sky.	FAO-PHPA	KSDA (2000)
	4/8/91	Low water level, dark moon, clear sky.	FAO-PHPA	KSDA (2000)
	4/12/01	High water level (flood), but most of s urvey distance the river bank could be seen very clearly, strong water current, heavy cloudy sky, no moon shine. Survey started 1930 h, finished 2100 h.	LIPI-KSDA	Kurniati *et al* (2001)
	21/12/02	High water level, less than half of survey distance the river bank could be seen clearly, full moon, cloudy sky. Survey started 1930 h, finished 2305 h.	KSDA	KSDA (2002)
4	**Soi River**			
	26/4/89	High water level (flood), full moon, cloudy sky.	FAO-PHPA	KSDA (2000)
	18/7/91	High water level (flood), full moon, clear sky.	FAO-PHPA	KSDA (2000)
	5/12/01	High water level (flood), but most of s urvey distance the river bank could be seen clearly, dark sky, no moon shine, shower to heavy rain. Survey started 1825 h, finished 2005 h.	LIPI-KSDA	Kurniati *et al* (2001)
	22/12/02	High water level, less than half of survey distance the river bank could be seen clearly, full moon, cloudy sky. Survey started 1915 h, finished 2200 h.	KSDA	KSDA (2002)
5	**Sobaki Lake**			
	26/9/89	Low water level, dark moon, cloudy sky.	FAO-PHPA	KSDA (2000)
	14/7/91	High water level (flood), dark moon, heavy cloudy sky.	FAO-PHPA	KSDA (2000)
	5/12/01	High water level, only about half of survey distance the river bank could be seen clearly, dark sky, no moon shine, shower. Survey started 2215 h, finished 2300 h.	LIPI-KSDA	Kurniati *et al* (2001)
	23/12/02	High water level, less than half of survey distance the river bank could be seen clearly, full moon, cloudy sky. Survey started 1900 h, finished 2115 h.	KSDA	KSDA (2002)
6	**Kamika Lake**			
	27/9/00	Low water level, dark moon, clear sky.	KSDA	KSDA (2000)
	30/11/01	High water level (flood), about half os survey distance the lake bank could be seen clearly, full moon, cloudy sky. Survey started 2130 h, finished 0040 h.	LIPI-KSDA	Kurniati *et al* (2001)
7	**Waropen lake**			
	28/9/00	Low water level, dark moon, clear sky.	KSDA	KSDA (2000)
	30/11/01	High water level (flood), full moon, shower. Survey started 1930 h, finished 2000 h.	LIPI-KSDA	Kurniati *et al* (2001)
	15/12/02	High water level, flooding, half of survey distance the river bank could be seen clearly, half moon, cloudy and dark sky. Survey started 1905 h, finished 2220 h.	KSDA	KSDA (2002)

No	River/Lake	Weather description	Personnel team	Reference
8	**Kweri Satu Lake**			
	29/9/00	Low water level, dark moon, cloudy sky.	KSDA	KSDA (2000)
	29/11/01	High water level (flood), full moon, clear sky. Survey started 1900 h, finished 2200 h.	LIPI-KSDA	Kurniati *et al* (2001)
	16/12/02	High water level, flooding, half of survey distance the river bank could be seen clearly, half moon, clear sky. Survey started 1850 h, finished 2140 h.	KSDA	KSDA (2002)
9	**Kweri Dua Lake**			
	30/9/00	Low water level, crescent moon, clear sky.	KSDA	KSDA (2000)
	1/12/01	High water level (flood), full moon, shower.	LIPI-KSDA	Kurniati *et al* (2001)
	15/12/02	High water level, flooding, less than half of survey distance the river bank could be seen clearly, half moon, cloudy sky. Survey started 1900 h, finished 2120 h.	KSDA	KSDA (2002)
10	**Apuse Lake**			
	30/9/00	Low water level, dark moon, cloudy sky.	KSDA	KSDA (2000)
	2/12/01	High water level (flood), full moon, clear sky.	LIPI-KSDA	Kurniati *et al* (2001)
	16/12/02	High water level, flooding, half of survey distance the river bank could be seen clearly, half moon, cloudy sky. Survey started 1900 h, finished 2140 h.	KSDA	KSDA (2002)
11	**Bernekam Satu Lake**			
	1/10/00	Low water level, crescent moon, clear sky.	KSDA	KSDA (2000)
	2/12/01	High water level (flood), but most of s urvey distance the lake bank could be s een clearly, dark sky, no moon shine, cloudy sky. Survey started 1805 h, finished 2050 h.	LIPI-KSDA	Kurniati *et al* (2001)
	17/12/02	High water level, flooding, half of survey distance the river bank could be seen clearly, half moon, heavy cloudy sky. Survey started 1830 h, finished 2130 h.	KSDA	KSDA (2002)
12	**Bernekam Dua Lake**			
	1/10/00	Low water level, dark moon, cloudy sky.	KSDA	KSDA (2000)
	1/12/01	High water level (flood), full moon, cloudy sky. Survey started 1815 h, finished 2010 h.	LIPI-KSDA	Kurniati *et al* (2001)
	17/12/02	High water level, flooding, less than half of survey distance the river bank could be seen clearly, half moon, heavy cloudy sky. Survey started 1830 h, finished 2130 h.	KSDA	KSDA (2002)
13	**Cabang Tiga Satu Lake**			
	2/10/00	Low water level, dark moon, cloudy sky.	KSDA	KSDA (2000)
	29/11/01	High water level (flood), full moon, cloudy sky, shower. Survey started 1900 h, finished 2200 h.	LIPI-KSDA	Kurniati *et al* (2001)
14	**Cabang Tiga Dua Lake**			
	2/10/00	Low water level, dark moon, cloudy sky, heavy rain.	KSDA	KSDA (2000)
	29/11/01	High water level (flood), full moon, cloudy sky, shower. Survey started 1900 h, finished 2200 h.	LIPI-KSDA	Kurniati *et al* (2001)
	18/12/02	High water level, flooding, less than half of survey distance the river bank could be seen clearly, half moon, cloudy sky, shower. Survey started 1905 h, finished 2215 h.	KSDA	KSDA (2002)

Table 3. Spotlight counts of *C. novaeguineae* in the Memberamo and Rouffaer River systems. H= hatchling; J= Juvenile; A= adult; EO= eyes only.

Date of Survey	Site	Area System	Boat/ Canoe	Distance (km)	Sightings				Total Crocodiles Sighted	Total Density (N/km)	Non-hatchling density (NH/km)
					H	J	A	EO			
1/10/87	Memberamo Mati River	Memberamo	?	4	8	14	0	26	48	12.00	10.00
20/2/88			?	4	4	0	1	7	12	3.00	2.00
3/10/89			?	4	2	2	0	8	10	2.50	2.50
6/4/90			?	4	0	0	0	4	4	1.00	1.00
1/10/90			?	4	1	0	0	0	1	0.25	0.00
12/10/92			?	4	0	1	2	5	8	2.00	2.00
26/11/01			boat	20	0	0	1	40	41	2.05	2.05
20/12/02			boat	22	1	1	0	53	55	2.50	2.45
24/9/89	Jaro River	Memberamo	?	47	4	0	0	14	18	0.38	0.30
30/7/91			?	47	0	0	0	6	6	0.13	0.13
29/11/01			boat	20	0	0	1	41	42	2.10	2.10
19/12/02			boat	20	0	2	0	39	41	2.05	2.05
30/9/89	Baso River	Memberamo	?	2	1	0	0	3	4	2.00	1.50
4/8/91			?	2	0	0	0	2	2	1.00	1.00
4/12/01			boat	20	0	0	0	20	20	1.00	1.00
21/12/02			boat	20	0	0	0	24	24	1.20	1.20
3/5/89	Soi River	Rouffaer	?	0.5	2	1	0	9	12	24.00	20.00
20/7/91			?	0.5	9	0	0	2	11	22.00	4.00
5/12/01			boat	15	0	0	0	44	44	2.90	2.90
22/12/02			boat	12	0	0	0	39	39	3.25	3.25
26/9/89	Sobaki Lake	Rouffaer	?	5	1	1	0	10	12	2.40	2.20
14/7/91			?	5	0	0	0	12	2	0.40	0.40
5/12/01			boat	5	0	0	0	2	2	0.40	0.40
23/12/02			boat	6	0	0	0	14	14	2.33	2.33
27/9/00	Kamika Lake	Memberamo	canoe	4	0	2	0	32	34	8.50	8.50
30/11/01			boat	30	0	47	41	316	404	13.46	13.46
14/12/02			boat	25	4	20	6	287	317	12.68	12.52
28/9/00	Waropen Lake	Memberamo	canoe	5	0	9	0	23	32	6.40	6.40
30/11/01			canoe	1	8	0	0	13	21	21.00	13.00
15/11/02			canoe	2.5	0	3	0	32	35	17.50	17.50
29/9/00	Kweri Satu Lake	Memberamo	canoe	3	0	11	0	16	27	9.00	9.00
29/11/01			boat	3.5	23	0	0	36	59	16.86	10.28
16/12/02			boat	4	1	2	0	47	50	12.50	12.25
30/9/00	Kweri Dua Lake	Memberamo	canoe	3.5	2	15	0	37	54	15.43	14.86
1/12/01			boat	5	20	0	0	29	49	9.80	5.80
15/12/05			boat	5	1	4	0	45	50	10.00	9.80
30/9/00	Apuse Lake	Memberamo	canoe	3	0	12	0	22	34	11.33	11.33
2/12/01			canoe	4	6	0	0	17	23	5.75	4.25
16/12/02			canoe	3	0	3	0	23	26	8.67	8.67
1/10/00	Bernekam Satu Lake	Memberamo	canoe	2.5	4	17	0	83	104	41.60	40.00
2/12/01			boat	10	0	0	7	27	34	3.40	3.40
17/12/02			boat	6	0	4	0	32	36	6.00	6.00
1/10/00	Bernekam Dua Lake	Memberamo	canoe	3.4	2	9	0	29	40	11.76	11.18
1/12/01			canoe	4	4	0	0	20	24	6.00	5.00
17/12/02			canoe	3.5	0	4	0	30	34	9.71	9.71
2/10/00	Cabang Tiga Satu Lake	Memberamo	canoe	3	1	2	0	58	61	20.33	20.00
29/11/01			canoe	15	13	0	0	49	62	4.13	3.27
18/12/02			boat	8	0	5	1	43	49	6.13	6.13
2/10/00	Cabang Tiga Dua Lake	Memberamo	canoe	2.5	6	11	0	57	74	29.60	27.20
29/11/01			canoe	10	27	0	1	93	121	12.10	9.40
18/12/02			canoe	6	3	10	1	74	88	14.67	14.17

Memberamo Mati River

Data for the Memberano Mati River span 14 years, from 1987 to 2001 (Table 3). Two spotlight surveys were carried out in 1990 (April, October) - the April (end of wet season) data were used in the analysis (Table 3). Since 1988 density (total and non-hatchlings) has been stable. Excluding the 1987 data, there was no significant relationship between density and time, for either total crocodile density (r^2= 0.00, p= 0.99, n= 6; Fig. 1a) or non-hatchling density (r^2= 0.11, p= 0.53, n= 6; Fig. 1b). Non-hatchling density decreased from a high of 10.0/km in 1987 to 2.0/km in 1988 (Table 3; Fig. 1b), and has stayed relatively stable since that time [mean= 2.18 NH/km (1988-2002)].

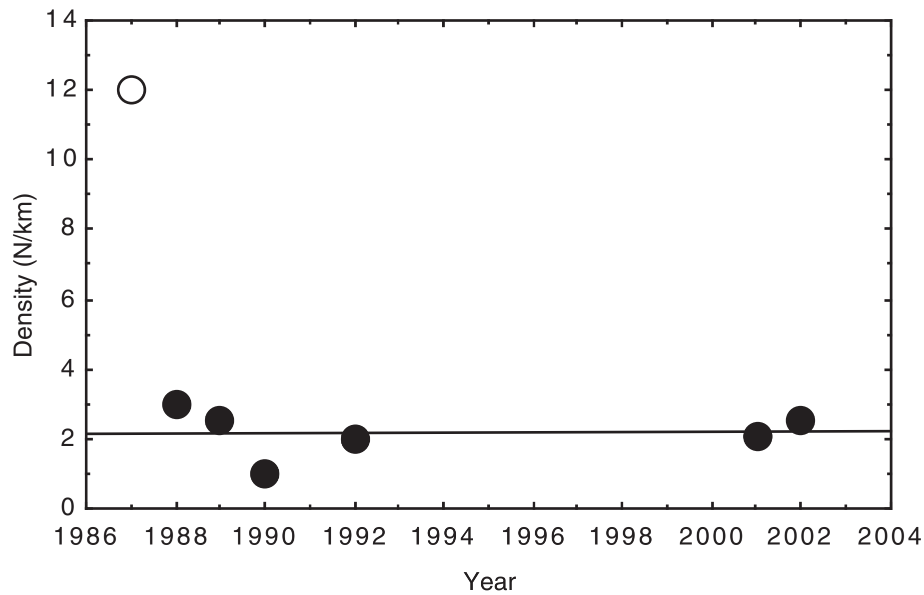

Figure 1a. Total density of *C. novaeguineae* sighted during spotlight surveys in the Memberamo Mati River, 1987-2002. Solid line indicates the non-significant trend between 1988 and 2002 (see text).

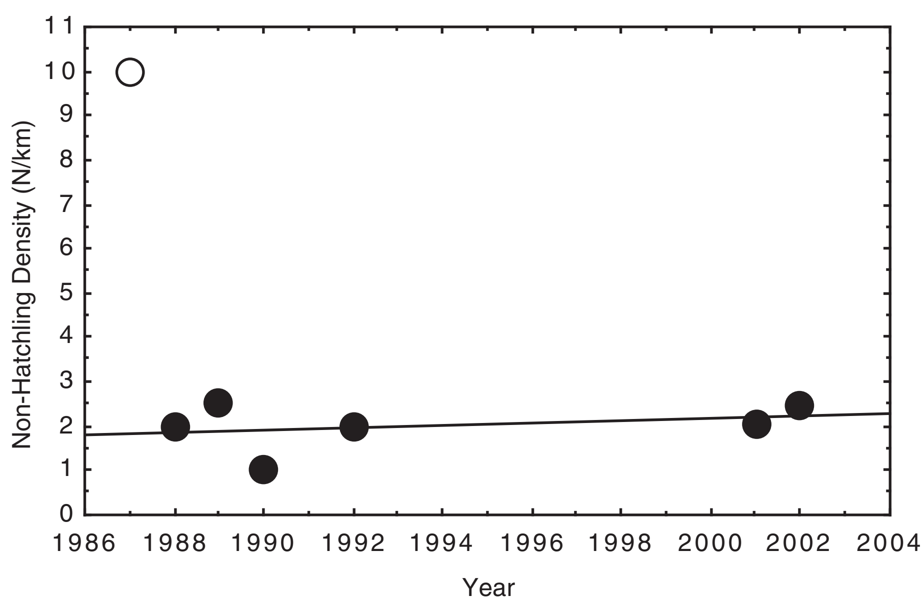

Figure 1b. Non-hatchling density of *C. novaeguineae* sighted during spotlight surveys in the Memberamo Mati River, 1987-2002. Solid line indicates the non-significant trend between 1988 and 2002 (see text).

Jaro River

Notwithstanding the limited number of surveys carried out in the Jaro River, there has been a significant increase in total crocodile density (r^2= 0.947, p= 0.027, n= 4; Fig. 2a) and non-hatchling density (r^2= 0.959, p= 0.021, n= 4; Fig. 2b) over time. The trends in the intervening period between surveys (10 years) are unknown. Certainly high densities have been recorded in both of the most recent surveys. Given the high water levels at the time of these surveys, the increase may actually be greater than recorded.

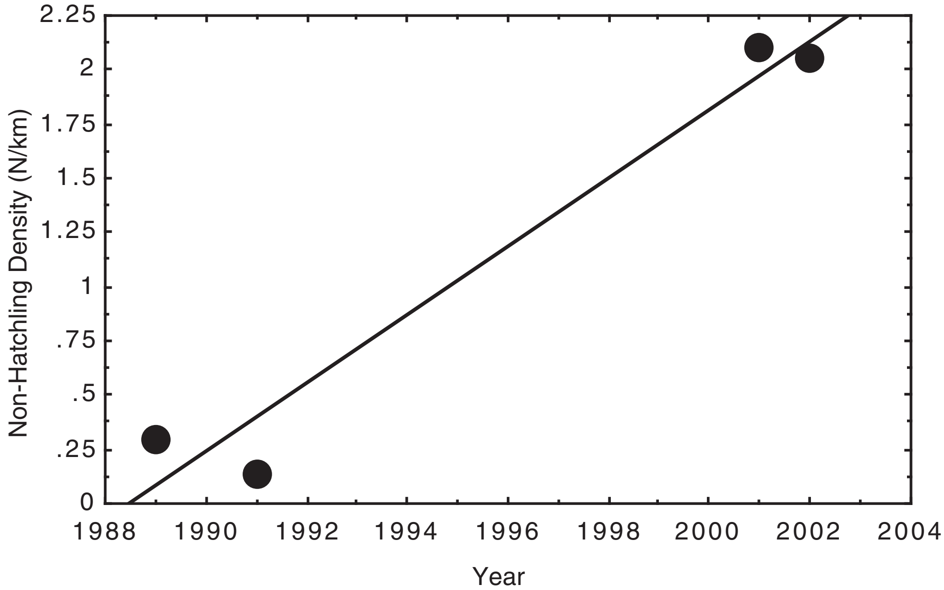

Figure 2a. Total density of *C. novaeguineae* sighted during spotlight surveys in the Jaro River, 1989-2002. Solid line indicates the significant linear regression trend (see text).

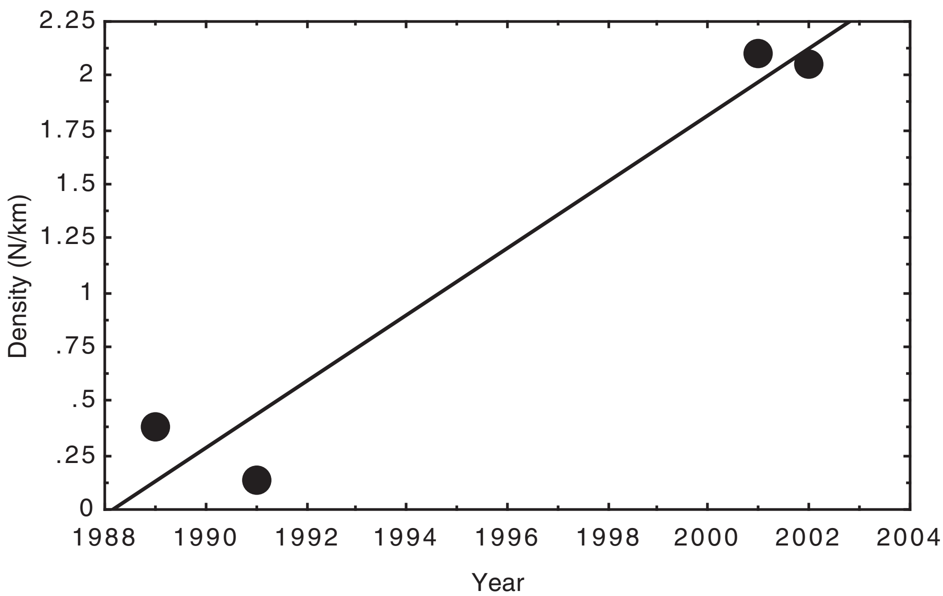

Figure 2b. Non-hatchling density of *C. novaeguineae* sighted during spotlight surveys in the Jaro River, 1989-2002. Solid line indicates the significant linear regression trend (see text).

Baso River

The four surveys undertaken in the Baso River span a 12-year period (1989-2001), and indicate that the density of *C. novaeguineae* is relatively low and stable. Non-hatchling and total crocodile densities in the Baso River have not changed significantly over time [r^2= 0.330, p= 0.425, n= 4 (Fig. 3a) and r^2= 0.2, p= 0.553, n= 4 (Fig. 3b) respectively].

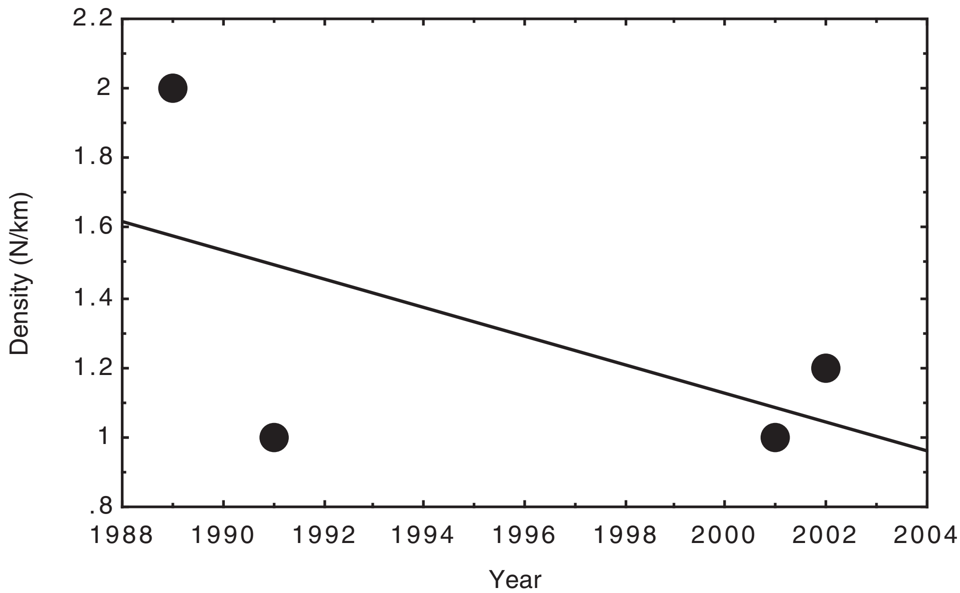

Figure 3a. Total density of *C. novaeguineae* sighted during spotlight surveys in the Baso River, 1989-2002. Solid line indicates the non-significant trend (see text).

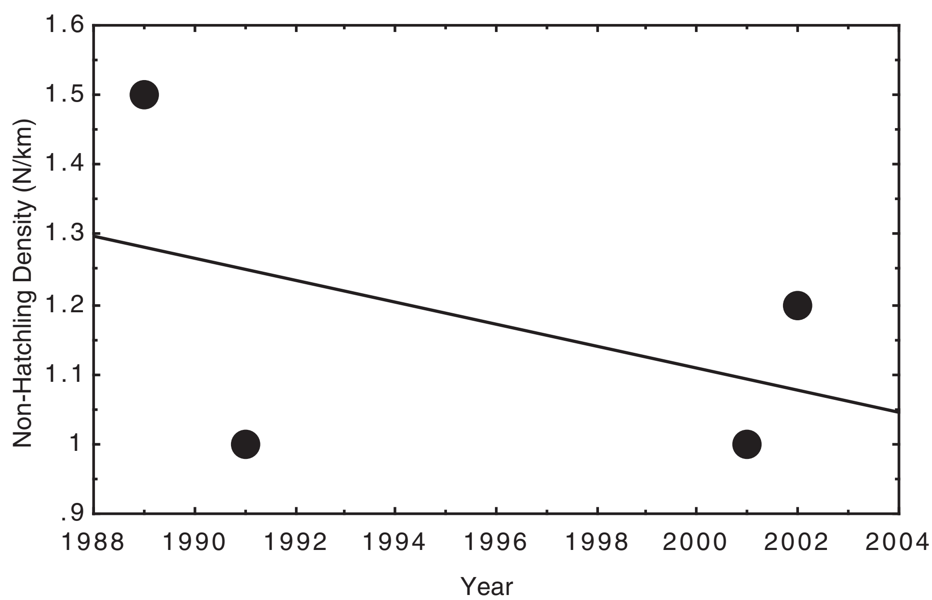

Figure 3b. Non-hatchling density of *C. novaeguineae* sighted during spotlight surveys in the Baso River, 1989-2002. Solid line indicates the non-significant trend (see text).

<u>Soi River</u>

Data for Soi River span 12 years, from 1989 to 2001 (Table 3). Regression analysis indicated a significant relationship between total crocodile density and time (r^2= 0.992, p= 0.004, n= 4; Fig. 4a), but not for non-hatchling density (r^2= 0.501, p= 0.292, n= 4; Fig. 4b). Analysis of results from the Soi River are complicated by the first two surveys involving a very short section of the river (0.5 km), and the later surveys a much longer section (20 km). The results for non-hatchling density (Fig. 4b) are more relevant here, and they indicate no change over time. Future monitoring will establish long-term population trends for the river.

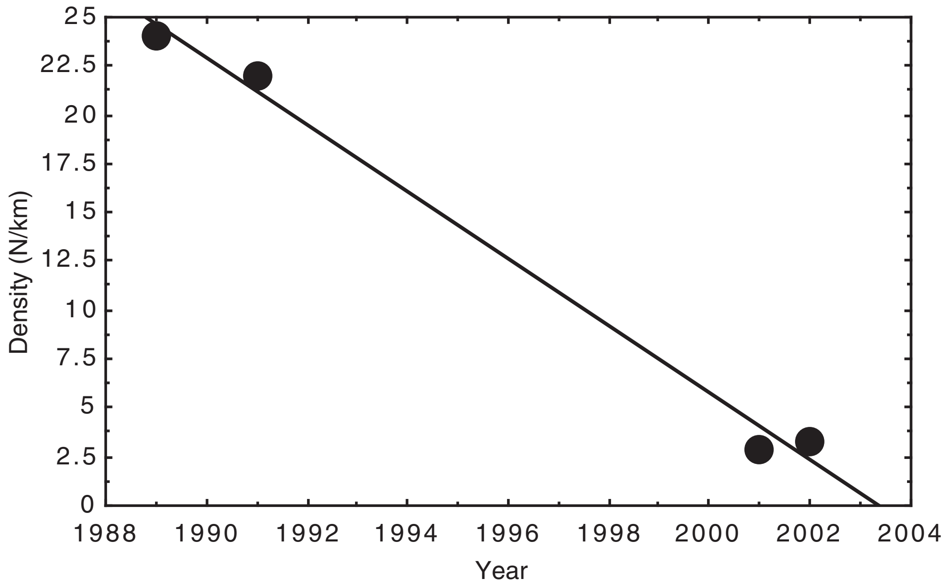

Figure 4a. Total density of *C. novaeguineae* sighted during spotlight surveys in the Soi River, 1989-2002. Solid line indicates the significant linear regression trend (see text).

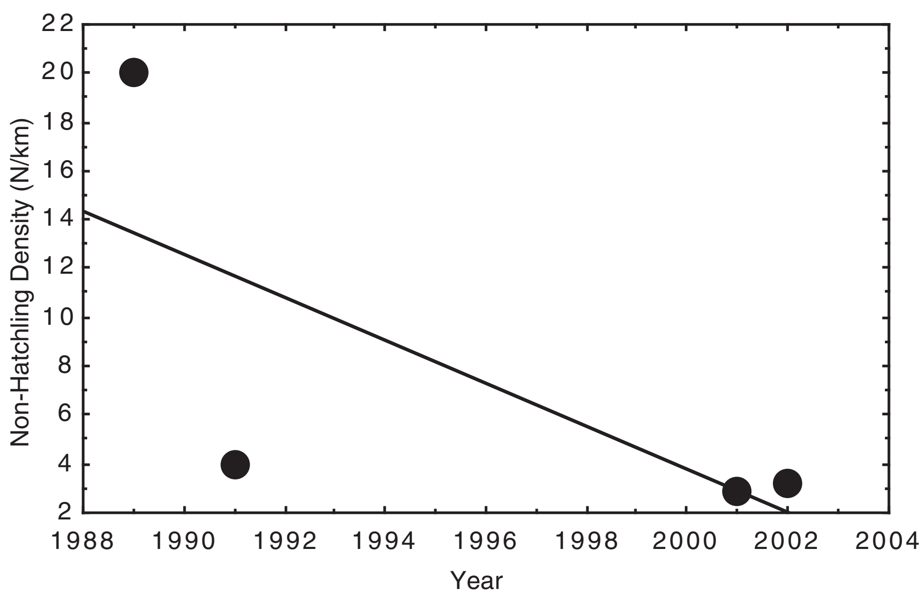

Figure 4b. Non-hatchling density of *C. novaeguineae* sighted during spotlight surveys in the Soi River, 1989-2002. Solid line indicates the non-significant trend (see text).

Sobaki River

There was no significant relationship between density and time for either total numbers (r^2= 0.004, p= 0.937, n= 4; Fig. 5a) or non-hatchlings (r^2= 0.000, p= 0.996, n= 4; Fig. 5b).

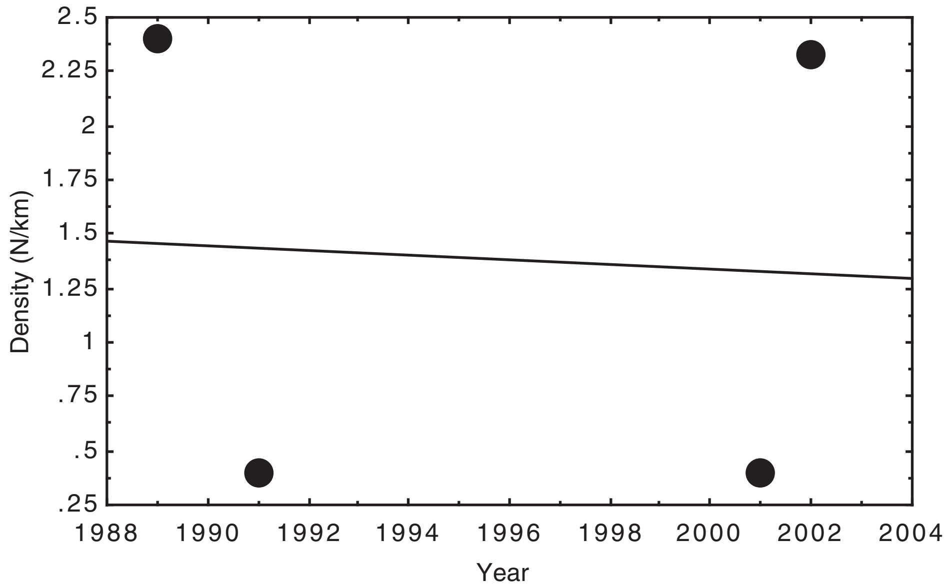

Figure 5a. Total density of *C. novaeguineae* sighted during spotlight surveys in the Sobaki River, 1989-2002. Solid line indicates the non-significant trend (see text).

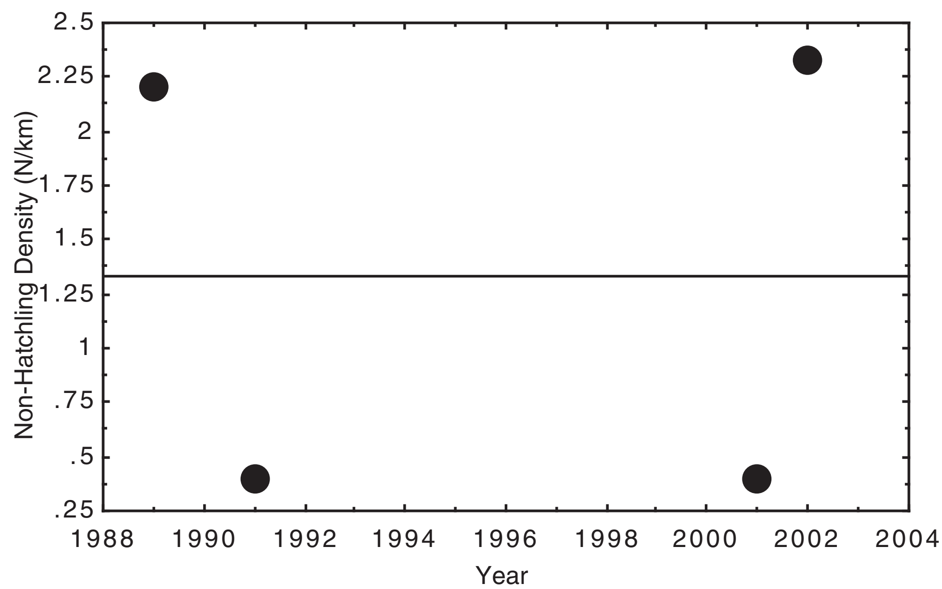

Figure 5b. Non-hatchling density of *C. novaeguineae* sighted during spotlight surveys in the Sobaki River, 1989-2002. Solid line indicates the non-significant trend (see text).

Kamika Lake

Survey data for Kamika Lake are available for three recent consecutive years (2000-2002). They indicate that total and non-hatchling densities have remained somewhat high (mean= 13.0 NH/km) and stable over the 3-year period (r^2= 0.614, p= 0.427, n= 3 and r^2= 0.582, p= 0.448, n= 3 respectively).

Waropen Lake

There was no significant relationship between density and time for either total numbers (r^2= 0.530; p= 0.481; n= 3) or non-hatchlings (r^2= 0.988, p= 0.069, n= 3). The trend in non-hatchling densit is positive, increasing from 6.4 NH/km to 17.5 NH/km (Table 3). Additional surveys are required to confirm whether this increase is indeed significant. The first two kilometres of survey distance contained a high density of *C. novaeguineae* (see Table 3), and increasing survey distance for Waropen Lake may reduce the overall density.

Kweri Satu Lake

Densities in Kweri Satu Lake have remained stable over the period 2000-2002 [total numbers (r^2= 0.196, p= 0.347, n= 3), non-hatchling (r^2= 0.985, p= 0.078, n= 3)]. The trend is towards increasing densities, from 9.0 NH/km in 2000 to 12.3 NH/km in 2002 (Table 3).

Kweri Dua Lake

Densities in Kweri Dua Lake have remained stable over time [total numbers (r^2= 0.722, p= 0.353, n= 3), non-hatchling (r^2= 0.311, p= 0.624, n= 3)]. Although the trend indicates decreasing densities, it is not significant.

Apuse Lake

Like Kweri Satu and Kwer Dua Lakes, data from Apuse Lake indicated no significant relationship between density and time for either total numbers (r^2= 0.227, p= 0.684, n= 3) or non-hatchlings (r^2= 0.138, p= 0.757, n= 3). Mean non-hatchling density is 8.1 NH/km.

Bernekam Satu Lake

A relatively high density (40.0 NH/km) was recorded in 2000, but subsequent surveys have indicated much lower levels (Table 3). Kweri Satu and Kweri Dua also showed similar trends, although the decrease was not as evident as it is for Bernekam Satu Lake. Water levels were higher in 2001 and 2002 (Table 2), and surveys were undertaken by boat rather than canoe (Table 3). These factors, particularly high water levels, could be expected to lead to lower sight ability of crocodiles. Nonetheless, the limited indicate no significant relationship between density and time for total numbers (r^2= 0.695, p= 0.372, n= 3) and non-hatchlings (r^2= 0.693, p= 0.374, n= 3).

Bernekam Dua Lake

Regression analysis for Bernekam Dua Lake indicated no significant relationship between density and time for total numbers (r^2= 0.123, p= 0.772, n= 3) and non-hatchlings (r^2= 0.052, p= 0.857, n= 3). Densities of *C. novaeguineae* are relatively stable and high (mean = 8.6 NH/km) in this area.

Cabang Tiga Satu Lake

There was no significant relationship between density and time for either total numbers (r^2= 0.646, p= 0.406, n= 3) or non-hatchlings (r^2= 0.601, p= 0.436, n= 3). The results for this area, like others, indicate high densities in 2000 (20.33 N/km and 20 NH/km; Table 3) relative to 2001 (4.13 N/km and 3.27 NH/km; Table 3) and 2002 (6.13 N/km and 6.13 NH/km; Table 3). Again, conditions at the time of survey may be implicated in this trend.

Cabang Tiga Dua Lake

Results for Cabang Toga Dua were similar to those for cabang Tiga Satu Lake (see above). Densities have been stable over time [total numbers (r^2= 0.624, p= 0.420, n= 3), non-hatchlings (r^2= 0.500, p= 0.500, n= 3).

Crocodile Harvest

Harvest data from two of the three legal companies that purchase live crocodiles and skins from the Memberamo River were not available. However, the harvest data from CV Bintang Mas (one of these companies) (Table 4) can be used as an index of the *C. novaeguineae* population in the Memberamo River. The numbers of live crocodiles traded by CV Bintang Mas has decreased significantly between 1995 and 2002 (r^2= 0.76, p= 0.005, n= 8; Fig. 6). The number of skins traded increased significantly between 1995 and 2001 (r^2= 0.70, p= 0.02, n= 7; Fig. 7). This relationship reaches non-significance with the inclusion of the 2002 data (2440 skins; Table 4) (r^2= 0.36, p= 0.116, n= 8).

Table 4. *Crocodylus novaeguineae* harvest data (live crocodiles and skins; 1995 to 2002) from mid-zone Memberamo River (Memberamo and Rouffaer River system), provided by CV Bintang Mas.

Category	1995	1996	1997	1998	1999	2000	2001	2002
Live (>60 cm TL)	6661	6076	4303	3519	273	1395	762	1804
Skin (30-51 cm belly width)	2234	1868	1762	2101	3998	3400	4379	2440
Live + skins	8895	7944	6065	5620	4271	4795	5141	4244

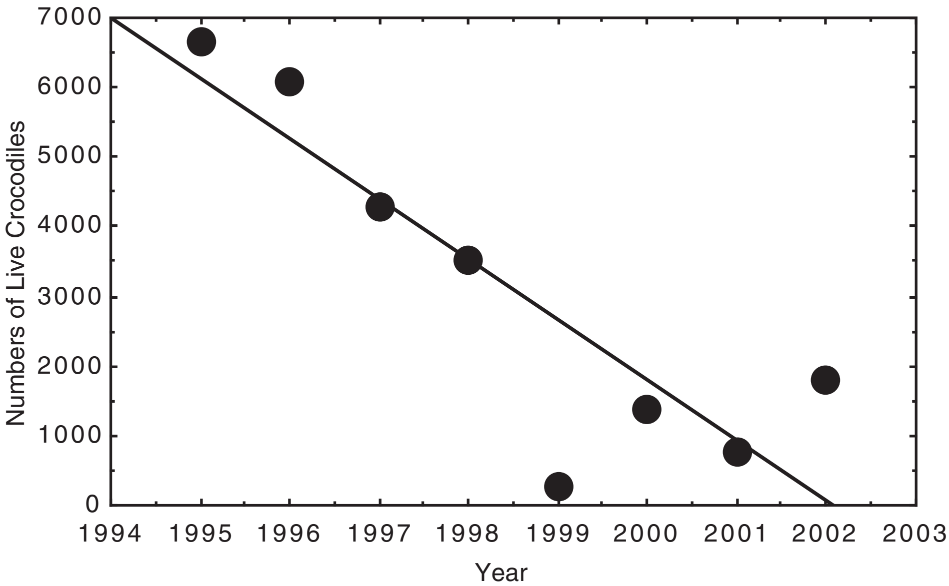

Figure 6. Numbers of live *C. novaeguineae* purchased by Bintang Mas (see Table 4). Line indicates significant linear regression trend (see text).

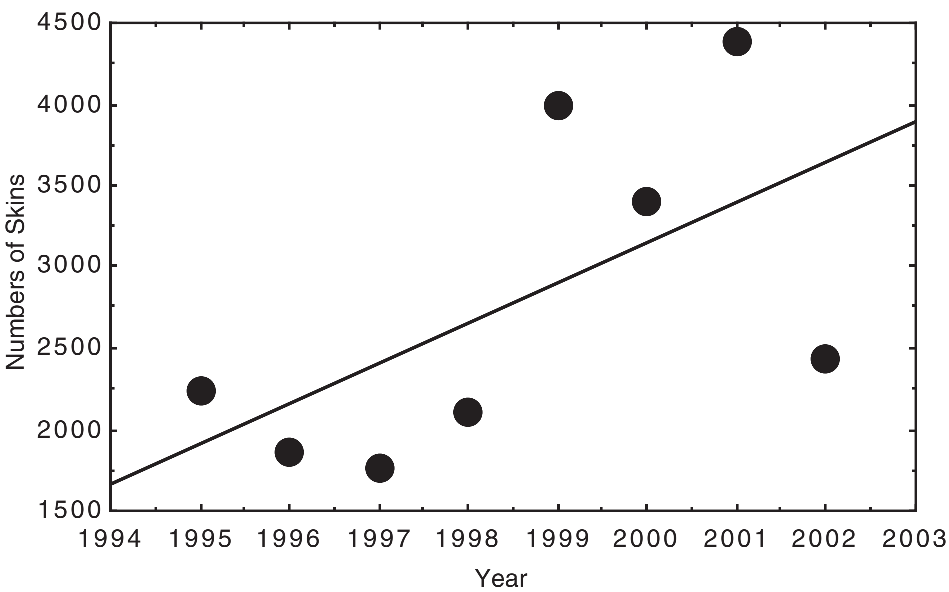

Figure 7. Numbers of *C. novaeguineae* skins purchased by Bintang Mas (see Table 4). The inclusion of 2002 data renders the trend non-significant (line) (see text).

Discussion

Survey results from 13 of the survey areas (Memberamo Mati River, Jaro River, Baso River, Sobaki Lake, Kamika Lake, Waropen Lake, Kweri Satu Lake, Kweri Dua Lake, Apuse Lake, Bernekam Satu Lake, Bernekam Dua Lake, Cabang Tiga Satu Lake, Cabang Tiga Dua Lake) indicated no significant trends between non-hatchling density of *C. novaeguineae* sighted, and time. For one area, the Soi River, there has been a significant increase in non-hatchling density (Fig. 4b).

High water levels (flooding) in 2001 and 2002 were not optimum for spotlight surveys, and most likely affected the sight ability of crocodiles (Messel *et al.* 1981). Trends in some areas (eg Bernekam Satu Lake, Cabang Tiga Satu Lake, Cabang Tiga Dua Lake), with high densities in 2000 followed by lower densities in 2001 and 2002, probably reflect the suboptimal conditions under which the latest surveys were carried out. Surveys in 2000 were carried out in the dry season (Table 2). Notwithstanding the effects of high water levels, population trends of *C. novaeguineae* in Mid-zone Memberamo River generally indicate stability from 2000 to 2002.

There has been an overall decrease in total harvest (live and skins) between 1995 and 1999, followed by a general increase after 1999 (Table 4). These trends are attributable to factors unrelated to the status of the crocodile resource. Specifically, the following factors need to be taken into account in assessing these harvest data:

- From 1994 and 1996, Indonesia had a moratorium in place, and the demand for live crocodiles for raising on farms, decreased.
- Demand for wild skins during the moratorium period also decreased, as many companies possessed stockpiles of skins purchased from Papua Province, which could not be sold.
- Between 1996 and 1999, the price of crocodile skins on the international market dropped, which did not encourage companies to sell accumulated skin stocks. It also discouraged live crocodile purchases.
- From 1999-2002, skin prices on the international market improved, and the demand for live crocodiles increased as a result.
- The drop in harvested skins purchased by CV Bintang Mas, from 4379 in 2001 to 2440 in 2002, was the result of an annual quota (2440 skins) imposed on the company by the Indonesian Ministry of Forestry.

Spotlight surveys are not always good indicators of population trends in heavily vegetated habitats (Montague 1983), and nest counts may perhaps be better indices of the population in heavily vegetated swamps associated with the Memberamo River, which are not conducive to spotlight survey (Hollands 1987). However, nest count surveys in Papua Province would require substantial financial resources, which simply cannot be afforded at this time. Monitoring through spotlight surveys still offers an index of the *C. novaeguineae* population in the Memberamo River, Papua Province, and will be the prime monitoring method for the wild population in the short-term.

Acknowledgements

We are grateful to the many people who supported this survey and were instrumental in its success. Many thanks to Mr. Gandhi Gan, Mr. Billy Gan, Mr. Raymon Gan, Mr. Andi Antariksa and Mr. Ibrahim Mile from CV Bintang Mas, and to Mrs. Ay Cu from The Indonesian Crocodile Farmers Association. The surveys were made possible by the outstanding contribution of Ir. B.G. Resubun [Head of Subdivision of Natural Resources Conservation Papua I (KSDA)] and his staff, Ir. Arijan Prasodjo, Ir. Nanang Hari Murdani, Mr. Oktavianus Howay, Mr. Alpianus Kilungga, Mr. Derek Makabory, Mr. Kristian Marandey, Mr. Hasudungan Hutaeyan Mr. Nixon Nauw, Mr. Untung Ginting and Mr. Teguh Mujiarto.

Literature

Bayliss, P. (1987). Survey methods and monitoring within crocodile management programmes. Pp 157-175 *in* Wildlife Management: Crocodiles and Alligators, ed. by G.J.W. Webb, S.C. Manolis and P.J. Whitehead. Surrey Beatty and Sons: Chipping Norton.

Hollands, M. (1987). The management of crocodiles in Papua New Guinea. Pp. 73-89 *in* Wildlife Management: Crocodiles and Alligators, ed. by G.J.W. Webb, S.C. Manolis and P.J. Whitehead. Surrey Beatty and Sons: Chipping Norton.

KSDA (Division of Natural Resources and Conservation Irian Jaya, I) (2000). Monitoring and survey report of crocodile population in Memberamo Zone, Irian Jaya. Jayapura. ii+18 pp. [Balai Konservasi Sumber Daya Alam Irian Jaya I (KSDA). (2000). Laporan monitoring dan survai populasi buaya di Zona Memberamo, Irian Jaya. Jayapura. ii+18 pp.]

KSDA (Division of Natural Resources and Conservation Papua I.) (2002). Survey and monitoring of crocodile population in Pagay (Kaureh District) and Dabra (Memberamo Hulu District), Jayapura Regency, Papua Province. Jayapura. v+14 pp+16 appendices. [Balai Konservasi Sumber Daya Alam Papua I (KSDA). (2002). Survey dan monitoring populasi buaya di habitat alam di Pagay (Distrik Kaureh) dan Dabra (Distrik Memberamo Hulu) Kabupaten Jayapura, Propinsi Papua. Jayapura.v+14 pp+16 lampiran].

Kurniati, H., Prasodjo, A., Murdani, N.H., Howay, O., Kilungga, A., Makabory, D. and Marandey, D. (2001). Crocodile spotlight surveys in mid-zone Memberamo River (Memberamo and Rouffaer River systems), Irian Jaya, Indonesia. Indonesian Institute of Sciences, Cibinong. 12 pp.

Messel, H., Vorlicek, G.C., Wells, A.G. and Green, W.J. (1981). Surveys of Tidal River Systems in The Northern Territory of Australia and Their Crocodile Populations. Monograph 1. Pergamon Press: Oxford.

Montague, J.J. (1983). Influence of water level, hunting pressure and habitat type on crocodile abundance in the Fly River drainage, Papua New Guinea. Biological Conservation 26: 309-339.

Webb, G.J.W. and Jenkins, R.W.G. (1991). A proposal to transfer the Indonesian population of *Crocodylus porosus* Schneider (1801) from Appendix I to Appendix II of CITES. Pp. 36 *in* Management of Crocodilians in Indonesia: a Review with Recommendations.

Report on the Activities and Actual Situation of the Crocodylia in Mexico

Recompilation of information by Manuel I. Muñiz
(Credit to authors in Annex)

Sociedad para el Estudio y Conservación de los Cocodrilos en México a.c. (SECOCOM a.c.) Dec. 2003., Apartado Postal 41– 601, Lomas de Chapultepec, c.p. 11000 México D.F. México.
moreletii@mx.inter.net or moreletiimuniz@hotmail.com

Abstract

Decades have passed studying, investigating and producing articles related to caimans, *Caiman crocodilus chiapasius* (not yet confirmed as subspecies), American crocodiles, *Crocodylus acutus* and Morelet's crocodiles, *Crocodylus moreletii* in our country, but little of these results are known outside Mexico. That's why the main objective of this work is to let know in a synthesis the situation and activities done around the crocodilians that inhabit in Mexico. We can find in 16 of the 32 States in Mexico crocodilians. From Sinaloa to Chiapas along the Pacific coast; from Tamaulipas and San Luis Potosí all the way to the Peninsula of Yucatan and in inland at the central depression of Chiapas State.

The Mexican authority in charge of environment regulations is SEMARNAT, through the Wildlife General Direction (DGVS), CITES Administration; CONABIO CITES scientific; PROFEPA inspection; and INE.

All three specie are protected by Mexican laws under the category **Special Protection**, which permits and allows sustainable use. Registered are 35 farms and more than 80 zoo and collections with crocodilians, 3 of them have CITES permits and 1 farm working with caimans, which doesn't need to register with CITES for exports. Mexico has imported more than 250,000 hides of caiman and other crocodilians which are transformed and re-exported as finished products and sub-products to the rest of the world.

Introduction

Decades have passed studying, investigating and elaborating articles related with Caimans, American crocodiles and Morelet's crocodile in our country, but little of these results are known outside Mexico. That's why, the main object of this work is to let know in a synthesis the situation and activities done around the crocodilian that inhabit in Mexico. As is known, in Mexico live 3 of the 23 species of crocodilians (Ross 1998) that we actually can find in our world. The caiman, *Caiman crocodilus chiapasius* (not yet confirmed as subspecies); American crocodile, *Crocodylus acutus*; and Morelet's crocodile, *Crocodylus moreletii*.

Mexican species, as with most crocodilians, are threatened because of the destruction, transformation, contamination and invasion of their habitat. In addition they are victims of hunting and illegal trade. With great effort between the communities, cooperatives, investigators, private investors, ONG, Government entities, academics and institutions had invert and minimize these threats. But still a lot of work has to be done to guarantee the recovery of the three crocodilian species in Mexico.

Because of their ecological value and keystone importance in their habitat, the Mexican crocodilians are considerate as charismatic and priority species, acquiring with this, a high priority for their conservation and protection.

From the 32 States of the Mexican Republic, in 16 of them, we can find crocodilians. From Sinaloa to Chiapas along the Pacific cost; from Tamaulipas and San Luis Potosí all the way to the Peninsula of Yucatan and in inland at the central depression of Chiapas State.

Also during the last four year Mexico has imported more than 250,000 hides of caiman and other crocodilians which transform and re-exports finish products and sub-products to the rest of the world.

En Español

Décadas han transcurrido estudiando, investigando y elaborando artículos en torno a los caimanes, a los cocodrilos de río y a los cocodrilos de pantano en nuestro país, pero poca de estos resultados se han dado a conocer a nivel internacional. Por ello, el objetivo principal de este trabajo es dar a conocer en síntesis la situación y actividades que se realizan en torno a los cocodrilianos que habitan en México.

Como se conoce en México existen 3 de las 23 especies de cocodrilianos (Ross 1998) que actualmente se pueden encontrar en el planeta. El caimán, *Caiman sclerops chiapasius,* el cocodrilo de río, *Crocodylus acutus*, y el cocodrilo de pantano, *Crocodylus moreletii*.

Como la mayoría de las especies de cocodrilianos las especies mexicanas se encuentran amenazadas por la destrucción, transformación, contaminación e invasión de su hábitat, además son victimas de cacerías y comercio ilegales. Con grandes esfuerzo entre las comunidades, cooperativas, investigadores, iniciativa privada, organizaciones no gubernamentales, entidades gubernamentales, académicos, e instituciones se han vertido y minimizado algunas de estas amenazas. Sin embargo falta mucho más por realizar para garantizar la recuperación de las tres especies de cocodrilianos en México.

Por su valor ecológico y eslabón importante en su hábitat, los cocodrilianos son considerados en México como especies carismáticas y prioritarias. Adquiriendo con ello una mayor importancia para su conservación y protección.

De los 32 estados en la República mexicana, en 16 de estos, podemos encontrar cocodrilianos. Desde Sinaloa hasta Chiapas por toda la costa del pacífico, desde Tamaulipas, pasando por San Luis Potosí y toda la península de Yucatán y adentrándose hasta la depresión central del estado de Chiapas.

Además en los últimos cuatro años México a importado más de 250,000 pieles de caimán y otros cocodrilianos que transforma y re-exporta productos y subproductos terminados al resto del mundo.

Situation with the Three Species of Mexican Crocodilians

Identification of the three species

Even though, in other part of the distribution of these species, other authors mention some characteristic, here we're going to describe and resemble the characteristics and the observation made by Mexican investigators and scientists.

Caiman sclerops chiapasius

Described by Bocourt, the *Caiman sclerops chiapasius* is now know as *Caiman crocodilus fuscus* because there's not enough information and studies on the species and subspecies of caimans to validate this data. But Álvarez del Toro and Luis Sigler (2001), mentioned and described the subspecies. Medem (1962) analysed, suggested, classified and described the subspecies of caimans, mentioning the *Caiman sclerops chiapasius*. Also some DNA studies suggest that *Caiman crocodilus fuscus* and *Caiman crocodilus chiapasius* are in the same natural group (Ross 1998). Though, we've seen morphological differences between the Mexican caiman, (*Caiman sclerops chiapasius* or *Caiman crocodilus chiapasius*) and the morphological descriptions made by other authors for the *Caiman crocodilus crocodilus* and *Caiman crocodilus fuscus*.

Sizes: Adults' maximum sizes registered, 2.50 m. Adults sizes 1.20 m minimum (females) up to 2.00 m or more (males). Hatchlings average sizes 22.5 cm. Minimum 18.5 cm, maximum 25.5 cm. Females lays an average of 30 eggs, maximum 37 eggs. (Lopez M, per.com.)

Conformation: Snout is short, triangular and round tip. Measuring between 1.2 to 1.5 times as long as broad at the level of the front corners of the eyes. A transverse bony ridge connects the anterior points of the orbits. On the upper eyelid presents a high point or tubercle. On the lower jaw presents two, three or more blotches.

Scutellation: The belly skin of caimans has osteoderms and without the integumentary sensory organs (ISO) (Richardson, Webb and Manolis 2002). Postoccipitals: One or two transversal row of 6 to 8 scales irregularly arranged. Nuchals: 4 to 5 transverse rows of 4 to 2 scales, continuous with the dorsal scales. Collar: A single row of enlarged scales on collar.

Distribution: The species, *Caiman crocodilus* could be found from the south Pacific coast of Mexico all the way to north of Argentina. The subspecies *Caiman sclerops chiapasius*, as described by Medem, is registered to the Tonala region, northwest cost of Chiapas, so we believed that the distribution of this subspecies could find from the south of Oaxaca state throw all the pacific cost of Chiapas as high as 50 meter above sea level (Álvarez and Sigler 2001). Not knowing is the distribution of this subspecies goes on Central America. Some authors mention it all the way to Panama.

Crocodylus acutus

Sizes: Adults' maximum sizes registered, 7.00 m (Brazaitis 1973). In Mexico, is the biggest of all three species, Alvarez del Toro (1974) measured one specimen with a total length of 6.25 m, and also Pérez Higareda and friends (1991) reported one specimen of the same sizes. Nowadays, it's difficult to find specimens larger than 5 m. Adults sizes 2.10 m minimum (females) up to 5.00 m or more (males). Hatchlings average sizes 28 cm minimum 24 cm, maximum 31.5 cm. Females lays an average of 34 eggs, maximum 51 eggs. Eggs average sizes 7.69 cm (+4.2) x 4.69 (+1.6) cm (Valtierra 2001; Sigler *et al*. 2000)

Conformation: Skull and snout are slender and elongate, 1.8 to 2.5 times as long as broad at the level of the front corners of the eyes. Adults develop a hump on the snout anterior to the orbit. Iris is greenish and eyelid wrinkled.

Scutellation: The scutellation is highly irregular and variable among individuals. The dorsal scales are enlarge and high. Postoccipitals: A single transversal row of 1 to 6 enlarged scales medially divided by soft skin. Nuchals: The nuchal rosette has 4 enlarged scales in a quadrant and 2 scales flanked by the sides. Variability in pattern is great among individuals.

Distribution: *Crocodylus acutus* is found from south Florida, United States, Northwest Pacific coast of Mexico all the way to Venezuela and Colombia. Also found in Cuba, Jamaica, Dominique Republic, Antilles, and almost in all small island and cay in the Caribbean Sea. In Mexico, could be found from the north Pacific coast as high as La Bojonea, north of El Fuerte River all the way to the Suchiate River at the border of Guatemala and Mexico, inhabiting all mayor costal mangroves, marshes, swamps, rivers, lagoons, and larger ponds (Ponce 2002; Valtierra 2001; Sigler 2000). Also reported in the coast, cay and small islands of Quintana Roo State in the Yucatan Peninsula. It hasn't been confirm to inhabit the Gulf of Mexico specially at Tabasco and Campeche States (Carballar J., 2002). There's a particular isolated population of *Crocodylus acutus* living in the Grijalva River between massive high hydroelectric damps and high mountains, the Sierra Madre cordillera, in the middle of Chiapas State (Sigler 1998, 2000, 2002).

Crocodylus moreletii

Sizes: Adults' maximum sizes registered, 3.50 m. Adults sizes 1.30 m minimum (females) up to 3.10 m or more (males). Hatchlings average size of 26.2 cm, minimum 20 cm. maximum 31.5 cm. Females lay an average of 35 eggs, maximum 50 eggs. Eggs average 6.8 cm x 4.3 cm (Muñiz 2000).

Conformation: Snout short, massive and wide, not more than 1.5 times as long as broad at the level of the front corners of the eyes.

Scutellation: The belly skin of Morelet's crocodile is highly prized because it has few osteoderms (Richardson, Webb and Manolis 2002). Presents inclusions on tail and on ventral skin. Postoccipitals: a single transversal row of 4 to 6 scales divided by smooth skin. Nuchals: Presents a nuchal rosette with one row of 4 scales and another row with 2 scales.

Distribution: Occurs on the Atlantic side in the Gulf of Mexico from centre as high as Laguna Madre, 42º 30' latitude north in Tamaulipas State down throw Veracruz, San Luis Potosí, Oaxaca and Tabasco to Campeche, Yucatan and Quintana Roo in the Peninsula of Yucatan (Hinojosa 2001; Bautista 1999; Dominguez 2003; Figueroa *et al*. 2000; Gómez 2002; Pani 1999; Pérez 2002; Merediz 1999; Cedeño 2002). Inland as far as in the Huasteca Potosina at San Luis Potosi State in the north and in the south occurs in the northwest and northeast of Chiapas State, El Caracol lagoon, El Aguacate lagoon; Lacanja river and River Lacantún at Montes Azules biosphere reserve, Usumacinta river (the border between Guatemala and Mexico) up to 800 m above sea level. (Martinez 2001; Sigler *et al*. 2001; Sigler 2002). Also occurs in Belize and north of Guatemala (Platt 1997; Castañeda 2001).

Status of the Three Species

National and international list, laws and conventions that affects and controls Mexican crocodilians.

- NOM-059-ECOL-2001: is the official norm that identifies the species and population that are in risk of wild flora and fauna in the Mexican Republic. Based on a list that corresponded to the risk, the criteria of inclusion, of exclusion and changes of categories. See Categories in chapter of legislation.

- CITES: Convention on International Trade in Endangered Species of Wild Fauna and Flora.

- IUCN (International Union for the Conservation of Nature and Natural Resources) - The World Conservation Union Red List.

- ESA: Endanger Species Act, United States law.

- CSG: Crocodile Specialist Group, Species Survival Commission, IUCN.

Caiman sclerops chiapasius: Considered as *Caiman crocodilus fuscus*.
NOM-059-ECOL-2001: Special Protection.
CITES: Appendix II
IUCN 1996: Not listed.
ESA: Not listed
CSG 1998: Availability of survey data - Adequate, Need for wild population recovery - low, Potential for sustainable management - highest. Though in Mexico requires more survey data - poor.
Threats: Habitat loss and illegal hunting.

Crocodylus acutus
NOM-059-ECOL-2001: Special Protection.
CITES: Appendix I
IUCN 1996: Listed. Vulnerable. Criteria A.1.a.c. inferred decline >20% in 3 generations, inferred from reduction in extent of occurrence.
ESA: Listed
CSG 1998: Availability of survey data - poor, Need for wild population recovery - high, Potential for sustainable management - moderate.
Threats: Habitat loss and illegal hunting

Crocodylus moreletii
NOM-059-ECOL-2001: Special Protection.
CITES: Appendix I and Special permits for 3 farms. Num. 501, 502 and 503
IUCN 1996: Listed.
ESA: Listed
CSG 1998: Availability of survey data – poor, Need for wild population recovery – moderate, Potential for sustainable management - moderate.
Threats: Habitat loss and illegal hunting

Responsible Government Agencies, Legislation and Internal Control System

Management and Scientific Authorities:

Secretaria del Medio Ambiente y Recursos Naturales, (SEMARNAT) is the Secretary of Environment and Natural Resources in charge of everything related with the environment and our natural resources.

CITES Management Authority: Dirección General de Vida Silvestre, SAGP, SEMARNAT, (DGVS); is the general direction of Wildlife Service.
Address: Av. Revolución no.- 1475, Colonia Tlacopac, México D.F.

CITES Scientific Authority: Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO) is the National Commission for the Knowledge and use of the Biodiversity.

Inspection, Control and Supervision

Procuraduria Federal para la Protección del Medio Ambiente (PROFEPA,) is the Federal Attorney's offices for environmental protection, is in charge of verifying the environmental legislation compliance, regarding natural resources and wildlife usage. In accordance, PROFEPA carries out a national inspection programme, which includes inspection of foreign trade of specimens, products and sub-products. It has inspection offices located in 65 ports, airports and borders where wild flora and fauna imports and exports permits are verified, as well as the sanitary conditions of wood and fishery products and sub-products, and also verifying all tips of Management units of wildlife. (UMAS)

Laws

In Mexico we have different laws that protects, conserves and establish the methods for the sustainable use of wild and captive flora and fauna.

Ley General de Vida Silvestre

The most important law in Mexico related with the wildlife, it's conservation, protection and sustainable use is the "General Law for wildlife" = "Ley general de Vida Silvestre", published on July 3, 2000 by the president Dr. Ernesto Zedillo Ponce de Leon. It's related to the conservation and sustainable use of wildlife and their habitat in the Mexican territory and the zones where the nation has jurisdiction. With the exception of the sustainable use on timber forest and fishery, who have their own law.

Ley General del Equilibrio Ecológico y la Protección al Ambiente. (LGEEPA) (1996)

This is the General Law for the Ecological Equilibrium and Protection of the Environment. Includes all laws related with the environment, our natural resources and its protection. Since December 1996, the article 87 of this law (LGEEPA) permits the use of wild flora and fauna species, when privates grants a controlled reproduction and growth in captivity or semi-captivity, including a management plan to conserve and protect their populations.

NOM-059-ECOL-2001
Norma Oficial Mexicana, NOM-059-ECOL-2001, Protección ambiental – especies de flora y fauna silvestres de México. Published in March 2002, this norm has for object to identify the species and subspecies of wild flora and fauna in the Mexican Republic that are in risked or endangered to disappear. Through the integration of a list, which categories, classifies, and determines the risk and threats of the wildlife.

Categories of risk:

1. Probablemente extinta en el medio Silvestre = Probably extinct in the wild

   Those species natives of Mexico whose individual had disappear from the wild in the Mexican territory, from which, we have the knowledge that specimens live in captivity or outside the country.

2. En peligro de extinccion = In danger of extinction

   Those species which distribution areas and population in the national territory had been diminish dramatically. (This category coincided partially with the categories of critically endanger and in danger of extinction on the IUCN classification).

3. Amenazada = Threatened

   Those species or their populations, that could get in danger of extinction or disappear in a short or middle period of time if the threats on the species continue. (This category coincided partially with the categories of low risk on the IUCN classification).

4. Protección especial = Special protection

   Those species and their population that could get threaten by negative factors, and has determined the need to recover, conserve or both the habitat and populations associated with the species and species. (This category could included the low risk IUCN classifications).

Other laws

Apart from the above laws, the hunting law, the Fishing Law, the Forest Laws and other are the fundaments for the conservation, protection and sustainable use of wildlife.

Inspection and Supervision

PROFEPA, (Procuraduria Federal para la Protección Ambiental) is the authority in charge to see that the laws are carried out.

CITES, Authorised Farms for exportation of Hides

CITES has authorised 3 farms to commercialise Morelet's crocodile hides, products and subproducts.

Cocodrilos Mexicanos s.a. de c.v., Cocomex - Mex
Number: A-MX-501 or Mx / 12.2002/ 1-1
With an estimated production of 12,000 eggs per year, it's commercialising at present between 6500 and 4800 hides per year. Principal markets Mexico, Japan and Spain.

Industrias Moreletii sa de cv
Number: A-MX-502 or Mx / 12.2002/ 1-2
With an estimated production of 3000 to 4000 eggs per year, it's commercialising at present around 1500 hides a year. Principal markets Mexico, Spain and Italy.

Cocodrilos de Chiapas s.a. de c.v.
Number: A-MX-503 or Mx / 12.2002 / 1-3
Not working at present and no data obtained.

Caimanes y Cocodrilos de Chiapas
Does not need a CITES permit to export. With an estimated production of 1000 eggs per year, it's commercialising *Caiman crocodilus*.

No other farms had applied for registration, though there are a few other farms that in a few years will apply.

Activities and Work done in each Region of the Mexican Republic

To simplify the work, the Mexican Republic has been divided in four regions. Each region has different climate and vegetation even though sharing some characteristic in the habitat. List of principal investigation projects and farms in each region are (in Spanish):

1. North Pacific Coast, including Sinaloa, Nayarit, Jalisco, Colima and Michoacán States

   The north Pacific coast characterized by a long dry season and low jungle (Caducifolian forest) with a thin line of mangrove cost and short rivers. Only *Crocodylus acutus* occurs.

   - Abreu, Alberto *et al*. Evaluación genética del hato reproductor en Cocodrilos mexicanos y establecimiento de un programa de selección genética.

   - León, Francisco *et al*. Chirricahueto Lagoon, Culiacán City, Sinaloa State.

   - Huerta, Sara *et al*. Dinámica Poblacional del "Caimán" (*Crocodylus acutus* Cuvier 1807, Crocodylidae), en Jalisco, México.

   - Huerta, Sara *et al*. Interacción Hombre-Cocodrilo en la Costa de Jalisco, México.

- Ponce, Paulino *et al*. Relación de factores físicos del hábitat con eventos reproductivos del "Caimán" *Crocodylus acutus* Cuvier, 1807.

- Ponce, Paulino *et al*. Distribución y situación actual de las poblaciones del "caimán" *Crocodylus acutus* en Jalisco

- Curul, Fabio *et al*. Ecología y Conservación del Cocodrilo de Río (*Crocodylus acutus*) en Bahía de Banderas, Jalisco-Nayarit, México

- Valtierra, Marciano. Ecología y Conservación del cocodrilo americano (*Crocodylus acutus*) en la Reserva de la Biosfera Chamela-Cuixmala, Jalisco, México.

- Navarro, Carlos. Determinación del estatus poblacional de *Crocodylus acutus* en el extremo norte de Sinaloa y sur de Sonora, México.

- Cocodrilos Mexicanos, Culiacán City, Sinaloa State. Is the biggest farm in Mexico with more than 25,000 crocodiles. Reproducing and commercializing, *Crocodylus moreletii*.

- Reptilario Cipactli, in Puerto Vallarta city, Jalisco State. Houses around 20 crocodiles at the Puerto Vallarta University, it's for exhibition, investigation, conservation and reproduction of *Crocodylus acutus*.

- Cocodrilos del Pacífico, Lagunas de Monte Negro, at Coquimatlán, Colima State. Houses more than 250 *Crocodylus acutus*. It's for investigation, reproduction and sustainable use.

2. South Pacific Coast, including Guerrero, Oaxaca and Chiapas States

   Caimanes y Cocodrilos de Chiapas, CAICROCHIS, in Tapachula City, Chiapas State. Breed *Caiman crocodilus, Crocodylus acutus* and *Crocodylus moreletii*. Housing over 1000 crocodilians. It has also South American species and African tortoise.

   - Sigler, Luis *et al*. Cañón del Sumidero Project, Chiapas State, Zoomat.

   - López, María de la Paz *et al*. Crecimiento y Desarrollo poblacional del Caimán (*Caiman crocodilus*) en Estado de Chiapas México.

   - García, Jesús. Diseño de una estrategia de manejo del cocodrilo de río (*Crocodylus acutus*) en el estero La Ventanilla, Oaxaca.

   - Giselle, Claudia *et al*. Estado Actual de la Población del Caimán *Caiman crocodilus chiapasius* en la zona del "Castaño" Reserva de la Biosfera La Encrucijada, Costa de Chiapas, México.

   - López, Andrés *et al*. Estado Actual de la Población y dieta alimenticia del Caimán *Caiman crocodilus chiapasius* en la zona de Laguna de Chantuto, Reserva la Biosfera La Encrucijada, Costa de Chiapas, México.

   - Gordillo, Omar *et al*. Recuperación del *Crocodylus acutus* en la Reserva de la Biosfera La Encrucijada, Chiapas.

   - Martínez, Ivonne *et al*. SSS Guardianes de la Selva, Pico de Oro Chiapas.

3. Gulf of Mexico, Tamaulipas, San Luis Potosí, Veracruz and Tabasco States

   - Domínguez, Jerónimo *et al*. Contribución al conocimiento de la distribución del cocodrilo de pantano (*Crocodylus moreletii*) en el estado de San Luis Potosí.

   - Domínguez, Jerónimo *et al*. Determinación de la densidad poblacional del cocodrilo de río (*Crocodylus acutus*) en los cuerpos de agua de Ixtapa - Zihuatanejo. Guerrero.

   - Domínguez, Jerónimo *et al*. Desarrollo productivo y operacional en la UMA de cocodrilos paraíso Husteco Tanchachin, en el municipio de Auismón, San Luis Potosí.

- Domínguez, Jerónimo *et al*. Estrategias para el desarrollo productivo de cocodrilo de pantano en la Cienega de cabezas, municipio de Tamasopo, San Luis Potosí.

- Domínguez, Jerónimo *et al*. Exploración para la identificación y localización de poblaciones cocodrilanas en los estados de Querétaro e Hidalgo como nuevos sitios con presencia de los Crocodylia en México.

- Domínguez, Jerónimo *et al*. Análisis genético de fragmentos polimorficos de las especies de cocodrilianos mexicanos, (*Crocodylus acutus*, *Crocodylus moreletii* y *Caiman crocodilus fuscus*).

Industrias Moreletii, at Villahermosa City, Tabasco State. Is the second biggest farm in Mexico, with more than 5000 crocodiles, reproducing and commercializing *Crocodylus moreletii*.

Reptilario Cipactli, in Puerto Vallarta city, Jalisco State. Houses around 20 crocodiles at the Puerto Vallarta University, it's for exhibition, investigation, conservation and reproduction of *Crocodylus acutus*.

4. Yucatan Peninsula, including Campeche, Yucatan and Quintana Roo States

- Carballar, Javier *et al*. Estudio, Manejo y Conservación de las poblaciones de cocodrilos en el Sistema Lagunar Nichupte, Cancún, Quintana Roo, México.

- Carballar, Javier *et al*. Monitoreo de cocodrilos de la reserva de la biosfera banco chinchorro.

ª Romero, Miriam *et al*. Proyecto cocodrilo CETMAR Campeche importancia, justificación, avances y metas.

- Gómez, Yadira. "Hábitos alimentarios de *Crocodylus moreletii* y *Crocodylus acutus* en la zona norte de la Reserva de la Biosfera Sian Ka'an"

Cocodrilos Maya, in Ciudad del Carmen, Campeche State. Houses around 250 crocodiles, it's for investigation, reproduction and commerce of *Crocodylus moreletii*.

Crococun, in Cancún City, Quintana Roo State. Houses around 100 crocodiles, it's for exhibition, investigation, conservation and reproduction of *Crocodylus acutus* and *Crocodylus moreletii*.

CET-MAR Univerisity, in Campeche City, Campeche State. Houses around 250 crocodiles at the CET-MAR University, it's for exhibition, investigation, conservation and reproduction of *Crocodylus moreletii*.

Commerce, Importations, Exportations and Domestic Market

The sustainable use of wildlife, has establish the way to conserve and protect the environment and the wild population in the world.

But Mexico has become more an importer than a producer, in crocodilian terms. We're producing around 8000 to 10,000 legal skins per year between all 12 commercial farms including the *Caiman* species and *Crocodylus moreletii*; this compared with the 250,000 hides imported every year is a big difference, because none of these imports provide incentives for the protection and conservation of the three species of crocodilians that live in our country.

These hides are transformed and 60 to 75% of the finished products are re-exported, mostly to the United States, with smaller quantities to Spain, Italy, Japan and southeast Asia (León, pers. comm., DGVS 2002).

Most imported hides come from Venezuela and Colombia, some other come from the United States, Argentina and Brazil in South America, Zimbabwe in Africa and Australia (Table 1, Fig. 1).

Principal tannery cities in Mexico are Leon in Guanajuato State, Juarez City and Chuihuahua City in Chihuahua State, Guzman City in Sonora, Guadalajara City in Jalisco State, Tapachula City in Chiapas State and in Mexico City.

Most of the goods are cowboy boots, belts, bags, wallets, and smaller products.

Table 1. Imports of crocodilian hides into Mexico, 1993-2001.

Species	1993	1994	1995	1996	1997	1998	1999	2000	2001	Total
A. mississippiensis	526	1377	758	795	7615	14,683	16,830	10,056	4385	57,025
C. c. crocodilus				10		143				153
C. c. chiapasius				13,972						13,972
C. c. fuscus	181	899	5650	43,334	24,550	74,374	146,165	260,018	243,334	798,505
C. yacare		210	9396	15,450	23,713	3999	600	1500	5444	60,312
C. johnstoni			2		40					42
C. niloticus				8190	204	4550	19,536	9898	6102	48,480
C. moreletii					35	70	50			155
C. novaeguineae	1						165			166
C. porosus		100	152						20	272
Totals	708	2586	15,958	81,751	56,157	97,819	183,346	281,472	259,285	979,082

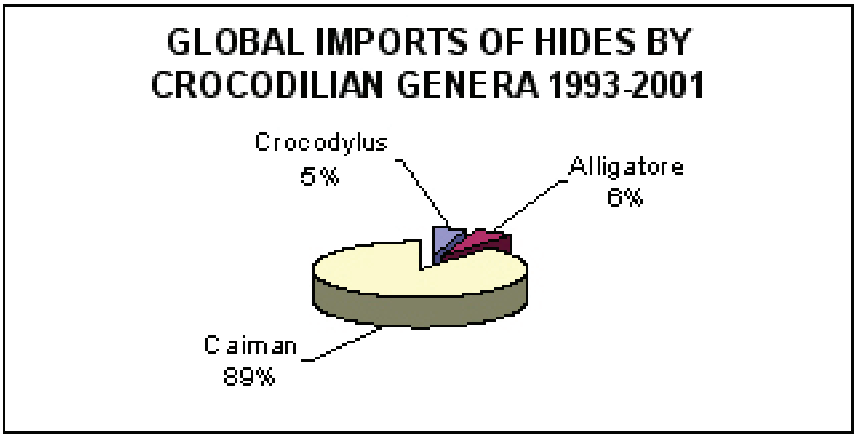

Figure 1. Imports of hides into Mexico, by genus, 1993-2001.

Some “Gavials” are *Tomistoma schlegelii*

Franklin D. Ross and Norna R. Ross

Department of Vertebrates, Nationaal Natuurhistorisch Museum,
PO Box 9517, 2300 RA Leiden, the Netherlands

Abstract

While examining older anatomical literature about the morphology of the trachea in crocodilians, we discovered an error in an original text, which caused a considerable amount of confusion. When reexamining the data about the tracheal rings and the rings of the exposed bronchi, and the total number of cartilage rings in the windpipes system, we conclude that the data supports contradictory systematic arrangements.

Introduction

When the anatomist Heinrich Rathke (1866) used the word "Gavialen" in the context that the number of tracheal cartilage rings in the gavials is smaller than the number in the genus *Crocodylus*, it contradicted the corresponding data in his accompanying table. What Rathke meant to say is that *Gavialis schlegelii*, now called *Tomistoma schlegelii*, has fewer tracheal rings than has any species within the genus *Crocodylus* or the species *Gavialis gangeticus*. The reason for Rathke's unfortunate wording is not known. However, because Rathke's (1866) dissections of a taxonomically diverse series of the modern Crocodylia were so completely and well reported and illustrated, his works have become an often cited source.

When Professor C.K. Hoffmann at Leiden University wrote the reptiles section in H.G. Bronn's prestigious "Klassen und Ordnungen des Thier-Reichs" book series, he relied heavily on Rathke for details and conclusions about many anatomical subjects, including the cartilage rings in the extra-pulmonary windpipes-system commonly known as the trachea and the two exposed bronchial tubes. Hoffmann's (1890: 1034) assertion that the number of tracheal rings "bei den Gavialen am kleinsten, bei den Crocodilen (ie bei der Gattung *Crocodilus*) am grossten" is remarkably similar to Rathke's (1866: 150) statement that "bei den Gavialen am kleinssten, hingegen bei den Thieren der Gattung *Crocodilus* am grossten" in that both phrases assert that the "Gavialen" (plural of gavial) have fewer cartilage rings in their tracheas, while normal crocodiles such as *Crocodylus* have the most.

In comparison with Rathke's fairly rare set of papers (1857 material listed, 1866 windpipes reported), Bronn's edited book-series containing Hoffmann's (1890) summaries of crocodilian anatomy has become widely circulated. Some authors like Reese (1915a-b) have attributed Hoffmann's (1890) German paraphrases of Rathke's original German to Rathke or to Bronn, without mentioning Hoffmann (see quotation of part of Reese's text, below); and, in this particular case, if Reese had consulted the original Rathke publication, he would have noticed the table saying that *Gavialis gangeticus* has more tracheal rings than any of the *Crocodylus* species.

Later and independently, when Steven F. Perry (1998: 36) said about "the number of cartilaginous rings in the trachea and extrapulmonary bronchi" that "the number is least in *Gavialis*, and greatest in *Crocodylus* (Hoffmann, 1890)", Perry and his editors fell into the trap of equating "Gavialen" with *Gavialis gangeticus*, the only living *Gavialis* species today. Their compound error not only misrepresented *gangeticus*; but, it also misrepresented *schlegelii* because, in 1998, the genus *Gavialis* did not include *schlegelii* as a species.

Discussion

The German "Gavialen" was a compound group in 1857-1866, when Rathke (in the 1866 table especially) put *schlegelii* and *gangeticus* in the same genus; and, it is interesting to note that Reese in 1915 was also using "gavials" to include *Gavialis gangeticus* and *Tomistoma schlegelii* together, following a classification system popularized by Ditmars and based on feeding habits or "the shape of the head" as Reese (1915a: 1-3) put it. Thus it is even more difficult to understand Reese. However, Perry in 1998, is very clear wording; and, because it appeared in the "Biology of the Reptilia" series, edited by Carl Gans, it is both highly respected, and globally circulated. In its final (1998) form, the statement is totally false; because, Rathke's data in the table showed *Tomistoma* to have the least number of trachial rings, followed then by the alligators and caymans as a group, followed in turn by the *Crocodylus* species; and, finally, with the most rings in the trachea is *Gavialis gangeticus*, as shown below in Column A, of Table 1.

Table 1. Rathke's (1866: 149-150) table, with original species names in their published order. Column A is cartilage rings in the main tracheal stem. Column B is the number of rings in the exposed left bronchus. Column C is the number in the right bronchial tube. Note that column A is the trachea ("dem Stamme") which has the "Gavialen" at the extremes of variation. Note also that columns B and C are left and right branches of the "more or less" symmetrical bronchial tubes, which are later averaged together to produce the mean bronchial numbers in Table 2, below. See other tables for translated material; and, see Table 4 especially for modern equivalents of Rathke's genera and species names.

	A	B	C
Embryo von *Gavialis Schlegelii*	51	36	37
jungen *Allig. punctulatus*	63	22	24
Embryo von *Allig. Sclerops*	60	20	19
jungen *Allig. Sclerops*	62	19	20
alteren *Allig. Sclerops*	66	18	19
jungen *Allig. Lucius*	66	12	14
alteren *Allig. Lucius*	60	16	16
Embryo vn *Allig. cynocephalus*	65	25	25
jungen *Allig. cynocephalus*	74	25	26
alteren *Allig. cynocephalus*	74	19	18
jungen *Allig. palpebrosus*	70	17	18
etwas alteren *Allig. palpebrosus*	12	16	18
jungen *Croc. vulgaris*	85	35	35
alteren *Croc. vulgaris*	84	32	34
nicht reifen Embryo von *Croc. acutus*	87	19	18
eben so alten Embryo von *Croc. acutus*	89	24	24
reifen Embryo von *Croc. acutus*	88	30	30
jungen *Croc. acutus*	79	33	32
alteren *Croc. acutus*	88	32	33
jungen *Croc. biporcatus*	102	36	36
etwas alteren *Croc. biporcatus*	96	39	40
noch alteren *Croc. biporcatus*	104	40	40
jungen *Gav. gangeticus*	116	29	25

Note that the statements made by Rathke and Hoffmann and Reese are not only not-true about the gavials in the broadest and simple descriptive sense (those two super-long snouted piscivores); but, their assertions are only describing the number of cartilage rings in the main and single-tube stem of the trachea, before the windpipes system (larynx, trachea, syrinx in crocs, bronchial pair, lungs) bifurcates posteriorly into the two exposed bronchi. If Rathke had summarized the left bronchus (Column B, Tables 1-2), for example, he would have said that the alligators have the fewest (12-25), followed by the true gavial of India (29), and that the species of *Crocodylus* have the most (19-40), with *Tomistoma* (36) in the range of *Crocodylus*; and, in essence, the same is true of the right bronchus, as shown in Column C, in Tables 1-2. A similarly different result is also obtained by averaging the left and right bronchi into a single "bronchial" mathematical mean, as shown in Column D, in Table 2 (where B + C, divided by 2 = D).

In Figures 2-4, the specimens identified as "Meckel" and "Cuvier" are second-hand data which Rathke obtained from the literature. The uncommon synonym (*Alligator punctulatus*) of the common cayman used by Rathke (1857, 1866) is distinguished from Rathke's *Alligator sclerops* by following Hoogmoed and Gruber (1983: 378-9) in the opinion that Jacaretingapunctulatus Spix = *Caiman c. crocodilus* (L.). The current names and classification of all of the specimens reported in Rathke (1866: 149-150) are made clear in Table 4, including Column F.

In Column D, in Table 2, the simple picture is that some Alligatoridae have the fewest cartilage rings in their bronchi, followed by chaos. In a theoretical sense, the number of rings in the trachea is no more important than the number of rings in the bronchi; or, even combining the two data sets for the number of rings in the whole windpipes system, as shown in Column E of Table 3 (where A plus C = E).

Table 2. Bronchial data from Rathke (1866: 149-150) including some second-hand data, with the names of all species updated; and, rearranged in order of column D. Column B is just the left bronchus (as in Table 1, but arranged here by bronchial mathematical means from column D). Column C is just the right bronchus, arranged by bronchial average (from column D). Column D is the average or mean value of the two bronchial tubes together as one number.

	B	C	D
young *Alligator mississippiensis*	12	14	13.0
older *Alligator mississippiensis*	16	16	16.0
older *Paleosuchus palpebrosus*	16	18	17.0
young *Paleosuchus palpebrosus*	17	18	17.5
Meckel's *Alligator mississippiensis*	no data	no data	18.0
older *Caiman latirostris*	19	18	18.5
ripe embryo *Crocodylus acutus*	19	18	18.5
older *Caiman crocodilus* ssp.	18	19	18.5
young *Caiman crocodilus* ssp.	19	20	19.5
embryo *Caiman crocodilus* ssp.	20	19	19.5
Meckel's *Caiman crocodilus* ssp.	no data	no data	20.0
young *Caiman crocodilus crocodilus*	22	24	23.0
embryo same age *Crocodylus acutus*	24	24	24.0
embryo *Caiman latirostris*	25	25	25.0
young *Caiman latirostris*	25	26	25.5
young *Gavialis gangeticus*	25	26	27.0
Meckel's *Crocodylus acutus*	no data	no data	30.0
Cuvier's *Crocodylus niloticus*	32	32	32.0
young *Crocodylus acutus*	33	32	32.5
older *Crocodylus acutus*	32	33	32.5
older *Crocodylus niloticus*	32	34	33.0
young *Crocodylus niloticus*	35	35	35.0
young *Crocodylus porosus*	36	36	36.0
embryo *Tomistoma schlegelii*	36	37	36.5
somewhat older *Crocodylus porosus*	39	40	39.5
even older *Crocodylus porosus*	40	40	40.0

In Column E, it is significant that Tomistoma does not have the fewest. We think that Rathke's arrangement of the species by tracheal rings (see Column A in Table 1) was following the science of his time by considering anterior (closer to the head) most important; and, also thinking larger (and single in this case) is better than smaller. He was likely not intending to make a taxonomic statement; but, was happy to be able to construct a short summary sentence because he had his "Gavialen" at both ends of the tracheal variation data. All that is clear is that Rathke's act of overlooking *Gavialis gangeticus* in his text statement was significant; and, that Hoffmann (1890) failed to catch Rathke's (1866) error. Part of Hoffmann's problem in summarizing Rathke's conclusions is the number of synonyms involved; for example, *Gavialis gangeticus* was called "*Gav. gangeticus*" and "*Gav. tenuirostris*" and "*Gav. tenuirostris* (*gangeticus*)" and also "*Rhamphostoma tenuirostre* (*Gavialis*)"in the first half of Rathke's (1866: 143-164) "Respiration organs" chapter.

Finally, Reese (1915a: 198-199) added some numbers to the tracheal-rings question (and put our famous "Gavialen" phrase into context), saying "The number of tracheal rings varies not only in different species but also in different individuals of the same species. There are between fifty and sixty in *A. mississippiensis*. According to Rathke the number of rings in the individual animal most certainly does not increase with age. The number of rings is smallest in the gavials and greatest in the crocodiles (genus *Crocodilus*). The number of rings in two divisions of the trachea does not increase with age except, perhaps, *C. acutus* and *biporcatus*. ... According to Rathke and others most of the tracheal rings are closed, but a varying, though at most small, number are open on the dorsal side. These openings become wider as the larynx is approached. The transverse muscle fibers which are found in the most anterior and largest of these breaks in the tracheal rings were found, says Rathke, in embryos after the middle period of incubation."

Table 3. Total windpipes-system data from Rathke (1866 incl. lit. cites) arranged in strict numerical order of column E. Column A and Column C are the same data as in Tables 1-2, though slightly expanded, and rearranged in order of column E. Column E is the total of the whole windpipes-system (trachea and bronchial-average combined).

	A	C	E
alteren *Alligator mississippiemis*	60	16.0	76.0
jungen *Alligator mississippiensis*	66	13.0	79.0
Embryo von *Caiman crocodilus* ssp.	60	19.5	79.5
jungen *Caiman crocodilus* ssp.	62	19.5	81.5
older *Caiman crocodilus* ssp.	66	18.5	84.5
jungen *Caiman crocodilus crocodilus*	63	23.0	86.0
Embryo von *Tomistoma schlegelii*	51	36.5	87.5
jungen *Paleosuchus palpebrosus*	70	17.5	87.5
etwas alteren *Paleo. palpebrosus*	72	17.0	89.0
Embryo vn *Caiman latirostris*	65	25.0	90.0
alteren *Caiman latirostris*	74	18.5	92.5
Meckel's (70-80) *Alligator mississippiensis*	75	18.0	93.0
Meckel's (70-80) *Caiman crocodilus* ssp.	75	20.0	95.0
jungen *Caiman latirostris*	74	25.5	99.5
nicht reifen Embryo *Croc. acutus*	87	18.5	105.5
jungen *Crocodylus acutus*	79	32.5	111.5
eben so alten Embryo von *Croc. acutus*	89	24.0	113.0
Cuvier's (82) *Crocodylus niloticus*	82	32.0	114.0
alteren *Crocodylus niloticus*	84	33.0	117.0
reifen Embryo von *Crocodylus acutus*	88	30.0	118.0
jungen *Crocodylus niloticus*	85	35.0	120.0
Meckel's (90) *Crocodylus acutus*	90	30.0	120.0
alteren *Crocodylus acutus*	88	32.5	120.5
etwas alteren *Crocodylus porosus*	96	39.5	135.5
jungen *Crocodylus porosus*	102	36.0	138.0
jungen *Gavialis gangeticus*	116	27.0	143.0
noch alteren *Crocodylus porosus*	104	40.0	144.0

For Reese's "between fifty and sixty" cartilage rings in the trachea data, we take the middle number of 55 tracheal rings, partly because it leaves the FALSE and TRUE "Gavialen" at the extremes of variation, as shown in Column A, in Table 4. This arrangement is so visually attractive that even we are willing to play with the numbers to keep gators (50 is less than 51) from having the fewest cartilage rings in their tracheas, though they apparently do.

Conclusion

The data in Rathke (1866) and in Reese (1915a) about cartilage rings in the trachea is probably meaningless in a taxonomic sense; because, arranging the species and genera by bronchial counts gives different results, as does arranging the data by the numerical variation in the total exposed windpipes system (tracheal and bronchus numbers combined). All assertions that the gharials or gavials or *Gavialis* have the fewest tracheal rings are most likely in error; because, *Tomistoma schlegelii* has very few, and is at the lower extreme of alligators; while, *Gavialis gangeticus* has the most, and is beyond the extreme of proper crocodiles.

Literature

Hoffmann, C.K. (1890). "Dr. H.G. Bronn's Klassen und Ordnungen des Thier-Reichs, wissenschaftlich dargestellt in Wort und Bild, volume 6, section 3: Reptilien. part 2: Eidechsen und Wasserechsen." = "Sechster Band. Ill Abtheilung Reptilien. II Eidechsen und Wasserechsen". C.F. Winter'sche Verlagshandlung, Leipzig, Germany, i-ii & 443-1399, & plates 49-107.

Table 4. Just the main-stem tracheal rings: Rathke 1866, and Reese 1915, combined and arranged in numerical order. Column F is subfamily (Tomistominae) or family (gators, crocs, gharial) classification. Column A is here expanded to include Reese's, Meckel's and Cuvier's data; and, here arranged in actual numerical order.

	F	A
Gavialis Schlegelii = Tomistoma schlegelii	Tomistominae	51
Reese (50-60) *Alligator mississippiensis*	Alligatoridae	55
Alligator Sclerops = Caiman crocodilus ssp.	Alligatoridae	60
Alligator Lucius = Alligator mississippiensis	Alligatoridae	60
Alligator Sclerops = Caiman crocodilus ssp.	Alligatoridae	62
Alligator punctulatus = Caiman c. crocodilus	Alligatoridae	63
Alligator cynocephalus = Caiman latirostris	Alligatoridae	65
Alligator Sclerops = Caiman crocodilus ssp.	Alligatoridae	66
Alligator Lucius = Alligator mississippiensis	Alligatoridae	66
Alligator palpebrosus = Paleosuchus palpebrosus	Alligatoridae	70
Alligator palpebrosus = Paleosuchus palpebrosus	Alligatoridae	72
Alligator cynocephalus = Caiman latirostris	Alligatoridae	74
Alligator cynocephalus = Caiman latirostris	Alligatoridae	74
M's *Allig. Sclerops = Caiman crocodilus* ssp.	Alligatoridae	75
M's *Alligator Lucius = Alligator mississippiensis*	Alligatoridae	75
Crocodilus acutus = Crocodylus acutus	Crocodylidae	79
C's *Crocodilus vulgaris = Crocodylus niloticus*	Crocodylidae	82
Crocodilus vulgaris = Crocodylus niloticus	Crocodylidae	84
Crocodilus vulgaris = Crocodylus niloticus	Crocodylidae	85
Crocodilus acutus = Crocodylus acutus	Crocodylidae	87
Crocodilus acutus = Crocodylus acutus	Crocodylidae	88
Crocodilus acutus = Crocodylus acutus	Crocodylidae	88
Crocodilus acutus = Crocodylus acutus	Crocodylidae	89
M's *Crocodilus acutus = Crocodylus acutus*	Crocodylidae	90
Crocodilus biporcatus = Croc. porosus	Crocodylidae	96
Crocodilus biporcatus = Crocodylus porosus	Crocodylidae	102
Crocodilus biporcatus = Crocodylus porosus	Crocodylidae	104
Gavialis gangeticus = Gavialis gangeticus	Gavialidae	116

Hoogmoed, M.S. and Ulrich Gruber (1983). Spix and Wagler type specimens of reptiles and amphibians in the natual history musea in Munich (Germany) and Leiden (The Netherlands). Spixiana (supplement 9) 319-415. Munich, Germany.

Perry, S.F. (1998). Lungs: comparative anatomy, functional morphology, and evolution. Pp. 1-92 *in* Biology of the Reptilia. Volume 19: Morphology G, ed. by C. Gans and A.S. Gaunt.

Rathke, H. (1857) "Untersuchungen über die Aortenwurzeln und die von ihnen ausgehenden Arterien der Saurier." Denkschriften der kaiserlichen Akademie der Wissenschaften (Mathematisch-Naturwissenschaftlich Classe) 13 (2): 51-142, including explanation pages for plates pp. 136-142, & plates 1-6. Vienna, Austria.

Rathke (1866). Untersuchungen über die Entwickelung und den Korperbau der Krokodile (= Investigations into the development and structure of the body of crocodiles). Friedrich Vieweg und Sohn: Braunschweig, Germany, i-viii, & 1-275 pp.; and, plates 1-11.

Reese (1915a). The Alligator and its Allies. G.P. Putnam's Sons: New York. 358 pp. & 28 plates 28.

Reese (1915b). The development of the lungs of the alligator. Smithsonian Miscellaneous Contributions 65 (2): 1-11. Not seen (J.P. Ross, pers. comm.).

Workshop Reports

Workshop 1: Skin Quality Workshop (Fritz Huchzermeyer)

A list of skin defects was established and discussed. It was agreed that the current secrecy surrounding this issue is entirely counterproductive and it was then decided to produce a document with a description and illustration of all known defects, with their causes if known, to be presented at the next CSG meeting in 2006. The participants promised to contribute knowledge and photographic material to this end.

Workshop 2: Human-Crocodile Conflict Workshop (Rich Fergusson)

There are now over 50 CSG members who have expressed interest in this groups' activities at either the 16th or 17th Working Meetings. It was agreed that the group needed to keep in close contact to ensure activity between CSG meetings. To facilitate this a number of regional co-ordinators offered to keep contact with interested people in their areas and to compile information and updates on HCC. The regional co-ordinators are:

USA	Allan Woodward (allan.woodward@fwc.state.fl.us)
Meso America	Miriyam Venegas (venegasm@naos.si.edu)
Australasia	Mark Read (mark.read@epa.qld.gov.au)
Africa	Rich Fergusson (zeahtco@zol.co.zw)
SE Asia	Nikhil Whitaker (kachugazl@hotmail.com) and
	S.M.A. Rashid (rashid@reptilesfarm.com)

The workshop agreed there was urgent need to deliver a number of products. It was agreed that these would be:

- Consolidated guidelines on the prevention and management of HCC that are approved by CSG that may be provided to government wildlife authorities that have the problem but no solution.

- A database of all attacks by crocodiles on humans and livestock. It was recognised that this maybe difficult to achieve in a globally compatible format but that within regions this should be achievable. There are two aims to this product - to formalise the capture and reporting of information on HCC incidents and to use this compilation to indicate how countermeasures may be applied.

- A "fact sheet" that combines information from the above two products. It is intended that this is provided to print and broadcast media pre-emptively and when HCC incidents occur, with the aim of minimising the hype that frequently appears, at least in the more developed countries.

It was further agreed that the group needed to improve the impact and reach of educational material and efforts. In this regard members of the group will react whenever possible to advise on the realities of HCC and its prevention, that "crocodile tourism" would be encouraged provided that this carries a strong message on responsible actions and that the African Educational Film Foundation would be approached to assist.

Finally it was agreed that more information and research was needed on crocodile populations, the incidence of HCC, factors increasing the risk of HCC, and countermeasures that can be employed, including harvesting to reduce crocodile populations. It was noted that harvesting alone was unlikely to provide a solution to HCC.

Workshop 3: Major Issues Affecting Sustainability of Crocodilian Trade (Don Ashley)

The results of this workshop are presented within the paper entitled " Major issues affecting crocodilian sustainable trade", pages 341-343 in this volume.

Workshop 4: CSG Core Business (Perran Ross)

CSG is at pivotal point in its development with substantial structural and financial strength, a strong record of achievement and success and good global credibility. Impending leadership change and new and previously unforeseen issues make consideration of Core issues and guidance for the immediate time frame of 2004- to the next Working Meeting, strategically important. The 17th Working Meeting provided a unique opportunity for broad member input to this process.

After discussion at Steering Committee and with core CSG leaders, a draft 'statement of core business' was proposed and a process to examine the views and opinions of meeting participants on this important issues was developed.

'The core business of the CSG is crocodilian conservation'

However, the exact direction, details and structure to achieve this general goal requires elaboration. To accomplish this, a simple questionnaire was developed and distributed to all participants on the first day of the meeting with the request that people would respond within 24 hours. Responses were collected, collated and a small team attempted a general analysis of the responses received. The questionnaire is attached as Appendix 1.

The questionnaire attempted to identify respondents according to their membership in CSG and knowledge about the relationship of CSG and its parent IUCN-SSC and then explored preferences of respondents regarding general themes and specific actions that CSG should be concerned with in the intermediate time frame of 1-3 years.

The results and analysis are severely limited by some inherent biases and constraints that must be considered before accepting the results as 'truth'. However, we believe that this exercise did allow a free and open discussion and contributions by the respondents and should be carefully considered as a general guide to future CSG strategy development. Biases and limitations of the process include the following:

- The questionnaire was ad-hoc and not scientifically designed and therefore represents the biases of its drafters.
- The respondents are neither a random sample of CSG members nor were they a fully representative sample. Respondents were a group of CSG members and non members, present at the meeting and choosing to provide responses. The result should be interpreted with these evident biases in mind.
- A specific and obvious bias was that a large proportion of meeting participants, and therefore of respondents, was Australian and reflects their national preoccupations and concerns. This bias is clearly evident in some of the results.
- The analysis we conducted was informal and hasty, not statistically robust.

For all these reasons the results should be taken as a general indication of opinions from a specific subgroup of CSG interests and should not be generalized. Rather they should serve as a foundation for further inquiry.

We distributed questionnaires to 230 meeting participants and received 81 responses, a creditable 35% response rate. We divided the responses according to whether the respondents were CSG members or not and whether they indicated their interaction with CSG was positive and useful (Question 2) or otherwise. Five responding CSG members suggested their interaction with CSG was negative. The reasons for this discontent needs to be identified and rectified.

	CSG members	Non-Members
CSG +ve	27	14
CSG neutral or -ve	18 (5 -ve)	17

The first question we analyzed was respondents understanding of SSC-IUCN. There was a clear difference in response between members and non- members. Most CSG members claimed to understand IUCN while many non-members did not.

The estimation of the value of the association with IUCN also segregated strongly by CSG membership and less so by positive feelings about CSG. As the Chairman of SSC was present in the audience, we are gratified that overall - most members appear to value the association with SSC.

	CSG members		Non-Members	
	CSG +ve	CSG -ve	Non-Member +ve	Non-Member -ve
Question 5				
Understanding of IUCN				
YES	24	15	8	5
NO	3	3	6	12
Questions 6 & 7				
Value of IUCN				
+ve	24	15-17	3	5
Neutral	2	1	0-2	2
-ve	0	0	0-2	0

Question 12 asked respondents to choose from a list of possible 'Core business' topics. Some very clear positive and negative responses emerged. Percent of positive (supporting) responses are shown in the following table.

Core Business IS:
b. Develop conservation policy (92%)
a. Prevent extinction (83%)
c. Advising national agencies (77%)
h. Advising IUCN (65%)

Core Business is NOT:
g. Local activism (16%)
j. Political advocacy (35%)

Question 13 inquired about the most important structural elements that CSG needed to address. Again there were clear majority indications of both preferences and dislikes. The percentage of positive (supportive) responses is indicated against the sub-question of question 13 (see Appendix).

Strong support
b.i Fundraising for Core activity (71%)
k. Broader integration of topics (66%)

Weak support
g., h. Changing size (15%)
j (ii.) Narrow commercial focus (30%)
i. Increasing paid staff (38%)

Interestingly, on the question of number of members, many respondents checked both 'do not increase' and 'do not decrease' indicating they think the size of the group is about right.

Question 14 asked respondents to indicate preferences from a list of issues deemed to be important for CSG. Of interest is the poor support for some emerging and newly identified threats to crocodilians. However, traditional CSG interests remain strongly supported.

Most Important
e. Sustainable management (88%)
f. Habitat loss (80%)
g. Critically endangered species (79%)

Least Important
j. Wildlife disease (36%)
d. Increased use (38%)
h. Pollution (41%)

A sub-question of question 14 gave respondents an opportunity to express a preference for a species they considered of high priority. The results suggest that a few species have their dedicated proponents, but most respondents have no species priority.

- No species preference (48)
- *Tomistoma* (5)
- *C. porosus* (5)
- Gharial (4)
- *C. siamensis* (3)
- *A. sinensis, C. mindorensis,* (2)
- *Osteolaemus, Caiman yacare* (1)

The final question gave respondents the opportunity to write in their own expressions of priority issues. This section is important because it is not constrained by the structure or content of the questionnaire and might more accurately reflect respondent interests. Analysis of this question was difficult, but we grouped the 118 responses into broad categories defined by consensus of the analysis team and indicate the number of similar statements for each group.

- Critically Endangered species (26 responses)

Asia, new approaches, active programs, interaction with government

- National Conservation and Management (26 responses)

Habitat loss, monitoring, capacity building, CITES compliance. Australian safari hunting, reintroduction programs

- Research (18 responses)

Biology, human-croc conflict, skin quality

- Trade and Use (17 responses)

Linking use and conservation, monitoring trade, sustainability, farming guidelines, promoting use, Occupational Health and Safety regulations in Australia.

- Information Issues (17 responses)

Public awareness, encourage young people, exchange with diverse groups

It is unclear why a significant number of respondents suggested that increased public awareness of crocodilian issues was needed but this item received very low support in question 12.

Finally, question 9 addressed a number of possible structural rearrangements for CSG. A short and useful list emerged of issues that respondents felt CSG should address.

- Reorganize regionally (questionnaire support 46%)
- Improve contact to IUCN
- Avoid internal politics
- Diversify fundraising
- Improve networking
- Empower members

In conclusion, we feel that this exercise has provided rough but useful indicators of the sentiments of this important gathering of CSG members and supporters. It has given a large number of people the opportunity to express their views. The results, while rough, are intriguing and indicators for additional inquiry. Overall, the results suggest a rather conservative perspective by these respondents. They do not support or propose any drastic departures from current structure or activity. However, there are a number of issues, identified in the tables above that are suggested for further examination and action.

Thanks for assistance in data collection, compilation and analysis to Akira Matsuda, Sally Isberg, Col Stephenson, Amanda Rice, Laurel Converse, Vicki Simlesa and Nancy Fitzsimmons. Significant assistance with concept, the questionnaire and the development of the core business statement was provided by Harry Messel, Grahame Webb, Charlie Manolis and members of the CSG Steering Committee.

APPENDIX

Core Business Workshop Questionnaire

1. Are you a CSG member ? Yes….. No…..

2. Does CSG assist your work/business ? Yes….. Neutral….. No……

3. I contact CSG (please be honest) Often….. Sometimes…. Rarely…..

4. I learn of CSG activity from: (check as many as you like)
   a. CSG Newsletter Yes…..
   b. Other members Yes…..
   c. CSG Website Yes…..
   d. Direct correspondence Yes……
   e. Working Meetings Yes…….
   f. Other……………………… Yes…….

5. I understand CSG's relationship to IUCN and SSC and can answer questions 6 -11 below (if No , Please go to question 12) Yes…… No……

6. CSG's inclusion in IUCN is Valuable…Neutral….. Useless…

7. CSG's inclusion in SSC is Valuable…Neutral….. Useless…

8. IUCN policy defines my CSG activity? Yes…. No…..

9. IUCN Croc Red Listing helps my work Yes….. Neutral….. No…..

10. I am a member of other SSC groups Yes…. No…..

11. IUCN/SSC assists my work Yes….. Neutral No…..

12. CSG's "CORE" business is: (check as many as you like)
    a. Preventing crocodilian extinction? Yes……… No…..
    b. Developing conservation policy Yes….. No…..
    c. Advising national agencies Yes….. No…..
    d. Advising international treaties Yes….. No…..
    e. Biological Research Yes….. No…..
    f. Initiating conservation programs Yes….. No…..
    g. Local activism Yes….. No…..
    h. Advising IUCN and its members? Yes….. No…..
    i. Public education Yes….. No…..
    j. Political and advocacy Yes….. No…...
    k. Disseminating information Yes….. No…..
    l. Fundraising for croc conservation Yes….. No…..
    Other (please indicate)
    m. ……………………… Yes….. No…..
    n. ……………………….. Yes….. No…..

13. CSG can accomplish its Core business most effectively by the following actions, strategies and structure:

a. More active advocacy	Yes…..	No…..
b. More Fundraising		
i. For core CSG activity	Yes…..	No…..
ii. To fund member activity	Yes…..	No…..
iii. Support member fundraising	Yes…..	No…..
c. Funding member projects	Yes…..	No…..
d. Maintaining current structure	Yes…….	No…..
e. Reorganising current regions	Yes…..	No…..
f. Independent organizational status	Yes…..	No…..
g. Becoming smaller (fewer members)	Yes…..	No…..
h. Becoming larger (more members)	Yes…..	No…..
i. Increase paid staff	Yes…..	No…..
j. Narrower focus		
i. On endangered species	Yes…..	No…..
ii. On commercial species	Yes…..	No…..
iii. On economic/social factors	Yes…..	No…..
iv. On habitat/landscape issues	Yes….	No….
k. Broader focus integrating i.-iv.above	Yes….	No…..
l. Support independent member action	Yes…..	No…..

14. The most important issues facing CSG in the immediate future are:

a. Trade regulation	Yes…..	No…..
b. This species (please indicate)		
…….………………………..	Yes…..	
c. Human-croc conflicts	Yes…..	No…..
d. Increased commercial use	Yes…..	No…..
e. Sustainable management	Yes…..	No…..
f. Habitat loss	Yes…..	No…..
g. Critically endangered species	Yes…..	No…..
h. Pollution effects	Yes…..	No…..
i. Public perception of crocs	Yes…..	No…..
j. Wildlife disease	Yes…..	No…..
k. Inadequate biological knowledge	Yes…..	No…..
l. Inadequate national programs	Yes…..	No…..
m. Other (please indicate)		
…………………………………..	Yes…..	

CSG's three main priorities for the next 2-3 years must be :

#1………………………………………………………………………………………

#2 ………………………………………………………………………………………

#3 ………………………………………………………………………………………