Crocodile Specialist Group, Species Survival Commission

CROCODILES

Proceedings of the 24th Working Meeting of the Crocodile Specialist Group
Skukuza, South Africa, 23-26 May 2016

(Unreviewed)

2016

IUCN

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24th Working Meeting of the Crocodile Specialist Group
of the Species Survival Commission of the IUCN
convened at Skukuza, South Africa, 23-26 May 2016

Top left: *Crocodylus suchus*, Burkina Faso (Photograph: Christine Lippai); Top right: *Osteolaemus tetraspis*, Gabon; Centre left: *Mecistops* sp. nov. cf. *cataphractus*; Centre right: *Osteolaemus* sp. nov., Côte d'Ivoire (Photographs: Matthew Shirley); Bottom: *Crocodylus niloticus* (Photograph: David Kirshner).

(Unreviewed)

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Front cover: Nile crocodile, *Crocodylus niloticus*. © David Kirshner.

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The Crocodile Specialist Group

The Crocodile Specialist Group (CSG) is a worldwide network of biologists, wildlife managers, Government officials, independent researchers, non-government organization representatives, farmers, traders, tanners, manufacturers and private companies actively involved in the conservation, management and sustainable use of crocodilians (crocodiles, alligators, caimans and gharials). The CSG is supported financially through the International Association of Crocodile Specialists Inc. (IACS), and operates under the auspices of the Species Survival Commission (SSC) of the International Union for Conservation of Nature (IUCN). The CSG members in their own right are an international network of experts with the skills needed to assess conservation priorities, develop plans for research and conservation, conduct surveys, estimate populations, provide technical information and training, and to draft conservation programs and policies. The CSG itself keeps its members updated on international events with crocodilians, conducts reviews of country programs, and tries to track and prioritise issues in forums such as CITES that encourage legal trade and discourage illegal trade. CSG Working Meetings are generally held every two years.

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Foreword

The attendance of over 250 people at the CSG's 24th Working Meeting at Skukuza Rest Camp says something about synergism between the passion and dedication of CSG members, and the international allure of Kruger National Park, one of the world's most famous nature-based tourist destinations.

It was a wonderful meeting and the organising committee and event coordinators are to be commended for their splendid efforts. The host, South Africa National Parks (SANParks), with the help of various sponsors, made this a truly important and memorable meeting. Both the formal and social aspects of the meeting were carried out at the highest standards.

Despite South Africa being at the southern limit of the range of Nile crocodiles (*Crocodylus niloticus*) in Africa, and the wild populations being modest, South African researchers have played a pivotal role in our global understanding of crocodilian biology in the wild and in captivity. They were also present at the first CSG Working Meeting in New York, in 1971.

Tony Pooley (1938-2004) was South Africa's first real leader in crocodilian conservation. He was a tenacious researcher, naturalist and observer, who worked mainly in the then province of Natal. Amongst other things, including early efforts at developing farming technologies, he was one of the first to describe the intricacies of parental care by adult female crocodiles of eggs, nests and hatchlings, which does not occur in any other group of reptile.

With the advent and expansion of crocodile farming within South Africa, another great South African, Fritz Huchzermeyer (1930-2014), would make a lasting contribution nationally and internationally. A prominent veterinarian and scientist, Fritz became immersed in crocodile issues, both in the wild and in captivity. His book "Crocodiles: Biology, Husbandry and Disease" (2003) remains a pivotal reference work for people working on crocodilians around the world.

We were truly fortunate to have Tony and Fritz's wives, Elsa Pooley and Hildegard Huchzermeyer, attend the meeting. And it was heartening to see their children following in fathers' footsteps and work with crocodiles - through Simon Pooley and David and Philippa Huchzermeyer.

But this leadership also exists today. In 2007 wild Gharials in India mysteriously started to die, and a research mission to India, led by Fritz Huchzermeyer, were able to implicate metabolic disfunction and extreme "gout" as the cause. When mortality rates started to climb within the Nile crocodile population in Kruger National Park in 2008, SANParks initiated a diverse and intensive research program, that continues today. It appears metabolic disfunction and pansteatitis are the causes, but understanding how the status of a previously secure and safe wild crocodilian population could change so dramatically in such a short time, has far reaching ramifications for crocodilian conservation.

So in holding our 24th CSG Working Meeting in South Africa, we were embedded amongst giants, and truly within an environment where science and evidence-based policies about crocodilian conservation, management and sustainable use, are the norm rather than the exception. The meeting itself was rich in new insights and diverse findings, in both the formal presentations and posters. It was preceded by a two-day workshop of the CSG Future Leaders Working Group (19-20 May), a highly successful Veterinary Workshop (21 May), and CSG Executive (21 May) and Steering (22 May) Committee meetings.

The Proceedings of the 24th CSG Working Meeting will once again provide a unique compendium of current information on crocodilian research, conservation, management and sustainable use. It will serve as an important reference source for CSG members and non-members with an interest in crocodilians. My personal thanks to everyone for making this meeting such a great success.

Professor Grahame Webb, CSG Chair.

Summary of the Meeting

The CSG's 24th Working Meeting was convened at the Nombolo Mdluli Conference Centre at Skukuza Rest Camp, Kruger National Park, South Africa, from 23-26 May 2016. It was attended by 292 delegates and 60 accompanying family, who took over 95% of the Camp. South African National Parks (SANParks), represented by Dr. Danie Pienaar, hosted the meeting, and many sponsors provided financial support. The principal sponsors were Exotic Leather South Africa (ELSA; Stefan van As) and Wildlife Ranching South Africa (WRSA; Rob Reader).

The theme for the meeting was "Crocodiles, Communities and Livelihoods", and included a strong emphasis on crocodile-related issues from the southern African region. Presentations covered: species conservation, veterinary disease and research, markets and trade, human-crocodile conflict, and crocodilian husbandry. The organising committee (Christine Lippai, Howard Kelly, Alison Leslie, Danny Govender, Xander Combrink, Silke Pfitzer, Jan Myburgh) ensured that the venue, program, sponsors, entertainment, etc., were in place. An event organiser, "Inside Edge" (Tersia Tegmann, Hendrik Tegmann, Angus Morton), was contracted to assist with the complex logistic arrangements required to bring delegates from 43 countries (Argentina, Australia, Benin, Brazil, Burkina Faso, Cambodia, Colombia, Costa Rica, Côte d'Ivoire, Czech Republic, Denmark, France, Gabon, Germany, Egypt, Ghana, Guatemala, Hong Kong (China), India, Indonesia, Ireland, Israel, Italy, Japan, Mexico, Mozambique, Namibia, Nepal, Netherlands, Nigeria, Norway, Panama, Papua New Guinea, Philippines, Slovakia, South Africa, Spain, Taiwan, United Arab Emirates, United Kingdom, United States of America, Venezuela, Zimbabwe), and SANParks and the organising committee are especially grateful to Inside Edge.

The CSG's Future Leaders Working Group (FLWG) took the opportunity to hold a 2-day workshop on 19-20 May 2016, where a number of senior CSG members were able to "mentor" some younger CSG members in the history of the CSG, and how it operates under the IUCN, involvement with CITES, etc.

The working meeting was preceded by a Veterinary Workshop on 21 May 2016 in the Elephant Reception Room at the Game Processing Plant (Skukuza). Unfortunately, participation had to be capped at 50 participants. Enormous thanks are extended to Dr. Danny Govender (SANParks) for organising the venue and coordinating the event together with Dr. Paolo Martelli and Dr. Cathy Shilton (Vice Chairmen of CSG's Veterinary Science Group), Dr. Jan Myburgh and Dr. Silke Pfitzer. The Veterinary Workshop concluded with a safari drive and "sundowners" atop a rock in the bush.

The CSG Steering Committee meeting was held on 22 May 2016 and was attended by 30 Steering Committee members and 64 observers. Prior to addressing the packed agenda, the Chairman called for 1-minute silence in memory of CSG members who had passed away since the previous meeting. The Steering Committee meeting was followed by a cocktail reception and welcome (Dr. Danie Pienaar), including loud and enthusiastic traditional drumming and dancing.

The opening of the working meeting on 23 May 2016 began with the CSG Chairman Professor Grahame Webb being led into the conference venue by Praise Singer Shadrack, who gave a traditional blessing for the meeting in three languages - English, Zulu and Shangaan. The South African National Anthem was beautifully performed by infant children from the Skukuza Village School. The meeting was opened by Professor Webb following an official welcome by Mr. Glen Phillips, the Managing Executive of SANParks.

In addition to the oral presentations, working groups and thematic groups met during the meeting [CSG IUCN Red List Authority (Dr. Perran Ross), Veterinary Science (Dr. Paolo Martelli, Dr. Cathy Shilton), Public Education and Community Participation (Dr. Myrna Cureg), Zoos (Dr. Kent Vliet), Future Leaders Working Group (Dr. Matt Shirley)], as did members interested in human-crocodile conflict (HCC). A particularly interesting non-crocodilian side event presentation on Rhino Poaching & Rhino Health in Kruger National Park was given by Markus Hofmeyer (SANParks).

The first day of the meeting was dedicated to "Africa's Crocodiles: From Kruger to Beyond", beginning with a keynote address from Dr. Danie Pienaar on the Research History in the Kruger National Park and ending with a variety of presentations from the North, West and Central Africa regions. For some presenters, this was their first time participating in a CSG meeting, and some made presentations in English, which is not their primary language. An afternoon dealing with HCC culminated in a PECP-dedicated session which then continued in a working group meeting at the end of the first day.

A thematic day focused on Veterinary, Genetics and Behavioural Ecology began with a keynote address on the "Wicked Problem of a large scale crocodile mortality event" by Dr. Danny Govender, and several presentations explored the veterinary and dietary aspects of the pansteatitis crisis, the environmental health, chemical pollution and exotoxicology in the Olifants River of South Africa, and the possibility of using non-invasive means to measure physiological stress as a biomonitoring tool. Pollution and poisoning were highlighted through two presentations of research carried out on American alligators, as well as through poster presentations.

By special request, and following an interactive e-mail dialogue between CSG members, Larissa McLeod spoke about the Hidden Danger in Working with Crocodiles - several images will linger in many delegates' memories for quite some time!

Presentations on the fascinating response of crocodilian mothers to the calls of their offspring, as well as the acoustics of the American crocodile in Belize, kicked off the session on Behavioural Ecology, which also showcased the new research being carried out on crocodilian ventral colour change in response to exposure to visible light (Agata Staniewicz) and adaptation to its environment (Dr. Mark Merchant).

An afternoon session dedicated to Reproductive Biology, Genetics and Immunology, and Captive-breeding & *In-situ* Conservation included a diversity of presentations, including: variations in nest temperature; semen collection; hatching success and nest inundation; genetic diversity in the Okavango; cave isolation and ecological/evolutionary divergence in the African Dwarf crocodile; preserving Orinoco crocodiles; and, Siamese crocodile conservation in Cambodia.

A session dealing with Crocodilian Conservation Biology saw presentations on the Conservation and Spatial Ecology of the American alligator, American crocodile, Philippine crocodile, Morelet's crocodile, Saltwater crocodile and Mugger. This was followed by an intensive session devoted to the Gharial, including another captivating update on the Chambal Gharial Ecology Project by Dr. Jeff Lang.

As a lead-in to a day dedicated to Husbandry, Production and Trade, an afternoon session dealt with Sustainable Use and case histories from India and Mexico. The Husbandry session included a keynote address on research-based approaches to developing best practices along the value chain, particularly in relation to improving sustainability of South Africa's exotic leather industry. The current perspective of animal welfare at slaughter was discussed, as well as an example of a new model for sustainable use at an intensive Morelet's crocodile breeding farm in Mexico. Production and Trade examined the fashion industry's desire for sustainably sourced exotic skins for the luxury market and the use of information technology in the crocodile industry to promote traceability and value chain integration. The final session of the meeting, a Panel Discussion on 'The Classics in Trade and Conservation', was opened by Mr. Rob Davies, South African Minister of Trade & Industry, who highlighted the opportunities for trade and investment that the Ministry was providing to the crocodile industry. The Panel was chaired by Jimmyson Kazangarare from Zimbabwe.

The poster session was coupled with a Cheese & Wine event, which encouraged delegates to spend quality time at each of the posters and connect with the authors who could elaborate on their work. Following the poster session, a buffet dinner was served and prizes were handed out to students for oral presentations (Theresa Cantu, Agata Staniwiecz, Jonathan Warner, Lauren Augustine) and posters (Joey Brown, Donné van der Westhuizen, Evelyn Lopez Fernandez, Maria Laura Romito). Howard Kelly announced Enrico Chiesa (Italy) as the winner of the "International Golf Tournament", although he modestly claimed that his win was most likely a result of poor maths! The CSG Chairman's Encouragement Award was presented to Nathalie Kpera (Benin). Following the presentation of awards, the CSG Chairman announced that the CSG Executive Committee would include three new Deputy Chairmen - Perran Ross, Charlie Manolis and Christine Lippai.

The traditional CSG Auction then commenced, and was once again skilfully choreographed by Carlos Piña and Joe Wasilewski. A variety of items were on offer and a total of $US11,944 was raised, with 50% allocated to the Orinoco Crocodile program (Venezuela) and 50% to the Olifants River crocodile program (South Africa).

The African Bush Gala Banquet was a highlight of the social agenda, with a procession of safari vehicles taking everyone on a 2-hour game drive with spectacular sightings such as Lesser Galago, White Rhinoceros, African Elephant, Giraffe, Nile Crocodiles and even Spotted Hyena at the Bush Braai (BBQ) venue. An amazing setting for a candle-lit banquet in the bush, telescopes at the ready for some southern sky star-gazing, bottles of white and red wines on each table (courtesy of Villiera Wine Estate), a Mbira band and traditional dancers.

In a brief speech, the CSG Chairman honoured the late Tony Pooley and Fritz Huchzermeyer, who had contributed so much to conservation, management and research on South African crocodiles. It was good to see the participation of Elsa Pooley and Hildegard Huchzermeyer at the meeting, and to see their respective children, Simon Pooley, David and Philippa Huchzermeyer, follow in their fathers' footsteps and work with crocodiles. The culmination of the banquet was the presentation of the Castillo Award for Crocodile Conservation to Dr. Alison Leslie (Stellenbosch University, South Africa) for her (often) behind-the-scenes but untiring dedication to crocodile research and nurturing of future generations of crocodilian scientists.

Unfortunately, due to recent rhino poaching incidents in Kruger National Park, participants were returned to Skukuza by 10 pm, where many continued to party and dance until the early hours. As with every CSG Working Meeting the degree of camaraderie is a delight for old and new participants! Enormous thanks go to Louw the Audio-Visual Technician for setting up the music system in the reception area!

Host, Sponsors and Donors

Host
- South Africa National Parks (SANParks)

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- South Africa National Parks (SANParks)
- Exotic Leather South Africa (ELSA)
- Wildlife Ranching South Africa (WRSA)

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- Villiera Wines
- Chrisal Probiotic Hygiene
- Zambezi Grace

Organizing Committee
- Xander Combrink
- Danny Govender
- Howard Kelly
- Alison Leslie
- Christine Lippai
- Jan Myburgh
- Silke Pfitzer

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- Inside Edge, especially Tersia Tegmann, Hendrik Tegmann Angus Morton
- Louw, the audio-visual technician, for setting up the music system in the reception area
- Charlie Manolis, for editing and compilation of the Proceedings

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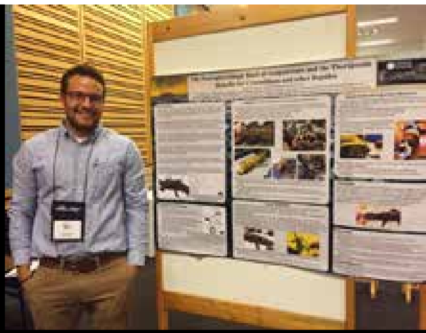



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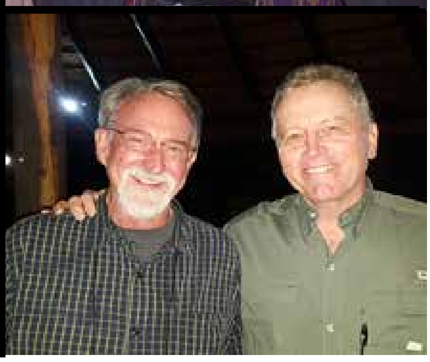

The History of the Crocodile Research Program in the Kruger National Park

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Abstract

In the Kruger National Park (KNP) crocodile research has been overlooked for many decades in favour of research on more "charismatic" animals like lion, elephant and other large mammals. From 1904 to 1960 at least 374 crocodiles were shot as part of the predator control program initiated to rebuild the depleted game numbers. The first scientist was appointed in KNP in 1950 and this was the start of a more systematic research program. Currently the KNP supports a vibrant research program with scientists from more than 30 scientific institutions conducting 200+ registered research projects and producing about 50 scientific papers per annum.

Crocodile aerial counts started in 1982 as part of the regular hippo surveys. During the 1980s and mid-1990s crocodile numbers in the 5 major rivers flowing through KNP varied between 1900 and about 3700. The sudden mass mortality in 2008 of more than 170 crocodiles in the lower Olifants and Letaba Rivers due to pansteatitis was the impetus for a focused research program on crocodiles and led to collaboration with a number of external scientists, including leading crocodile experts and the now late, Dr. Fritz Huchzermeyer and Dr. Lou Guillette Jr.

The demanding terrain in the Olifants Gorge offered a steep learning curve to the Kruger team of primarily mammal and terrestrial scientists but also offered opportunity for multidisciplinary collaboration with a wide network of research partners, many of whom are present at this meeting.

The research program resulted in the publication of 12 peer-reviewed research papers and numerous popular articles covering topics ranging from crocodile ecology and spatial dynamics to ecotoxicology, food web analysis and diagnostics development for the disease. The greatest achievement, however, of the Kruger Crocodile Research Program, has been that it has captured the minds of all South Africans and highlighted the unique role of these aquatic reptiles as sentinels of environmental health. It has also highlighted the threats to the species at its southern most range extent and also positively impacted funding streams towards research on the species in the wild.

The Nile crocodile population in South Africa is rapidly declining outside of protected areas and even in the relatively large KNP worrying crocodile declines are manifesting in the major perennial rivers. Changes in river morphology and water quality due to dam construction and consequent siltation associate with these declines. It is only through thoughtful and engaged scientific collaboration and learning to inform policy and considered land use planning that we will be able to halt or reverse the noted trends.

The Status of Limpopo and Mpumalanga Nile Crocodile Populations Outside the Kruger National Park

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Abstract

Monitoring of Nile crocodiles (*Crocodylus niloticus*) has been carried out irregularly in Mpumalanga and Limpopo in the past. The program was initially driven by the need to locate hippopotamus that were starving during drought periods and inform strategies to deal with these crises. Crocodiles were counted in addition to hippos, but initial figures were very low, this possibly reflecting a lack of focus on this species and the sporadic, piece-meal nature of many early surveys. With the advent of GPS devices it became possible to accurately plot the locations of individual crocodiles. We report here on the results of a number of aerial and spotlight surveys carried out in Mpumalanga and Limpopo, to the west of the Kruger National Park. Census results are presented and discussed in the context of changes in spatial distribution and potential drivers of population trends.

Status of Nile Crocodile in North-Eastern KwaZulu-Natal and Conservation Management Recommendations

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Abstract

Crocodilians are increasingly being viewed as "sentinel species" because, as top predators, they are good models for studying environmental threats to their respective ecosystems and the associated food web. Present threats to the major Nile crocodile *Crocodylus niloticus* populations in South Africa include pollution, habitat alteration/destruction and poaching. Concurrent studies of spatial and reproductive ecology, population status, and health of Nile crocodiles in Lake St Lucia, Ndumu Game Reserve and Pongolapoort Dam were undertaken. The sites differed in their ecology and the human pressures. The studies revealed numerous novel insights into the ecology, behaviour and health of Nile crocodiles in KwaZulu-Natal, South Africa, and some of the findings may be applicable to other crocodilian taxa. The Nile crocodiles were affected in different ways, illustrating that "one shoe" does not fit all. There are concerns about protection of nesting sites, increased anthropogenic disturbance, illegal poaching of crocodiles, and human/wildlife conflict. It is hoped that the results will guide the management and conservation of this threatened species and the waterbodies with which they are associated. In particular the following recommendations need be implemented at all study sites for Nile crocodile conservation: continuation of annual Nile crocodile aerial surveys; continuation of annual Nile crocodile nesting surveys; limitation of anthropogenic disturbance, especially destruction of riverine vegetation by unsuitable agricultural activities and the resultant destruction of nesting sites; prevention of illegal poaching of live crocodiles; and reduction in use of lead sinkers by fishermen to prevent lead poisoning of crocodiles. In addition, it is recommended that viable and unthreatened Nile crocodile populations be identified and protected. There is an urgent need for ecological, behaviour and health research of Nile crocodile populations in Kosi Bay, Lake Sibaya and Hluhluwe-Imfolozi Park.

Blood Lead Concentrations in Free-Ranging Nile Crocodiles (*Crocodylus niloticus*) from South Africa

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Abstract

Generally crocodilians have received little attention with regard to the effects of lead toxicity despite their trophic status as apex, generalist predators that utilize both aquatic and terrestrial habitats, thereby exposing them to a potentially wide range of environmental contaminants. During July-October 2010 we collected whole blood from 34 sub-adult and adult free-ranging Nile crocodiles (*Crocodylus niloticus*) from three separate populations in northeastern South Africa in order to analyze their blood lead concentrations (BPb). Concentrations ranged from below detectability (<3 µg/dL, N= 8) to 960 µg/dL for an adult male at the Lake St Lucia Estuary. Blood lead concentrations averaged 8.15 µg/dL (SD= 7.47) for females and 98.10 µg/dL (SD= 217.42) for males. Eighteen individuals (53%) had elevated BPbs (≥10 µg/dL). We assessed 12 general linear models using Akaike's Information Criterion (AIC) and found no significant statistical effects among the parameters of sex, crocodile size and population sampled. On average, crocodiles had higher BPbs at Lake St Lucia than at Ndumo Game Reserve or Kosi Bay, which we attribute to lead sinker ingestion during normal gastrolith acquisition. No clinical effects of lead toxicosis were observed in these crocodiles, even though the highest concentration (960 µg/dL) we report represents the most elevated BPb recorded to date for a free-ranging vertebrate. Although we suggest adult Nile crocodiles are likely tolerant of elevated Pb body burdens, experimental studies on other crocodilian species suggest the BPb levels reported here may have harmful or fatal effects to egg development and hatchling health. In light of recent Nile crocodile nesting declines in South Africa we urge further BPb monitoring and ecotoxicology research on reproductive females and embryos.

The Past, Present and Future of the Nile Crocodile in KwaZulu-Natal Province, South Africa

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Abstract

Historically, Nile Crocodiles (*Crocodylus niloticus*) were widespread throughout the rivers, floodplains, lakes and estuaries of the northern and coastal areas of KwaZulu-Natal Province (KZN), South Africa. Explorers, soldiers and hunters for the skin trade exterminated most crocodile populations, followed by efforts during agricultural expansion to extirpate the remaining crocodiles, listed as vermin until 1968. Tony Pooley, one of the founder members of the CSG, established a crocodile rearing and restocking station in Ndumo Game Reserve, later moving to St Lucia. Tony repatriated 824 crocodiles (5-32 months old) from 1967 to 1977 to formally protected, but mostly unfenced areas. This programme had a significant impact on re-establishing crocodile populations in KZN following the decades of killings and persecution. Initial population recovery for Ndumo and Lake St Lucia were virtually identical, 13% growth from around 350 observed individuals in the early 1970's to approximately 1000 individuals during the mid-1990s. Since then, both populations stabilised but in recent years have shown a marked decline. The threats at Ndumo and Lake St Lucia are ubiquitous for the other crocodile populations in KZN and mostly a result of conflict with expanding human populations competing for the same aquatic resources. This have resulted in the degradation and transformation of aquatic habitat, destruction of nest-sites, infestation of alien vegetation and cattle disturbance at nest sites, direct and deliberate illegal killings of crocodiles using wire snares and baited hooks, driven by the high value of crocodile products for witchcraft. Other causes of mortalities include fishtraps and gillnetting. Neighbouring communities continue to view crocodiles as a threat to life and livestock, competing for fish resources and damaging fishing equipment. Despite being Red Listed as Vulnerable in South Africa and protected by a plethora of legislation, limited capacity from committed, but severely understaffed conservation agencies, requires new and innovative management approaches. This should allow neighbouring communities to share in the benefits of conserving healthy crocodile populations, such as regulated egg-harvesting quotas. The success of market-based conservation ultimately depend on the socio-economic context and the institutional mechanisms in place, and may be the only mechanism to stop the decline, as well as head-start biologically extinct populations in KZN, such as Lake Sibaya and Kosi Bay.

The Wicked Problem of a Large-Scale Crocodile Mortality Event

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Abstract

When crocodiles started dying in 2008 of a disease known as pansteatitis in the Olifants River in the Kruger National Park, the most obvious place to look was the many known pollutant sources that characterize this overworked South African River. However, the epidemiological investigation that followed proved more complex than was initially anticipated and highlighted the wicked nature of environmental problems involving top predator collapse in a complex system. Even with long term river health monitoring in place, we could not predict this disaster. Though the absolute cause for the mortalities has been difficult to tie down, a number of drivers have been implicated including, an exotic species invasion, the impact of a large dam, and the persistent pollution threats.

The Significance of Discovering Pansteatitis in African Sharptooth Catfish (*Clarias gariepinus*, Burchell) in Explaining the Pansteatitis-Related Mass Mortality of Nile Crocodiles *Crocodylus niloticus*, Laurenti) in the Kruger National Park, South Africa

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Abstract

Repeated field studies, using detailed autopsy, histopathology, haematology and blood chemistry techniques, have demonstrated an increasing prevalence of pansteatitis in African sharptooth catfish, *Clarias gariepinus* (Burchell), in the Olifants Gorge, an area where significant numbers of large Nile crocodiles (*Crocodylus niloticus*, Laurenti) died of the same disease in 2008 and 2009. The relative instability of certain polyunsaturated fats found in some prey fish when consumed by catfish exert a level of oxidative stress capable of overwhelming the natural anti-oxidant defence mechanisms of the animal, in particular vitamin E. In experimental studies, it was demonstrated that pansteatitis lesions in catfish do not heal over time and that lesions may be cumulative and debilitating. A chain reaction, initiated by oxidative breakdown of fats in the adipose tissues, leading to death of fat cells and release of breakdown products of fat oxidation was responsible for the acute and chronic inflammation typical of the pathology observed in the catfish. Similar differences in fatty acid and stable isotope composition of tissues in catfish with and without pansteatitis were reflected in crocodiles with and without pansteatitis, and in the Olifants Gorge implicated a common trigger for the disease in these two species.

In the Kruger National Park, pansteatitis in sharptooth catfish was shown to be a serious problem in the inlets to large man-made lakes fed by rivers arising in the polluted catchments of the Olifants and Sabie Rivers. The results of this study present the first record of pansteatitis in both wild and farmed African sharptooth catfish and emphasize the ecological importance and complexity of nutritional oxidative stress in a disturbed aquatic environment. The author proposes that nutrient entrapment leading to eutrophication and the consumption by catfish and crocodiles of phytoplankton-feeding fish rich in polyunsaturated fats, particularly silver carp (*Hypophthalmichthys molitrix*, Valenciennes), a species alien to Africa but present in the Olifants River, were the dietary cause of the pansteatitis.

Related Literature

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Dietary Reconstruction of Nile Crocodiles (*Crocodylus niloticus*) in Kruger National Park Using Stable Light Isotopes

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Abstract

Pansteatitis is a dietary disease, and recent pansteatitis-related crocodile mortalities in Kruger National Park prompted different approaches to dietary reconstruction for the species. Direct observation of feeding is problematic because of the shy nature of animals in the wild and the low frequency occurrence of episodic feeding events. Post-mortem stomach-content analysis yielded insufficient identifiable material. Stable light isotope analysis (δ13C and δ15N) is a means of identifying the position of an organism in a foodweb, and while this does not facilitate species level identification of prey, it provides a time-integrated indication of the trophic level at which the animals forage. The technique requires the metabolic changes in the isotope values during tissue synthesis (the diet-to-tissue discrimination factor - DTDF). In order to determine the DTDF the isotopic values of skin, erythrocytes, blood plasma, scutes and claws from different age and sex cohorts of captive Nile crocodiles fed on a fixed diet were analysed. The results show that the DTDF for Nile crocodiles differs from published values from other crocodilian species. The values are used to interpret the isotopic values obtained from scute samples in the SANParks biobank and this suggests that wild crocodiles in Kruger National Park rely less on fish than was previously thought to be the case. Time series of claws from pansteatitis affected crocodiles suggest seasonal gorging on fish, possibly during spawning migrations. Additional samples of crocodiles from a mining complex dam show a very distinctive isotopic signature indicative of pollution nutrient sources in the foodweb.

Exploring Associations Between Chemical Pollution and Nile Crocodile Mortality Episodes in the Kruger National Park

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Abstract

One of the possible contributors to Nile crocodile mortalities in the Kruger National Park during the onset of the winters of 2008 and 2009 remains toxic chemicals. We investigated a wide range of organic and inorganic and toxicants in crocodile tissues, crocodile eggs, and sediments. These included dioxins, furans, DDT, other chlorinated, fluorinated and brominated organic compounds, and metals and metalloids. Clinical chemistry parameters in affected crocodiles were indicative of mitochondrial toxicity. We therefore also investigated the possibility of rotenone (a readily available non-selective piscicide) involvement. Association was investigated mainly by comparisons between river catchments with and without mortalities, as well as with reference to similar studies. In general, no strong associations were found, although there were some compounds with higher concentrations in the sediments of the Olifants River Gorge.

Environmental Health and Ecotoxicology of Nile Crocodiles in the Olifants River, South Africa

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Abstract

The Olifants River has experienced significant changes in water quality due to anthropogenic activities and increased population. For the past ten years, there have been various 'outbreaks' of the inflammatory disease, pansteatitis, in several aquatic vertebrate species within the Olifants catchment. Nile crocodile populations have been severely depleted in recent years primarily due to the reduction of riverine habitat caused by the construction of dams, weirs and irrigation schemes (Jacobsen 1988; Botha 2010; Botha *et al*. 2011). It is therefore a species of conservation concern, especially within highly populated regions of South Africa where increased anthropogenic pollution has perturbed population growth and success of the Nile crocodile (Botha 2010). The Olifants River is currently regarded as one of the most stressed catchments in South Africa (Havenga 2007). In the upper catchment, Lake Loskop has experienced a severe decline in its Nile crocodile (*Crocodylus niloticus*) population along with concomitant fish mortality. In 1979, crocodile surveys at Lake Loskop indicated an estimated 32 animals; however, this number has dropped dramatically to as few as 4 animals in 2010. The current population also appears to include only smaller crocodiles (below reproductive age), as opposed to an equal distribution among different size classes. Other portions of the Olifants system have similarly witnessed massive mortality events of wildlife species. In 2008, approximately 180 adult crocodile carcasses were discovered in the Olifants Gorge in Kruger National Park (KNP), some 530 km downstream from Loskop. Hypothesized causes for these significant mortality events include direct impacts of increased water pollution from local mines and agriculture, algal blooms, nonfunctioning wastewater works, and alteration/destruction of habitat in areas impacted by dams. Previous work in our group has sampled crocodiles at two specific locations within the Olifants region - Flag Boshielo Dam and the Olifants River Gorge in Kruger National Park. We have examined a total of 66 crocodiles in an on-going project with Mpumalanga Tourism and Parks Agency, Medical University of South Carolina, National Institute of Standards and Technology, and SANParks scientists. The ongoing project has three specific objectives: 1. investigate the contaminant profile, both legacy and emerging contaminants, in the plasma of Nile crocodiles at several locations along the Olifants River system and explore the impact of this burden on crocodile health; 2. identify potential disease-related biomarkers for crocodiles using blood chemistry, omics-related techniques, and prey species (eg fish) as a proxy for biomarker discovery; and, 3. to determine if there is a link between contaminant exposure and pansteatitis.

New Thinking on Human-Wildlife Conflicts, and the Crocodilians

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Abstract

Human-wildlife conflicts, and particularly those involving large predators, are on the increase in many parts of the world and have long been a challenge for crocodilian conservation. This is reflected in the creation of an IUCN Task Force on Human-Wildlife Conflicts which I've been invited to join. This paper will provide an overview of current thinking on human-wildlife conflicts, which draws on a range of disciplines to focus on human and crocodilian behaviour and interactions. It will be informed by recent work at WildCRU in Oxford by a multidisciplinary team I put together to think specifically about human-predator conflicts. This overview will provide a framework for a reflection on approaches to crocodile conflict mitigation, drawing on published papers and papers published in CSG Proceedings over the past decade.

Examination of Predictive Parameters for Pansteatitis in Nile Crocodiles and Prey Species along the Olifants River, South Africa: The Use of Targeted Lipidomics for Wildlife Health Assessments

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Abstract

Several aquatic vertebrates within the Olifants River system have had numerous outbreaks of the inflammatory disease, pansteatitis. At Loskop Dam, this disease has decimated the Nile crocodile population and several fish species. Pansteatitis is diagnosed histologically by increased deposition of immune cells in adipose tissue. While the disease predominately manifests in adipose tissue, subsequent breakdown and necrosis of adipose tissue releases several lipid and other metabolites into the plasma, thus resulting in the potential for several minimally invasive plasma-based biomarkers for the classification of the disease. However, the lipid distribution and biochemical changes associated with pansteatitis remain unknown for wild aquatic populations. Pansteatitis is primarily designated as a disease of adipose tissue inflammation; the tissue undergoes cellular breakdown from the infiltration of immune cells. As the adipose tissue undergoes dramatic cellular changes with respect to the disease, lipids distributed in this tissue and plasma can be utilized as biomarkers for pansteatitis. This study aimed to 1) develop a method to quantify the eicosanoid profile in healthy and pansteatitis-affected adipose tissue of a prey species of the Nile crocodile (Mozambique tilapia), 2) identify plasma-based biomarkers for future monitoring of both crocodilians and prey species, and 3) validate findings with a commercially available standard peroxidase assay kit (TBARS). Here, novel lipidomic approaches are utilized to investigate an inflammation-based disease in a wild population. Adipose tissue and plasma of tilapia (healthy and pansteatitis-affected, N= 39 plasma, N= 12 tissue) were collected from Loskop Dam, South Africa. Adipose tissue was cryo-homogenized and eicosanoids were extracted using a modified solid phase extraction method. Extracts were analyzed using a targeted eicosanoid liquid chromatography-tandem mass spectrometric (LC-MS/MS) method developed using an AB Sciex API 4000 triple quadrupole mass spectrometer. Measurement and quantification of each eicosanoid species was performed using standard calibration curves and the final concentrations were expressed as ng eicosanoid/g wet weight of biological material. Fatty acid methyl ester (FAME) analysis of adipose tissue and plasma was performed using a gas chromatography-flame ionization detector instrument. Lipid peroxidation in the plasma was measured by using a colorimetric TBARS assay.

In this study, healthy and pansteatitis affected adipose tissue and plasma from prey species (*Orechromis mossambicus*) of the Nile crocodile were collected from Loskop Dam, South Africa and analyzed for differences in fatty acid/eicosanoid composition. Two on-site veterinarians designated health status in field; for this study, tilapias were designated either healthy (vet score <1) or pansteatitis-affected (vet score ≥ 1). The need for in-depth necropsies (vet scores) to diagnose pansteatitis in aquatic vertebrates limits large-scale monitoring of the disease in remote locations along the Olifants River system. Nile crocodile plasma (N= 52) was also examined via fatty acid methyl ester analysis to determine if there were patterns to determine disease states between Flag Boshielo and Kruger populations. Plasma based-biomarkers and non-invasive techniques are needed to aid with monitoring and subsequent management of this disease in the Olifants River system.

Preliminary data indicates that pansteatitis-affected fish had significantly higher levels of arachidonic acid ($p= 0.004$) in adipose tissue, as well as significantly higher levels of oxidized metabolites of linoleic acid (13-HODE and 9-HODE, $p= 0.002$; $p= 0.04$). The adipose of pansteatitis-affected and non-affected fish had no significant differences in PGE2 ($p= 0.9942$) and PGF2α ($p= 0.5887$); diseased related differences in COX metabolites were not observed. Plasma peroxidation indicated higher MDA content in pansteatitis-affected fish ($p= 0.0370$), further validating that fish with pansteatitis had higher levels of oxidative stress. The use of targeted lipidomics (eicosanoids) in this study to distinguish pansteatitis biomarkers and physiological changes in wildlife as a determinant of environmental stress is novel given that lipidomics techniques are primarily used for human health assessments and biomedical investigations. Translating this work to environmental monitoring allows us to determine how disease patterns emerge in wild populations. Future work aims to examine the use of this biomarker panel to identify probable areas of concern for pansteatitis in both fish and Nile crocodiles within South African river systems, and investigate how prey items influence the progression of pansteatitis in wild crocodilians.

Using Non-Invasive Measurement of Physiological Stress in the Nile Crocodile as a Biomonitoring Tool

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Abstract

Physiological stress, as measured using faecal glucocorticoid metabolites (fGCMs), is often used to test specific hypotheses about social and ecological stressors that wild animals are challenged with. This would suggest that there may be potential for using fGCMs as biomonitoring tools for assessing ecosystem health. However, few studies have attempted to validate such a broad-scale application of standard fGCM techniques. We attempted to validate the use of fGCMs in the Nile crocodile (*Crocodylus niloticus*) to monitor freshwater health in southern Africa. We conducted a biochemical validation and a metabolite stability study, followed by a preliminary field study of approximately 40 sub-populations of wild Nile crocodiles at sites across South Africa. We found that fGCM levels remain stable over time, as crocodile faeces dry out, and thus that dry faeces can be used for monitoring Nile crocodile physiological stress. From a prior experiment comparing group- and singly-housed crocodiles, we found with 98% probability that group-housed animals exhibited higher physiological stress with an effect size of 0.27 μg/g dry weight (DW) fGCM, or a 0.35-fold (35%) increase in fGCMs. We determined that it was possible to easily, safely, and inexpensively collect faecal samples from wild crocodiles. We collected approximately 800 samples from more than 40 rivers and lakes, at a total of 73 sub-sites. Our mixed effects models of variability in crocodile fGCMs suggested that the size of a bolus (dry weight, and a proxy for animal size or age) was most important in explaining variability in fGCMs, with bigger or older crocodiles exhibiting higher stress levels. Other potential explanatory variables included whether animals were from estuarine sites or protected areas. From paired site comparisons, we found with 96% probability that wild crocodiles had higher physiological stress than captive animals, with an effect size of 0.25 μg/g DW fGCM or 1.3-fold (130%) increase. We found with 94% probability that crocodiles from a site degraded by an extreme cyanobacterial bloom had higher stress levels than crocodiles from a paired pristine site, with a small effect size of 0.12 μg/g DW fGCM but nonetheless a 0.34-fold (34%) increase (similar in relative magnitude to the effect of single- versus group-housing). Finally, comparing a site with historic pansteatitis-related mass mortalities to a paired site without mass mortalities, we found with 96% probability that crocodiles from the mass mortality site had higher stress levels even though only one of 60 observed animals at that site exhibited clinical signs of pansteatitis at the time of sampling. The effect size was 0.16 μg/g DW fGCM and represented a 0.7-fold (70%) increase in fGCM levels. The highest fGCM levels measured from wild crocodiles anywhere in our study were from sites with extremely degraded water quality, and these fGCM levels were approximately 8-fold or 800% higher than a putative baseline for captive animals. Our preliminary results suggest that using Nile crocodile fGCMs as a biomonitoring tool for assessing both crocodile and freshwater health may be feasible, albeit providing a very coarse metric.

Addressing Issues with Collecting Crocodile Attack Data in Africa

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Abstract

The Nile crocodile (*Crocodylus niloticus*) is likely responsible for more human deaths than all other crocodilian species, but obtaining detailed information on these attacks has proven difficult throughout much of the range of the species. While ample data exists for some countries (eg Namibia, South Africa, Zambia, Zimbabwe) in other countries, which are well-known to have very high attack frequency, attack data is either very limited (eg Botswana, Democratic Republic of the Congo, Madagascar, Mozambique, Uganda) or non-existent (eg Egypt, Ethiopia, Somalia, Sudan). It is likely that hundreds of Nile crocodile attacks go unreported and/or unrecorded annually. In our worldwide crocodilian attack database (CrocBITE) this paucity of data results in the Saltwater crocodile (*C. porosus*) appearing to be responsible for more attacks, although this is unlikely to be true. While portions of the Saltwater crocodile's range has attack data deficiency (eg New Guinea, Solomon Islands) it is still unlikely the Saltwater crocodile is responsible for as many deaths as the Nile crocodile. The best solution to this issue is better communication with local wildlife authorities and crocodile specialists, as well as sending "crocodile attack logging forms" to the relevant authorities.

Research-Based Approach in Developing Best Practice and Benchmarks throughout the Value Chain to Improve the Sustainability of the Exotic Leather Industy in South Africa

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Abstract

Exotic Leather South Africa is a vertically integrated value-chain cluster, established to overcome the challenges facing the domestic exotic leather industry and to seize the opportunities presented by the international luxury market. Supported and funded by the Department of Trade and Industry, Exotic Leather South Africa is a sub-national cluster and non-profit organisation, responsible for developing best practice and benchmarks to improve the long-term sustainability of the exotic leather industry, Nile crocodile and ostrich, in South Africa. To achieve these goals the University of Pretoria was appointed as Technical Partner to the sub-National Cluster to undertake and implement more that 30 industry identified interventions. The University of Pretoria through its Faculties of Veterinary Science and Natural and Agricultural Sciences has been involved in crocodile and ostrich health and production for more than 20 years and is uniquely positioned to meet the needs of the cluster. To fulfil the role of Technical Collaborator an Exotic Leather Research Centre (ELRC) with three associated research chairs viz. Chairs in Crocodile Health and Welfare; Crocodile Production; and Exotic Leather Marketing and Trade was established in 2015. An existing Chair in Poultry Health and Production in the Faculty of Veterinary Science, also associated with the Centre, takes responsibility of research and development of Ostrich Health and Production. The function of ELCR is too provide scientific support in the local beneficiation of exotic leather industry throughout the value chain, thereby increasing the industry's contribution towards skills development, job creation and increased export earnings; to establish a national research capacity in crocodile and ostrich health, welfare and production; to provide high quality internationally accepted research throughout the value chain of exotic leather production and in the manufacture and trade in exotic leather-goods; to provide human resource development both upstream and downstream; and to develop norms and standards that meet international standards of production, manufacture and trade in exotic leather and leathergoods. A brief overview will be provided of the background and justification for establishing the Centre and the interventions that the Cluster and Centre will be undertaking. There will be a specific focus on interventions and research to enhance crocodile husbandry in South Africa.

A Study on Crocodile Farming in South Africa

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Abstract

Currently there is no authoritative information on the number, size or the production statistics of the crocodile industry in South Africa. Surveys that have been done in the past are outdated with very limited information have being documented. More recent reports are in lay publications based on unverified data. A list of 78 crocodile farmers and contact details in South Africa has been compiled from different sources by the Exotic Leather Research Centre (ELRC) since 2014. This list is not regarded as complete and accurate but does appear to indicate that the number of commercial farms have increased sharply from the 35 commercial farms documented in 1992 (Luxmoore 1992). A comprehensive study of commercial crocodile farming in South Africa is currently being undertaken by the ELRC. The objectives of this study include, but are not limited to: (a) to describe the existing crocodile production systems and the number of crocodiles being produced in South Africa; (b) to establish the shortcomings, needs and opportunities of crocodile producers as well as the industry; (c) to document the downstream activities of crocodile farming operations; and, (d) the development of a benchmarking model for crocodile production operations. All information obtained during the study is managed highly confidentially and a confidentiality agreements is signed with each farming operation before any data is collected. Data is currently collected through a survey that is filled out by the farmer or a researcher, with the help of the farmer, who visits the farm once the survey is filled out in order to verify the data or alternately fills out the survey on farm with the farmer. The data is entered into a SQL data base which ultimately will be accessible to the farmers, for updating, monitoring and forward planning of production; and benchmarking within the farming operation. Selected and aggregated data will be feed into a National Crocodile Industry Registry to set national benchmarks which farmers can use to compare themselves to, and will also be used to make national and international market predictions.

Genetic Diversity and Population Genetic Structure in the Lower Kunene, Okavango and Lower Shire River System Nile Crocodile (*Crocodylus niloticus*) Populations in Southern Africa

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Abstract

With the distribution of a species over a large area, geographical barriers are commonly found to separate individual groups into sub-populations. Such isolated populations are particularly subjected to random genetic drift that may lead to random allele fixation or loss within populations. The Lower Kunene, Okavango and Lower Shire Nile crocodile populations are evaluated based on their mitochondrial control region and Short Tandem Repeat markers. The mtDNA sequences are compared to publically available sequences to evaluate their phylogeography and separation. The Lower Kunene and Okavango River systems showed low haplotype diversity with a single haplotype observed in a total of 65 individuals collected from four locations and no haplotype diversity observed among the two rivers ($h= 0$; $\pi= 0$). Interestingly, the Lower Shire River Nile crocodiles clustered separate to the Lower Kunene-Okavango populations. Short Tandem Repeats revealed two populations in Southern Africa and further populations structured relative to river basin formations. Contemporary diversity was comparable to neighbouring river systems and effective population size estimates were high for each river system population.

Introduction

Crocodiles (Subfamily Crocodylinae), or true crocodiles, are large aquatic, carnivorous reptiles that can be found across tropical and subtropical freshwater lakes, rivers and wetlands. Previous studies for *Crocodylus niloticus* in southern and eastern Africa considered a single river system with a large sample size or several river systems with one or no more than 17 individuals (Bishop *et al*. 2009; Hekkala *et al*. 2010, 2011; Cunningham *et al*. 2016).

The Lower Kunene and Okavango River systems are the most south-westerly distributed Nile crocodiles. Genetic diversity within a largely distributed population has been considered to decrease; the further the distance is from the site of population origin (Eckert *et al*. 2008). Additionally, the individuals were included from the Lower Shire River system in Malawi to estimate if any differences are genetically visible between largely separated Nile crocodile populations. Two different markers were considered for the analyses. The first being the control region of the mtDNA to determine whether different ecological niches are present in southern Africa. The second was to evaluate more recent Nile crocodile events for genetic diversity and structuring using Short Tandem repeats. We hypothesise the presence of two ecological speciated Nile crocodile species and the Lower Kunene and Okavango populations to have no genetic differences between the populations. However, the Lower Kunene and Okavango sampling sites will reveal two independent populations with limited gene flow between them.

Materials and Methods

<u>Sample collection and DNA extraction</u>

A total of 139 Nile crocodile samples were collected from wild and wild-caught, ranch held live specimens. The samples originated from the different countries and regions: Botswana (Okavango Delta, N= 29), Malawi (Lower Shire River North, N= 27; Lower Shire River South, N= 25), Namibia [Kunene, N= 12; Okavango River (Bwabwatwa National Park N= 20; Otjiwarongo Crocodile Ranch (known to descend from the Okavango population, N= 13] and South Africa (Izintaba Crocodile Farm, origin unknown, N= 13). Total DNA was extracted using a standard CTAB protocol (Saghai-Maroof *et al*. 1984).

<u>MtDNA sequences and analyses</u>

Primers were designed using publically available sequences of the *C. niloticus* control region. All PCR amplifications were performed in a total volume of 10 µl containing KAPA Taq ReadyMix (KAPA Biosystems, Cape Town, SA), 0.8 µM forward- and reverse primers each and DNA, as follows: initial denaturation at 95°C for 5 min, 35 cycles of 95°C for 15 sec, 56°C for 30 sec and 72°C for 80 sec and a final extension at 72°C for 5 min.

Standard diversity measures (number of haplotypes, haplotype diversity, nucleotide diversity, and average number of pairwise nucleotide differences) were estimated for each population using Arlequin software v3.5 (Excoffier and Lischer 2010). For the purpose of phylogeographic analyses, publicly available mtDNA sequences from previous studies and their geographical collection sites were retrieved from GenBank and Datadryad (Meredith *et al*. 2011; Hekkala *et al*. 2011). A median-joining network was constructed to illustrate the evolutionary relationships among haplotypes using Network software v4.6.3 (Bandelt *et al*. 1999) under default settings.

STR Selection and Analyses

Twelve loci were selected from previous publications for cross-species amplification in the Nile crocodile (Bishop *et al*. 2009; Miles *et al*. 2009) and amplified for each of the collected samples. These Short Tandem Repeats (STRs) were combined into three Polymerase Chain Reactions (PCRs) multiplexes based on annealing temperature, expected allele range and fluorescent labels. Multiplex amplifications were performed in 10 µl total volume containing KAPA2G Fast Multiplex PCR Kit (KAPA Biosystems, Cape Town, SA) 0.8 µM of each primer and DNA, as follows: initial denaturation at 95°C for 3 min, 35 cycles of 95°C for 15 sec, Ta for 30 sec, 72°C for 50 sec, and a final extension at 72°C for 80 sec.

Departure from Hardy-Weinberg equilibrium (HWE) (exact probability test, 500 batches, 10,000 iterations), number of alleles (An), expected (He) and observed heterozygosity (Ho) integrated over all STR loci and all STR loci per population were calculated using Arlequin software v3.5, and corrected for multiple tests using the Bonferroni correction. Allelic richness (Rs) and inbreeding coefficient (Fis) were estimated between populations in FSTAT v2.9.3.2 (Goudet 1995). Pairwise Fst (10,000 permutations) were integrated over all loci on a locus-by-locus basis, calculated in Arlequin.

Ancestral population structure was inferred using Structure software v2.3.4 (Pritchard *et al*. 2000). An initial analysis was conducted for K-values between 1 and 6, using the admixture model with independent allele frequencies. A second round of analyses was conducted to assess structure within the major clusters recovered from the first analyses. The K-values tested ranged from 1 to the number of different sampling sites in each subset (10 replicates for each K, 15,000 steps of burn-in period followed by 35,000 steps of MCMC).The estimated log probabilities for each K value were calculated using the rate of change in the log probability values, and plotted on a graph representing the uppermost level of structure in Structure Harvester software v0.6.93 (Earl and VonHoldt 2012).

Contemporary effective population sizes (Ne) were estimated by the linkage disequilibrium (LD) (0.02 critical value and 95% CI Jack-knife) and heterozygosity excess methods for each population indicated by Structure analyses, as implemented in NEESTIMATOR v2.01 (Do *et al*. 2014). Testing for recent bottlenecks or radial expansion was performed using the Wilcoxon signed rank test for significant deviation from heterozygosity excess and deficiency under the Infinite Allele Model (IAM), Single Mutation Model (SMM) and the two-phased model (TPM) implemented in BOTTLENECK v1.2.02 (Piry *et al*. 1999). BOTTLENECK analyses were performed using 1,000 replications at the 5% nominal level and a TPM composed of 70% SMM and 30% IAM (Piry *et al*. 1999).

Results

Mitochondrial analysis

Two lineages previously described across Africa (Schmitz *et al*. 2003; Hekkala *et al*. 2011) represent the two main Nile crocodile genetic clades, designated 'Eastern' and 'Western'. The sampled individuals from the Lower Kunene, Okavango and Lower Shire River systems clustered within the 'Eastern' clade. The Lower Kunene and Okavango showed strikingly low haplotype diversity with a single haplotype observed in a total of 65 individuals collected from four locations (Fig. 1), and no haplotype diversity observed among the two rivers ($h= 0$; $\pi= 0$). The Lower Shire River (Malawi) showed the highest haplotype diversity among the three river systems ($h= 0.332 \pm 0.083$; $\pi= 0.015 \pm 0.008$) with four haplotypes observed in a total of 47 individuals (Fig. 1). The Kunene and Okavango sequences clustered separately from the Lower Shire River sequences by two mutational steps.

Genetic diversity, effective population size, gene flow and structure

The two Lower Shire River populations (North and South) were the most diverse groups (He= 0.67, Ho= 0.63, and Rs= 5.53, averaged across the two groups) compared to the Kunene (He= 0.58, Ho= 0.50, and Rs= 4.10) and the three Okavango populations (He= 0.59, Ho= 0.58 and Rs= 4.46, averaged across groups) (Table 1).

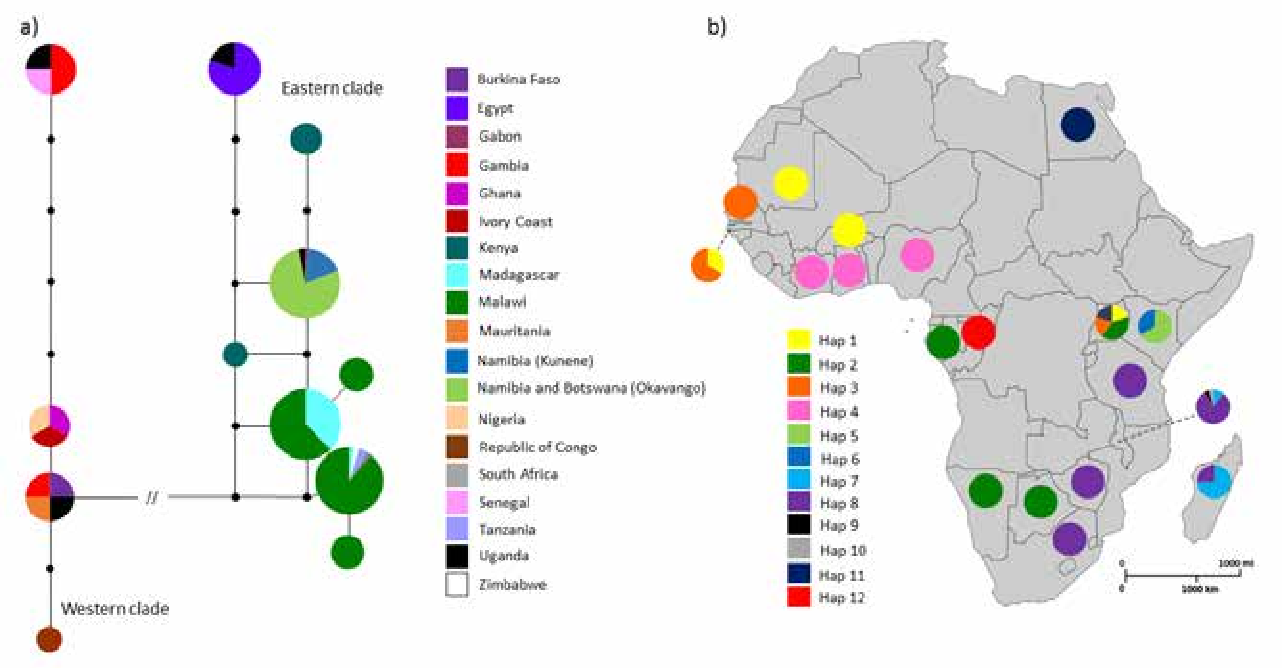

Figure 1 (a) The Medium-Joining haplotype Network depicting two groups of haplotypes, namely the Western and Eastern clades. Haplotype colours correspond to the countries where the samples were collected. Circles represent mtDNA haplotypes, lines connecting haplotypes represent a single substitution step, and black dots represent hypothetical haplotypes. // represents 15 mutational steps. (b), indication of the 12 haplotypes found within the Nile crocodile portrayed for each country of origin in Africa. Then samples within the study from the Kunene and Okavango share the same haplotype with Gabon and Uganda. Furthermore, the populations of Southern Africa show two different haplotypes among each other separating those of Kunene and Okavango from the Lower Shire River population, which shares haplotypes with the surrounding countries.

Table 1. Genetic diversity measures and Hardy-Weinberg Equilibrium test in Southern Africa Nile crocodile populations. N - number of individuals, An= number of alleles, He= expected heterozygosity, Ho= observed heterozygosity, HWE= Hardy Weinberg Equilibrium test (P-value), Rs= mean allelic richness, Fis= mean frequency of inbreeding coefficient. Values were estimated based on genotypes determined using 11 nuclear STR markers.

Population	N	An	He	Ho	HWE (P)	Rs	Fis
Panmixia	139	11.1	0.712	0.607	0.003	10.848	0.144
Kunene River	12	4.2	0.583	0.495	0.587	4.097	0.149
Bwabwatwa National Park	20	5.3	0.599	0.605	0.616	4.481	-0.009
Okavango Delta	29	6.0	0.624	0.613	0.442	4.749	0.011
Otjiwarongo Crocodile Farm	13	4.3	0.562	0.535	0.537	4.137	0.043
Okavango River (overall)	62	5.182	0.595	0.584	0.531	4.456	0.015
Lower Shire River (North)	27	6.9	0.664	0.617	0.337	5.519	0.098
Lower Shire River (South)	25	6.9	0.684	0.625	0.367	5.540	0.071
Lower Shire River (overall)	52	6.909	0.674	0.621	0.352	5.529	0.085
South Africa commercial crocodile farm	12	3.8	0.519	0.586	0.273	3.734	-0.104

Amongst the main four sampling locations (Lower Kunene, Okavango, Lower Shire and South Africa), all the pair-wise FST values were statistically significant ($P<0.05$) suggesting significant subdivision. However, within the two wild Okavango populations and the two Lower Shire populations FST values were non-significant ($P>0.05$) thus suggesting the occurrence of gene flow. The genetic distance (estimated as pairwise Fst value) between the Kunene and the Okavango (average Fst= 0.130, $P<0.05$) was the lowest between all pairs of populations. The Kunene-Lower Shire pair (average Fst= 0.211, $P<0.05$) and the Kunene-South Africa pair (Fst= 0.162, $P<0.05$) populations showed moderate genetic differentiation. The Okavango-Lower Shire pair (average Fst= 0.166, $P<0.05$) showed higher genetic differentiation compared to the Okavango-South Africa pair (average Fst= 0.142, $P<0.05$).

The Bayesian clustering analysis for the complete data set revealed the presence of two populations namely, Lower Kunene-Okavango and Lower Shire-South Africa. Interestingly, considering the Lower Kunene and Okavango revealed the presence of three populations of which two were found to overlap in the Okavango River (ΔK being the highest for K= 3) (Fig. 2).

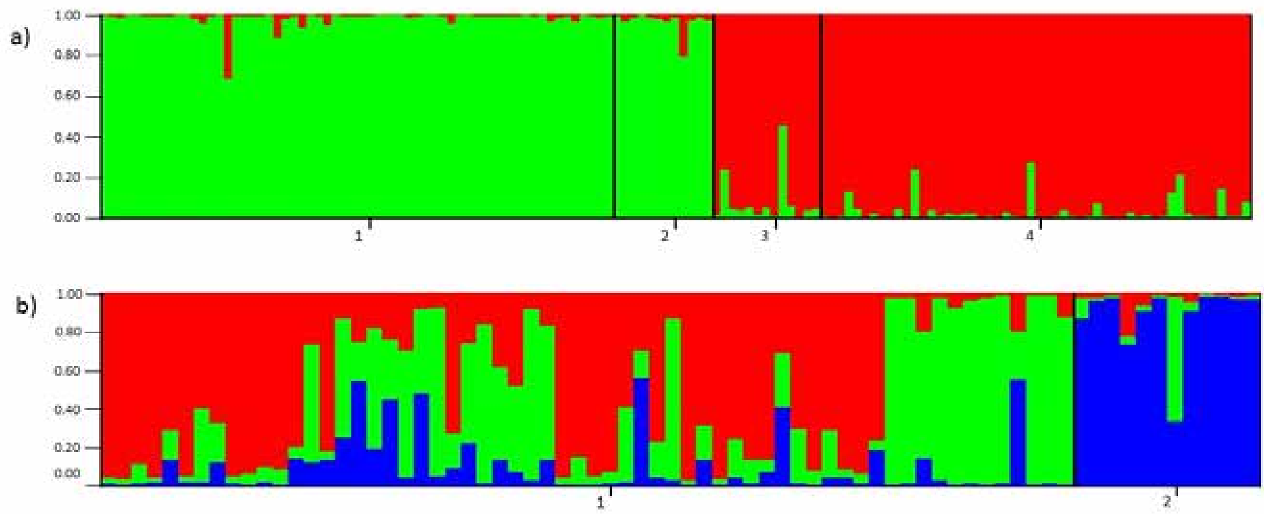

Figure 2 Genetic structure of *Crocodylus niloticus* populations based on Bayesian clustering analyses [Structure software v2.3.4 (Pritchard *et al*. 2000)] a) Genetic clusters in southern Africa (complete dataset) , K= 2 and (b) Genetic clusters in the Kunene and the Okavango samples. Populations: (1) Okavango, (2) Kunene, (3) South Africa commercial and (4) Lower Shire.

The effective population sizes, as assessed by the Linkage Disequilibrium (LD) estimates were generally high except for the Bwabwatwa National Park (38.6; 17.7-432.7) and Lower Shire (South) (43.4; 29.4-75.2) populations. Subsequently, due to the detection of gene flow within the Okavango and Lower Shire River systems, an overall LD estimate was determined for each river system, resulting in Okavango 404.6 (107.3-∞) and Lower Shire 143.5 (74.5-743.8). The Wilcoxon signed rank test indicated no presence of a recent bottleneck or population expansion ($P>0.05$) in any of the populations, based on the Two-phased model.

Discussion

Based on the limited sample set of the present study a single mitochondrial haplotype was observed among the Lower Kunene and Okavango samples, suggesting that not enough time has passed to fix population-specific mutations that render the two river systems divergent. Contrastingly, four haplotypes were found in the Lower Shire River sample, suggesting a more diverse population (Fig. 1).

The development of molecular genetic makers (STRs) for the Nile crocodile through cross-species amplification proved useful in assessing the levels of diversity and depicting the population genetic structure for this species in Southern Africa (Lower Kunene, Okavango and Lower Shire River systems, and a commercial crocodile farm in South Africa). The mean observed heterozygosity in all sampling locations were comparable to neighbouring river basins studied in previous work (Bishop *et al*. 2009; Hekkala *et al*. 2010). Interestingly, the Okavango population showed no evidence of a genetic bottleneck even though it has experienced a large reduction in size in the past due to over exploitation (Ross 1998). This result support a previous study conducted on the Okavango Delta Nile crocodile population Bishop *et al*. (2009) with no loss in effective population size reported. However, after comparison between effective population size and census size they found a limited number of adult Nile crocodile individuals contributing to the following generation and predicted a decrease in diversity for the future if the breeding numbers keep reducing.

It was previously shown that the Nile crocodile populations are structured relative to river basin formations (Hekkala *et al*. 2011), thus suggesting that no gene flow was to be expected between the Kunene and Okavango River systems. Indeed, our findings revealed significant population differentiation (pairwise Fst= 0.130, $P<0.05$ averaged across the Okavango river groups) between these river systems suggestive of a land barrier to gene flow (Fig. 2b). Furthermore, the Bayesian clustering analysis detected three populations, one in the Kunene and two in the Okavango River system. Genetic structure analysis among the Kunene, Okavango, and Lower Shire River systems and South Africa commercial populations revealed two populations with restricted gene flow (Fig. 2a).

Conclusion

The Nile crocodile populations in all three river systems conformed to the 'Eastern' Nile crocodile clade. Comparison of the Lower Kunene and Okavango samples to South Africa and Lower Shire indicated two separate populations, in accordance to a previous study that reported the occurrence of two genetic clusters in Southern Africa (Hekkala *et al*. 2011). Nile crocodile populations within the Lower Kunene and Okavango River systems did not show divergence, as indicated by no mtDNA mutational differences; however STR analyses showed that the Okavango sample was sub structured in two populations. The genetic diversity of Nile crocodile populations within the Kunene and Okavango River systems was comparable to previously published neighbouring river systems (Bishop *et al*. 2009; Hekkala *et al*. 2010).

Based on our findings, the Lower Kunene and Okavango River systems are isolated populations and will require population-monitoring specific to each river system. Sights of interest for future work will be to observe the range of the two common haplotypes observed in Lower Kunene-Okavango and Lower Shire populations and if secondary contact zones exist.

Acknowledgements

This research study was supported by Nedbank Go Green Namibia, the Melon Foundation, the Crocodile Specialist Group and the Russel E. Train Fellowship WWF-US. We thank the Ministry of Environment and Tourism for logistical support during the field work and sample collection in Namibia. Additionally, we would like to thank the Okavango Research Group in Botswana, the Shire River Crocodile Ltd in Malawi (Bruce Carruthers and Mike Fuller), Otjiwarongo Crocodile Ranch (Dieter Noelle and Victor Smit) and Dr Jan Myburg (South Africa) for providing Nile crocodile samples from Eastern and Southern Africa. Special thanks to Hans Swartbooi for field assistance in Namibia.

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Evaluation of the Efficacy of Ivorian Protected Areas to Protect *Mecistops cataphractus* Populations and an Update on Project Mecistops

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Abstract

In 2013 we initiated the Project Mecistops - a captive breeding and reintroduction program designed to facilitate the recovery of severely depleted populations of the Critically Endangered West African slender-snouted crocodile (*Mecistops cataphractus*). As part of this project we are using *Mecistops* as a model species to assess the efficacy of Ivorian protected areas in protecting threatened aquatic fauna. In Côte d'Ivoire, like throughout most of western Africa, most protected areas are delimited using natural boundaries such as rivers and lagoons - though often leaving these features outside of the core park area. We are conducting repetitive counts of crocodiles in/adjacent and completely outside of 7 protected areas (3 national parks, 3 classified forests and 1 local community area) and analyzing the data under two different quantitative models: 1) repeated occupancy point counts, and 2) habitat suitability/species distribution modeling. Our preliminary results found that in/adjacent protected areas *M. cataphractus* was encountered at a rate of between 0.08 crocodiles/km (Azagny National Park) and 1.307 crocodiles/km (Taï National Park). Most disconcerting was that in only one case did we find *Mecistops* outside of a classified protected area - in the vicinity of Taï National Park (0.35 crocodiles/km). The main threat to *M. cataphractus* in Côte d'Ivoire is by-catch in both subsistence and small-scale commercial fishing, though this species is hunted on a limited basis. We will present a status update of the breeding production and captive management. Future activities include a study on the movement ecology of wild *M. cataphractus* of the size of individuals we plan to reintroduce as part of the reintroduction planning process. We hope to start releasing captive-bred individuals in 2017.

Impact of Human Activities on the Distribution of Crocodiles within the Nazinga Game Ranch, Burkina Faso

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Abstract

We studied the distribution and abundance of crocodiles using direct and indirect observations, as well as ethno-zoological surveys, both within the Nazinga Game Ranch (NGR) classified forest and in neighbouring villages. We visited each sampling site every two months to conduct diurnal and nocturnal surveys. During our survey 412 crocodiles were collected. They were unequally distributed throughout 9 locations sampled in a total of 11 sites that make up the ranch. Two sites (Talanga and Poupanga) have not been sampled due to lack of water. The appraisal of the size and the age which was carried on 198 crocodiles made possible to divide up that population the following way: 103 juveniles (52.02%), 73 sub-adults (36.86%), and 22 adults (11.12%). Two hundred and fourteen (214) crocodiles were classified as "eyes only". Our results suggest that crocodiles are unevenly distributed in the Ranch, with the Akwazena, Akalon, and Barka Reservoirs having highest densities of individuals. Fishing, the main anthropogenic activity conducted in the Ranch, is permitted from December to May and represents a potential threat to the crocodile population. This is evidenced by the higher abundance of crocodiles in the Akalon and Akwazena Reservoirs where fishing is not practiced. Ethno-zoological surveys of 96 people, including local inhabitants and Ranch staff, suggest that that three crocodile species exist in the Ranch. However, our surveys only detected *Crocodylus suchus*, and it is as yet unclear whether this is a case of mistaken identity by the local people or a local extinction of Dwarf and Slender-snouted crocodiles. Ecological conditions in the ranch are favorable for crocodile population development; however, sustainable fisheries management will be required for protection and conservation of crocodiles in the long-term.

Introduction

In landscapes where natural habitats have been severely degraded due to anthropogenic pressures, the conservation of biodiversity is a growing issue, and the establishment of protected areas often forms the cornerstone of conservation strategies. Burkina Faso, one of several countries in the sub-Sahelian zone, contains an important biodiversity. More than 3801 animal species are identified, including 704 species of mammals, 497 species of birds and 60 species of reptiles (Chardonnet and Fritz 1995; Weesie and Belemsobgo 1997). However, these natural resources are facing environmental problems. Among others human activities that influence on the distribution of animals are of increasing concern. In this situation, protected areas appear to be the main shelter to animal's conservation. In order to protect this biological biodiversity the Reserve was established in 1975; built for water needs of animals. These Reservoirs are home to a large population of crocodile and fish. The form of fishing that takes place on these amounts of water remains traditional and constitutes many threats on crocodiles. The West African crocodile (*Crocodylus suchus*), recently separated from *C. niloticus* on the basis of genetic and molecular evidence (Hekkala *et al*. 2011; Shirley *et al*. 2013) is the only species found in the ranch currently. The distribution of *C. suchus*, covers all of West Africa as far east as Lake Chad and central Africa. This crocodile is smaller than Nile crocodile (*C. niloticus*) and less inclined to attack humans or livestock (Schmitz *et al*. 2004). The numbers of crocodiles are decreasing throughout the world, due to the human activities increasing (Treves *et al*. 2009). Fishing was recorded as major form of disturbance of the distribution of crocodiles during this study. Crocodiles play a major role in the functioning of aquatic ecosystems in which they live. So, it is important to conduct investigations to understand the dynamics and distribution of Nazinga crocodiles. It is therefore critical to develop effective management and conservation strategies to ensure their protection. This paper describes findings of our survey on distribution of crocodiles in the ranch. In addition, local communities' views related to crocodiles especially biology, ecology.

The purpose of the surveys is three-fold:

- Assess the distribution and population status of crocodiles in the reserve
- Identify human activities that influence the distribution and abundance of crocodile population
- Make recommendations for conservation

Study area

The present study has been carried out in the Nazinga Game Ranch (NGR). It is one of the largest reserves in Burkina

Faso. NGR lies in southern Burkina Faso, 200 km from Ouagadougou, between 11° 1' and 11°18'N and between 1° 18' and 1° 43'W. It covers an area of about 98,100 ha. A part of its southern boundary forms the border between Burkina Faso and Ghana (Fig. 1). The NGR forest was created in 1975. The main management objectives are to protect and conserve wildlife by controlling human impacts (eg agriculture, hunting and fishing). Important population of crocodile inhabiting the 11 waterbodies that occur in this reserve.

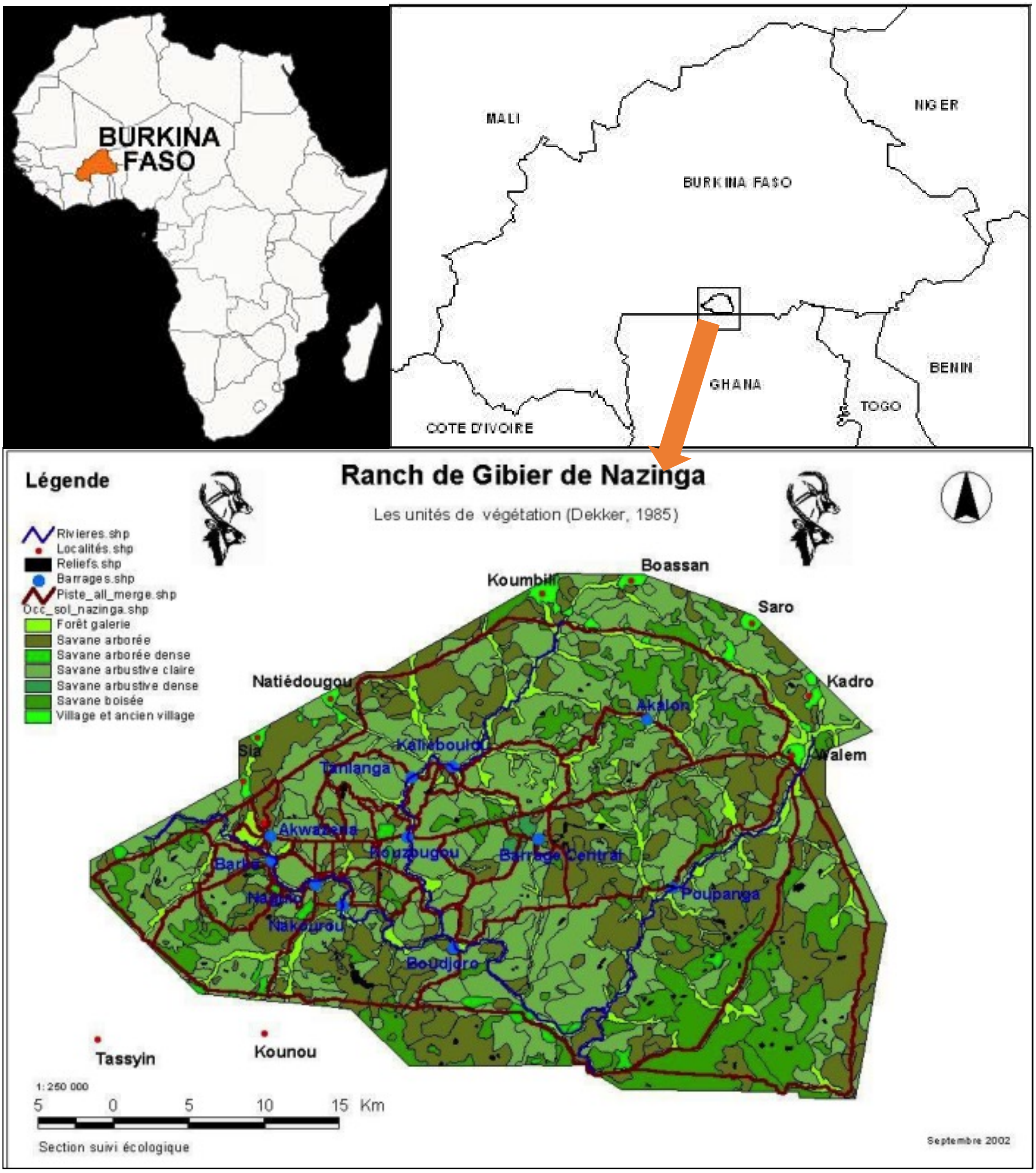

Figure 1. Map of study area highlighting the section surveyed in the Nazinga Game Ranch.

Methods

The study of the distribution and the evaluation of threats which crocodiles facing in the NGR have been done from 27 July 2015 to 20 April 2016. This work has been organized in two steps: ethno-zoological survey, then the crocodiles and the human activities inventory.

Ethno-zoological survey

Ethno-zoological survey has been carried out using direct interview with the local people living along the Ranch and sites managers. People were selected randomly and subjected to standard questionnaires. Interviews lasted 15-20 minutes and all respondents were above the age of 18 years. Local guides were employed when required in each of the survey sites to assist with translation and introductions. Any incentives or rewards have been offered to the respondents. Questions were designed to be simple and clear to elicit consistent responses. The questionnaire followed a logical progression and began with general questions on the crocodiles. Results of the interviews were analyzed in qualitative manner as well as summarizing the data in graph forms.

Crocodile surveys

Diurnal Surveys

Sites were surveyed by walking along the bank, by wading, or using dugouts. A teach survey, date, start time and local name of crocodile were noted. During the survey, recordings of the location were made for all individual crocodile sightings using a Global Positioning System (GPS). Between 0700 h and 1000 h when most of the crocodiles were basking we used binoculars and a telescope to facilitate counting and a camera for taking pictures. Direct observation was carried out on foot or kayaks depending on the size of the site and accessibility. At every sighting of crocodiles, their number, location, habitat and behaviour were noted. When is possible, the length of the animals was estimated visually. Once an individual crocodile was sighted and with its size estimated, it was assigned into standard size class following standard classification using the Bayliss (1987) method. But the indirect observations have consisted in recording of the presence indicator of animal on the site: traces; burrows, legs; droppings and nests. This method used to estimate the abundance of the population.

Nocturnal spotlight counts

We used spotlight count to census crocodilian population. The counting has been facilitated by the reflection of crocodile eyes to light. Each reflection of a pair of eyes corresponds to individual (Bayliss 1987; King *et al*. 1990). This methodology consisted to count crocodiles on shore or in water. This counting is performed in the evening between 1900 h and 2300 h, because at this time many crocodiles are active. Spotlight survey are generally conducted from a kayaks, but can also be done from land. Four categories were recognized: hatchlings (<0.5 m), juveniles (0.5-1.0 m), sub-adults (1.1-2.0 m), and adults (>2 m). It was not possible to determine the sex of the individuals in the field (Santiapillai and de Silva 2001). All crocodiles were categorized according to size class (hatchling, juvenile, sub-adult and adult). On occasions when observers were unable to accurately estimate size class, the sighting was recorded as eyes only (EO). All detected crocodilians were approached in order to characterize by species and size class. After the survey the following, we noted end time, end location, survey distance, crocodile sightings, encounter rate (crocodiles/km), the site name and additional important remarks.

Description of fishing activities

In order to characterize the impact of fisheries on the distribution of crocodiles in the Ranch, we submitted a questionnaire to fishermen. The questionnaire aimed to estimate the numbers of fishermen, the duration of their activities in the Reserve and finally the fishing techniques. Likewise interview was made to fishermen to estimate also the number of *C. suchus* accidentally trapped and killed in their nets. We noted that permits were issued for fishermen for activities related to fishing in the Ranch.

Results and discussion on preliminary investigation

Ethno-zoological survey

For this study an investigation by the local population to collect information was made. However, this method gave global number of crocodiles while data on their biology and ecology would be very beneficial. In total, 96 persons were interviewed in the 4 villages and also in the Ranch. They were consisted of 71% of men and 29% of women (Fig. 2a). There was a 3.12% refusal rate to participate in interviews and these cases were due to lack of time. As crocodiles could be easily found in almost all rivers in the Ranch, 99% of the respondents claimed that they have seen crocodiles in the wild. According to the people surveyed, 54% of them think that crocodiles are not endangered. Almost all respondents said they try to avoid crocodiles. However, if they encounter these animals, they would just carry on with their activities as normal, without disturbing the animals. During this study, all respondents never had any personal experience with human-crocodile conflicts; however majority of them had met or heard from people who have had an experience about humans attacked by crocodiles. Over 54% of respondents estimate that crocodiles are not endangered in the Ranch, conversely 30% considers that there are threatened (Fig. 2b). The questionnaire survey accounted for zero fatal attacks. Most of the attack occurred during canoe fishing.

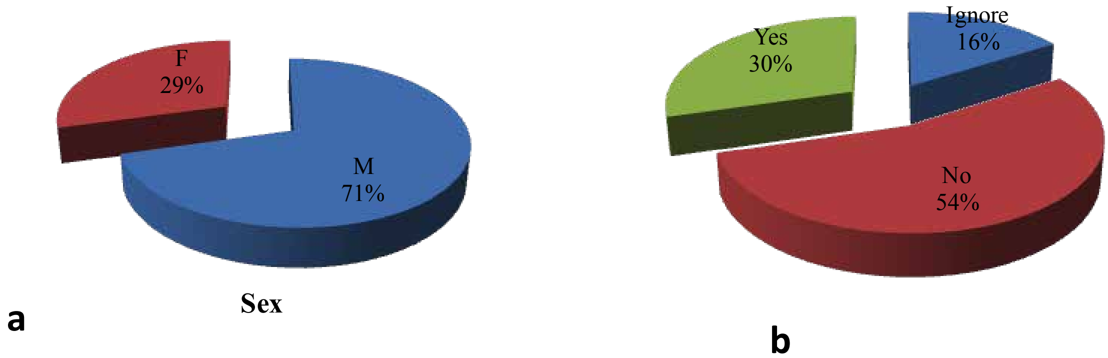

Figure 2. Percentage of people interviewed.

Crocodile Surveys

Census survey of crocodiles had been conducted in 9 different rivers in NGR. A total of 412 crocodiles were encountered. 198 *C. suchus* (formally identified) and 214 eyes only or unknowns (Fig. 3). Crocodiles are unequally distributed throughout 9 locations sampled at a total of 11 sites that make up the ranch. Two sites (Talanga and Poupanga) have not been sampled due to lack of water during the sampling period. During our surveys only detected one species *C. suchus* and it is as yet unclear whether this is a case of mistaken identity by the local people or a local extinction of Dwarf and Slender-snouted crocodiles. If we analyze the population structure only on size class on the *C. suchus* met during our inventory in the reserve. We have 52.02% of crocodiles as juveniles, 36.86% as sub-adults and 11.12% as adults.

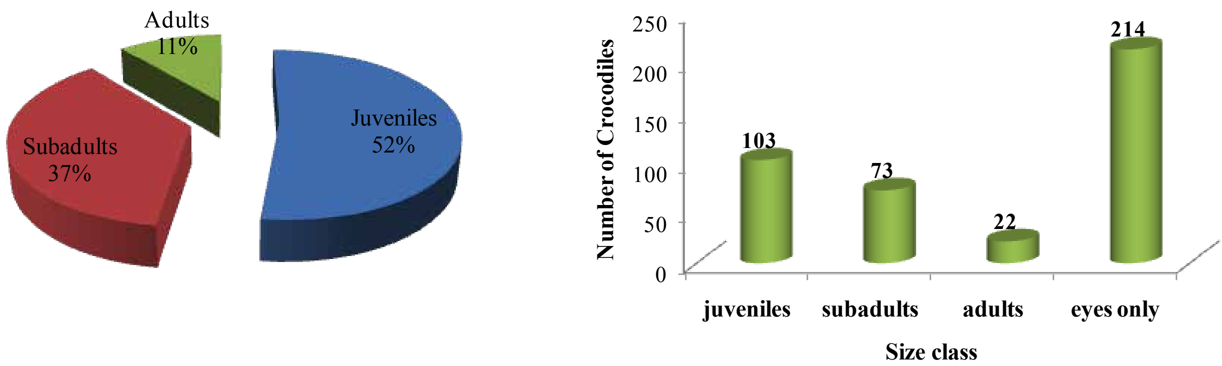

Figure 3. Size structure of the population.

Where there is no fishing, crocodiles are abundant and easily observable during the day and the night. Spotlight surveys found that crocodiles were present at very low densities in all visited site in the Ranch. Nocturnal surveys of the different dams confirmed the presence of crocodiles, though most individuals detected were juveniles. The distribution was not uniform among the different sites. Particularly high densities were found in some rivers such as Barka, Kalieboulou and Akwazena. Results show that Bodjoro and Nakuru (Fig. 4) have relatively low crocodile populations. This can be attributed to several factors, including the sampling effort, and detectability due to the presence of fishermen in these sites. Interestingly, our study indicates that the crocodile population in the Ranch is increasing. The high concentration of crocodiles in the Akalon and Akwazena Rivers (Fig. 4) can be attributed to suitable habitats. This study maintains earlier findings that suggested that Akwazena is an important river for the survival of crocodiles in the Ranch.

Regarding the structure of the population, we chose to group hatchlings and juveniles together. The study shows a higher population of juveniles compared to other classes (Fig. 3). It has been noted that other factors influence likely crocodile population size and structure. Sometimes fishermen fish for a long period during the day in water. They seem to be aware of the favored basking sites of crocodiles as well as their distribution in close proximity to preferred fishing areas. Crocodiles regularly feed on fish ensnared in gill nets and consequently destroy fishing equipment and interfere with fishing efforts (McGregor 2005). In this study, most of fishermen met reported damage to multiple nets within the rivers. In addition, 6 crocodile nests were located, one with the remains of eggshells indicating it was active in 2015 (Fig. 5)

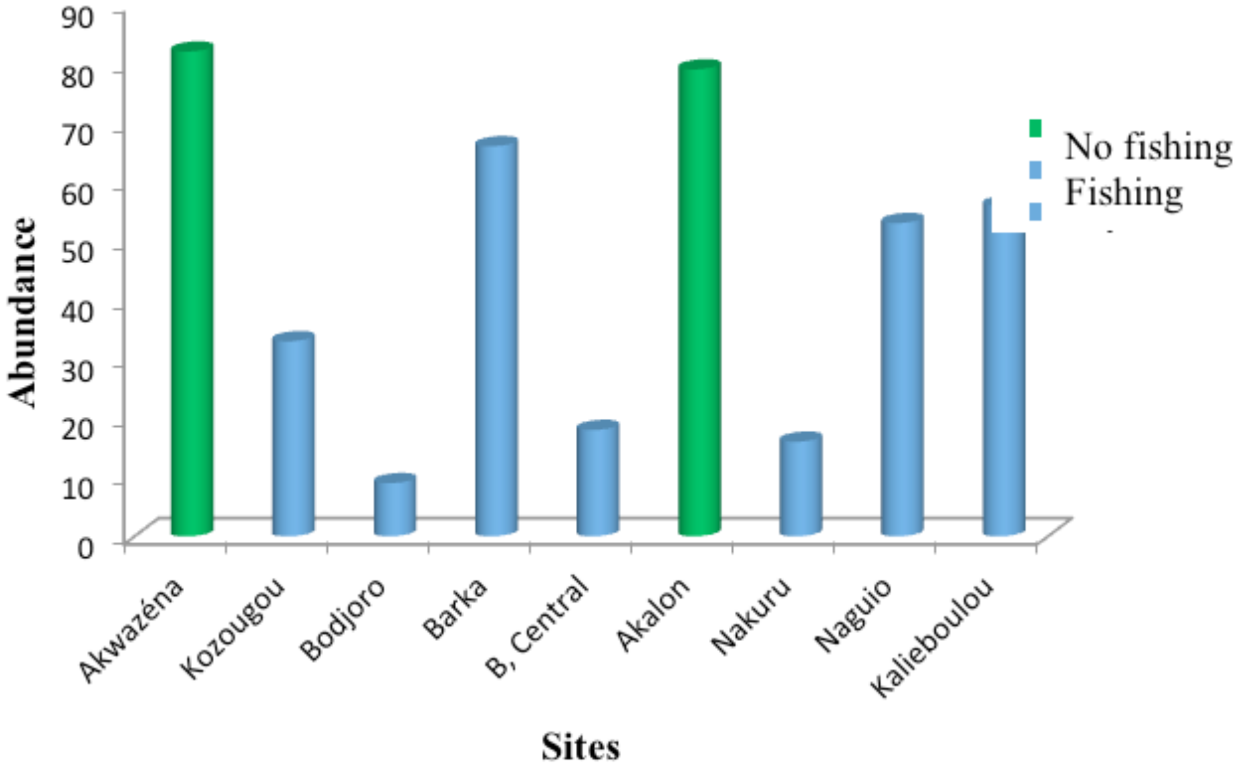

Figure 4. Distribution of *C. suchus* at 9 sites in NGR.

Figure 5. Indirect observation of crocodiles in NGR.

<u>Fisheries characteristics</u>

The main anthropogenic activities conducted in the Ranch, are permitted from December to May and represent a potential threat to the crocodile population. Two fishing camps were identified in the Ranch. A fishing camp was defined as a base camp on the Kozougou River where fishermen process their fish on smoking ovens; they sleep and keep their belongings on the site. Many people (60 fishermen) work in the Ranch rivers such as local fisherman who fish for subsistence (and sell excess catch to the local markets) and professional fisherman (Fig. 6). All fishermen have fishing authorization, but their activities are not rigorously followed. They even use illegal fishing methods as the use of seine nets. The fishing methods varied according to the nature of the water source and even the water retention capacity. A of total six different fishing techniques have been identified, each of which is aimed to exploit a particular species of fish, at a particular period of the year. Among the common fishing methods mentioned by respondents we can cite: fish-traps, funnel nets, angling, casting nets, drift nets, seine (use of non-prescribed fishing gear) and traditional fishing traps. Fishing activities increased generally during the dry season from December to May. In retaliation for damaging nets crocodiles of all sizes are vulnerable to this threat. Fishermen estimated that crocodile populations have increased dramatically in the Ranch. This situation has induced frequent conflicts between fishermen and crocodiles. Fishing affects indirectly, the distribution and abundance of crocodiles by reducing fish stocks and changing prey size and species composition. According to fishermen safety and freedom of movement are constrained in areas with crocodiles. In the ranch, besides the threats by incidental mortality from fishing nets. We noticed other threats such as eggs of crocodile predation by monitor lizards. The other humans activities in the Ranch is the tourism but this activities is regulated and no impact on the distribution of crocodiles

Figure 6. Team of fishermen.

Recommendations

- Monitor an regulate fishing
- Carry along stakeholders

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Status of Crocodile Conservation in Ghana

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Abstract

Ghana is home to three species of West African crocodiles: *Mecistops cataphractus*, *Osteolaemus tetraspis* and *Crocodylus suchus*. Unfortunately, crocodiles have received little research and conservation attention in Ghana mainly due to lack of expertise, perceived danger and funding difficulties. However, their national conservation status is increasingly aggravated by threats resulting from hunting pressure, habitat encroachments and depletion of prey resources. Although Ghana wildlife laws offer complete protection for all the three species, enforcement of these laws is weak making crocodiles more prone to illegal harvest. To respond to this wildlife management crisis, we have undertaken to develop crocodile research and conservation work in Ghana. Currently, there are 6 ongoing projects on various aspects of crocodile ecology and conservation. These projects are aimed at establishing population status, building local capacities and raising awareness to promote crocodile conservation. One of these projects has helped confirm the presence of *O. tetraspis* in Ankasa Conservation Area (ACA). In all project sites (Draw River Forest Reserve, Owabi Wildlife Sanctuary, Tano-Offin Forest Reserve, ACA, Bomfobiri Wildlife Sanctuary and Anwiafutu Community Forest) O. tetraspis were recorded while *C. suchus* was recorded only at the Bomfobiri Wildlife Sanctuary. Alarmingly, no *M. cataphractus* have thus far been encountered. Looking into the future, there are plans to achieve three major objectives: 1) conduct rigorous surveys in order to establish the population status of all three species in Ghana and develop a national crocodile conservation action plan; 2) build a strong network of crocodile researchers in Ghana; and, 3) establish an NGO dedicated to training upcoming crocodile researchers as well as raising awareness about the need for crocodile conservation in Ghana.

The Slender-Snouted Crocodile (*Mecistops cataphractus*) is (Almost?) Extinct in Southern Nigeria

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Abstract

An intensive survey of the Slender-snouted crocodile (*Mecistops cataphractus*) distribution and status was conducted across 12 southern states of Nigeria from September 2015 to April 2016 following the outcome of a previous general crocodilian survey of the country. These surveys were based on original field surveys in key habitats within 5 forest reserves. This was in combination with contact visits and specific night time spot checks which were conducted to locate the animal in Taylor Creek Forest, Nun Forest, Apoi Creek Forest, Edumanon Forest and Stubb's Creek Forest Reserves, bushmeat market surveys, questionnaire analysis and other indirect sources of data (bibliographic records, reliable sightings provided by other scientists, etc.). While the African Dwarf crocodile (*Osteolaemus tetraspis*) and West African crocodile (*Crocodylus suchus*) proved to be widespread in southern states, *M. cataphractus* could not be found in any of the known habitats surveyed. Field sites in swamp forests and extended marshes of the Niger Delta and mangrove marshes and mangrove areas of Benin River and tributaries of Forcados River did not yield any animal. Six coloured photographs showing different views of the animal were printed and handed over to hunters and experienced fishermen alongside monetary rewards (30,000 Naira for any live specimen and 15,000 Naira for any intact skull) were offered to known crocodile hunters in communities that were previously known to habour such animals. At the end of the survey only a very few, indirect, data were collected on *M. cataphractus*, which implies that the species is clearly an extremely rare and threatened species, probably almost extinct at the country scale. Currently, *M. cataphractus* is well known and clearly described by a minority of fishermen in Cross River State and in Rivers/Bayelsa States, but with no recent (after year 2005) field observations by scientists. Overall, we consider that *O. tetraspis* should be LC, *C. suchus* VU, and *M. cataphractus* CR at the country scale. A more intensive and widespread survey including use of camera traps in key sites should be carried out across seasons while posters depicting the animal should be produced and circulated across the Niger Delta region to create awareness.

Innovation Platform as a Conducive Space for Reducing Human-Crocodile Conflicts in Agro-Pastoral Dams in Benin

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Abstract

Agro-pastoral dams (APDs) - water reservoirs constructed in Benin to provide water for livestock and for agricultural development - face several conflicts including human-crocodile conflicts. The research aimed to reduce conflicts and to find way for optimal use and management of APD ecosystem services for the benefit of all the stakeholders involved including crocodiles. The research was built on the Integral Ecology framework, which helps to develop an integral understanding taking into account institutional, technical, socioeconomic, and environmental dimensions of APD's problems. Comparative case studies of the use and management of three APDs in northern Benin were explored from an interdisciplinary perspective. Several technical and institutional constraints hamper the use and the management of APDs that are used for multiple purposes. The involvement of human and non-human stakeholders (crocodiles and livestock) makes an APD a complex system, impeding agreement on common rules for their management. The fear that crocodiles engender and crocodiles' negative effects on local livelihoods and people's tranquility make all stakeholders to frame the presence of crocodiles as problematic. While some inhabitants (more tolerant towards crocodiles) have constructed informal rules and socially rooted practices that assist them to live in peace with crocodiles, other stakeholders have constructed particular informal institutions that allow them to deny formal rules and thus kill them. At the same time, the APDs' water quality is problematic, because of significantly ($P<0.01$) high levels of nitrite, nitrate, iron, and chemical oxygen demand and the contamination of waters with harmful bacteria. An innovation platform should be established that allows stakeholders to interact to solve problems, through which they acquire new skills, produce knowledge, develop relationships, and share local experiences about conflicting activities around APDs, water pollution, siltation of APDs, APD ecosystem sustainability, the role of crocodiles in a healthy ecosystem, food security, climate change, and poverty alleviation. The research concludes that living with crocodiles should be considered as part of the APD agro-ecosystem, and people should thus adapt their behaviours towards crocodiles in order to peacefully share APD ecosystem services with them.

Introduction

In the semi-arid tropics and subtropics, the recurrence of drought calls for water management approaches to reduce shortages in water supply systems. (Falkenmark 2013). The construction of dams in West Africa has been found to be a sound strategy to increase water storage capacities, regulate water flows, contribute to food security, increase livelihood resilience, and maintain or/and improve wetland ecosystem functions and services as important components to assist people to adapt to climate change (Sally *et al*. 2011; IUCN-PACO 2012; de Fraiture *et al*. 2014). This led also to the extension of wetlands, creating permanent green pasture and favourable ecosystems for the expansion of wildlife such as hippopotamus, crocodile, duck, crane, heron, eagle, cormorant, pelican, and so forth. Therefore, dams also contribute to the maintenance of biodiversity (Bazin *et al*. 2011).

In Benin, 250 Agro-pastoral dams (APDs) have been constructed since 1970 by the Government of the Republic of Benin to water livestock and to boost livestock production (Bouraima 2006; Gadelle 2010). An APD ecosystem is an aquatic ecosystem consisting of a water body with surrounding land used for a variety of functions. The multiple functions of APDs provide include: drinking water supply for humans, livestock watering place, fish farming, vegetable production, food cropping, cotton farming, cleaning, washing, swimming, cooking, small business water use, house and road construction (Kpéra *et al*. 2012). The stakeholders involved in the use and the management of APDs have been facing problems such as infrastructural deterioration, degradation of watersheds and river banks, poor organization of involved stakeholders, underutilization of the agricultural potential of the dams, environmental problems, invasion of dams by crocodiles, conflicts between stakeholders, and so on (Capo-Chichi *et al*. 2009; Kpéra 2009). Therefore, there is an urgent need to understand how and why APDs are used as they are used and to create conditions to optimize their use and management.

The research aimed to reduce conflicts and to find ways for optimal use and management of APD ecosystem services for the benefit of all the stakeholders involved including crocodiles.

Methods

Research area

A case study approach was used as the overarching research design. The research area covers three APDs and the local communities making use of these APDs (Nikki, Sakabansi and Fombawi) in Nikki District, Borgou Department, northeastern Benin (Fig. 1). Covering an area of 3171 km^2 and lying between 9° 56’ 2” N and 3° 12’ 16” E, Nikki District is home to 20 APDs constructed by the national Government to enhance users’ incomes by improving and supporting agricultural production (Capo-Chichi *et al*. 2009). Local communities engage mainly in farming, herding, and fishing, and receive a cash income from trading their products and from other activities.

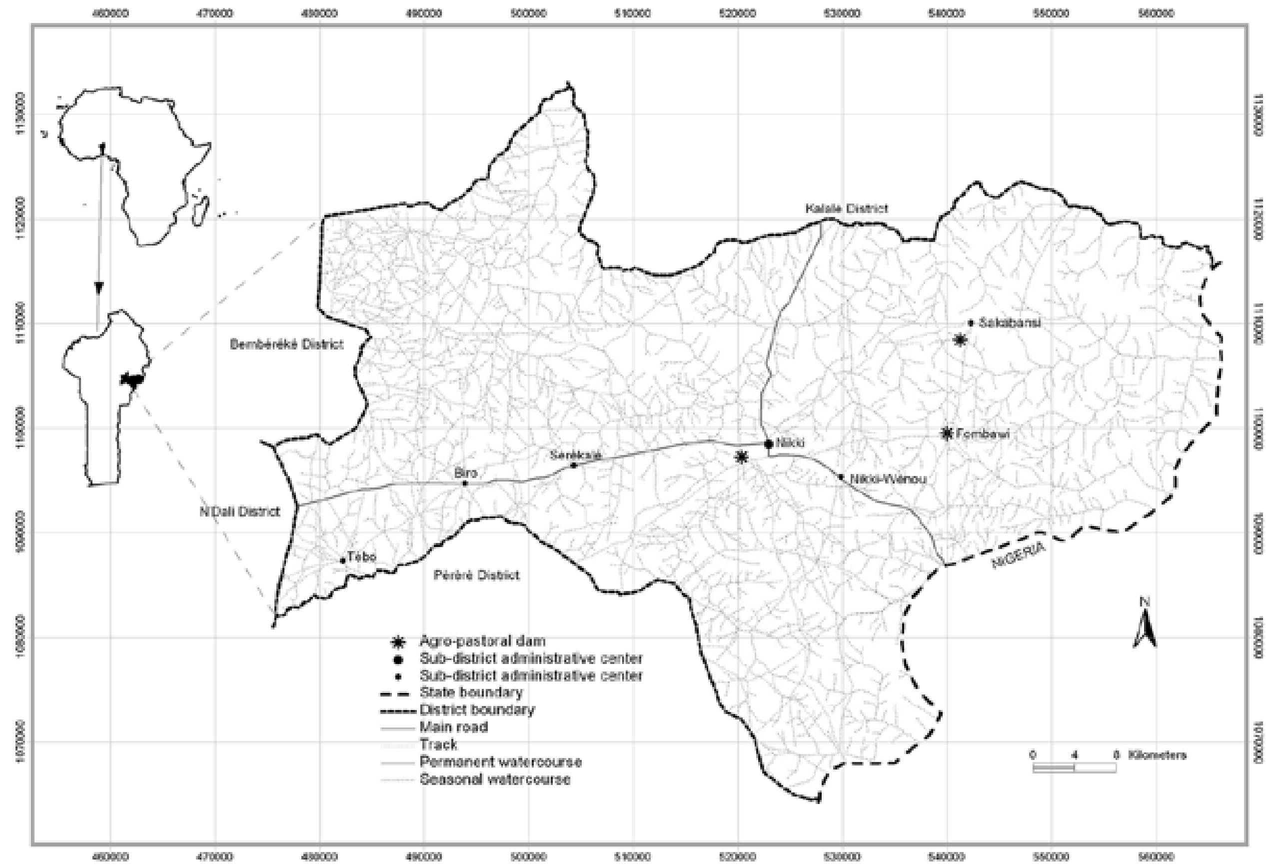

Figure 1. Location of Nikki, Sakabansi and Fombawi agro-pastoral dams (Source: Kpéra 2010).

Integral ecology framework applied to agro-pastoral dams

The research perspective is based on the integral ecology (IE) that is a framework with a global vision that contains various perspectives in a way that links, leverages, correlates, and aligns these perspectives (Wilber 1996). IE provides a comprehensive framework for considering multiple approaches to ecological and environmental phenomena, including our embeddedness in these (Esbjörn-Hargens and Zimmerman 2009). According to integral ecology theory, four irreducible perspectives (objective, interobjective, subjective, and intersubjective) must be considered when one is attempting to understand and remedy environmental problems. These perspectives are represented by four quadrants: the interior and the exterior of individual and collective realities. These four quadrants represent the intentional (“I”), cultural (“we”), behavioural (“it”), and social (“its”) aspects of ecological issues (Fig. 2).

The terrain of experience (UL)

The different stakeholders involved in the use and management of APDs have experiences and expectations that contribute to their problem perceptions and their appreciation of specific solutions. These include endogenous knowledge and people’s experiences (positive and negative) in addressing conflicts relating to the sharing of APD ecosystem services and to the presence of crocodiles. The relevant data are gathered by means of interviews.

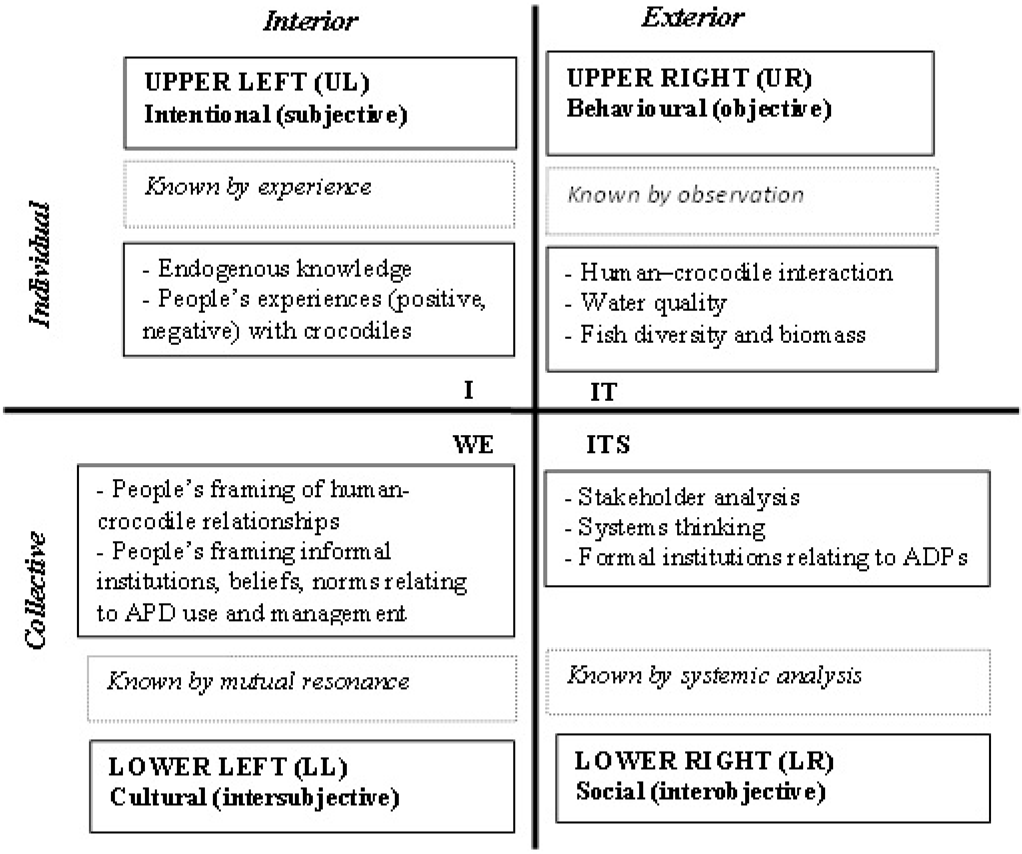

Figure 2. The four quadrants of integral ecology applied in APDs (Source: Esbjorn-Hagens and Zimmerman 2009).

The terrain of culture (LL)

People's views and related socially constructed and shared institutions are addressed. Emphasis is put on informal institutions (beliefs, norms, values, traditions, narratives, and discourses, an so forth) that collectively make sense and shape stakeholders' behaviours. In relation to the subjective and the intersubjective perspective, the concept of framing becomes relevant. Framing has to do with making sense, interpreting, and giving meaning to what is happening in the ongoing world. People make sense of situations for themselves and for others by means of certain perspectives or frames that they deploy in interaction (Dewulf *et al*. 2009). In conversations with others, realities are constructed by means of framing, including formal and informal rules that govern our daily practices.

The terrain of behaviour (UR)

From this terrain, we focus on tangible, empirically observable features, for instance the visible behaviours and practices relating to the use and/or management of the APDs. The interactions between people and crocodiles in different contexts are included. This terrain also points to important measurable features relating to water quality, the APD ecosystem biology, fish biodiversity and biomass. In other words, it is important to understand how users' behaviours during their daily activities around the dams contribute to environmental problems or affect APD ecosystem services and functions.

The terrain of systems (LR)

From this terrain, the formal association of stakeholders (who is involved, how do people relate to one another?) is studied, as also the formal institutions that guide practices, including professional stakeholders involved, more specifically the ecosystem biology (ie the relations between water quality, fish diversity and biomass, crocodiles, humans, and livestock).

Data collection and analysis

Activities and stakeholders in agro-pastoral dams

The aims were to (1) characterize the stakeholders involved in agro-pastoral dam use and management, and (2) identify important institutional and technical impediments and opportunities related to dams as perceived by the stakeholders. The data were collected through focus group discussions, semi-structured interviews, participant observations and participatory exercises (brainstorming and problem analysis) with diverse stakeholders. A stakeholder analysis (Stanghellini 2010) was carried out to identify the stakeholder categories. Finally, two stakeholder meetings with representatives of each category of stakeholders from the three APDs were organized.

Human-crocodile relationships

To elucidate how stakeholders frame the presence of crocodiles and how they use formal and informal institutions to deal with them, we focused on the framing of the issue at stake and the relationships involved. Issue frames reflect the meanings attached to events, phenomena, or problems in the relevant domain or context. Issue frames may contain problem, cause, and solution frames - in this case relating to humans and crocodiles sharing a dam - and are constructed to define the causes of problems as well as solutions for living together. When talking about the issue at stake, stakeholders disclose their own identities and their characterizations of others in their expressions (Gray 2003; Shmueli *et al*. 2006).

Water quality

To characterize APD water quality (physical, chemical, and microbiological composition). Physical and chemical parameters selected are among those suggested by WHO (2011) to assess water quality. These parameters include: temperature, pH, electrical conductivity (EC), nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), phosphates (PO_4^{3-}), sulphates (SO_4^{2-}), chlorides (Cl^-), bicarbonates (HCO_3^-), calcium (Ca^{2+}), magnesium (Mg^{2+}), total iron (Fe), chemical oxygen demand (COD) and total hardness. As the APDs are used as drinking water by both humans and livestock, microbiological analyses were performed to detect Total Coliform, *Escherichia coli*, spore of *Clostridium*, *Enterococcus feacalis*, *Salmonella typhi*, *S. typhimurium*, *S. enteritidis* and *Campylobacter jejuni* (WHO 2011).

Towards the setting of an innovative platform

An innovation platform is a space for learning and change. It is a group of individuals with different backgrounds and interests: farmers, traders, food processors, researchers, government officials, etc. The members come together to diagnose problems, identify opportunities and find ways to achieve their goals. They may design and implement activities as a platform, or coordinate activities by individual members.

Three stakeholder meetings with representatives of each category of stakeholders from Nikki, Sakabansi and Fombawi were carried out in Nikki. The first meeting was attended by 22 participants, the second by 28 and the third by 35. The participants included vegetable producers, food crop and cotton farmers, herders, fishermen, dam management committee members, members of the council of Nikki, daily users of the APDs, dam security guards, local officers of the ministry of agriculture, livestock farming and fisheries and local union of herders, and crocodile worshippers. The objective of the first meeting was to see how stakeholders collectively frame human-crocodile interactions as well as the management of APDs. The second meeting aimed at finding solutions to the problems posed by the stakeholders at the first meeting and to see how space for change could be created to reach a sustainable management of APDs based on converging the interests of all stakeholders. The third meeting sought to allocate responsibilities to each category of stakeholders to implement the process that was negotiated.

Results and Discussion

Activities and stakeholders in agro-pastoral dams

Agro-pastoral dams are used for multiple purposes such as providing drinking water for livestock and people, fish production, vegetable production, swimming, bathing, washing, house construction, food crop production and cotton farming. All these practices involve diverse stakeholders with different interests, backgrounds, knowledge, and assumptions. In addition, the dams are the main habitat for crocodiles, which thus can also be seen as key stakeholders. The use and management of the dams create conflicts among the stakeholders who all tend to reproduce their own 'truth' and to shift the responsibility for solving conflicts with others. Moreover, the water is becoming seriously polluted, which impinges on every stakeholder's interests.

Table 1 presents the stakeholders involved in APDs, their main interests, the main impediments they perceive and the tensions identified. Formal rules (relating to water and wildlife management) and informal rules related to the management and use of the dams exist. Informal rules do appear to guide stakeholders' behaviour in the APDs to some extent. They are well known by the dam users, however, this does not mean that they are automatically or universally obeyed. The traditional rules constituted by Fombawi culture that require that crocodiles are treated in a respectful way include: When stakeholders were asked why they do not always follow the formal and informal rules set by the council, which they appear to know so well, they answered that they see no reason to do so since the council itself does not meet its promises. This seems to suggest that the notion of a societal contract is latent in people's minds, and that there is a preparedness to act differently only if there were mechanisms to ensure the contract was honoured. This suggests that more attention should be paid to how sanctions are structured and enforced. Since our findings show that the dams are used for multiple purposes, involving diverse practices and stakeholders' interests, backgrounds, knowledge, and assumptions, the creation of an effective regime would seem to require an active policy of negotiation that includes representatives who can legitimately 'speak for the crocodiles'.

Figure 3. Watering cattle in Fombawi APD, April 2011 (Source: Kpéra 2011).

Figure 4. Fishermen setting nets in Nikki APD, March 2010 (Source: Kpéra 2010).

Our results furthermore show that the use and management of the dams create tension among the stakeholders, each of whom in effect treats the dams as an open access resource (Hardin, 1968), to reproduce their own 'truth' about who causes the tensions, and to shift the responsibility for conflict resolution to someone else.

The result of this institutional failure is a situation of 'collective irresponsibility' (te Velde *et al*. 2002). Meanwhile, the water is becoming seriously polluted and the dam infrastructure is deteriorating - problems that everyone recognizes. Everyone, however, is continuing to intensify exploitation because they receive a direct profit from their activities.

<u>Human-crocodile relationships</u>

Several species of wild animals occupy the dams, including snakes, monitor lizards, turtles and crocodiles. Crocodiles appear to be the most impressive of these animals, both in number and in size, and people give them full attention. Crocodiles, a protected species, share ecosystem services with local communities in agro-pastoral dams in northern Benin. They occur in all the three dams, but in different numbers. According to local people, crocodiles are rare in Nikki (less than 20), common in Sakabansi (20-100) and abundant in Fombawi (more than 200). Two crocodile species are said to occur in the dams: the West African crocodile (*Crocodylus suchus*) and the Dwarf crocodile (*Osteolaemus tetraspis*). Most of the dam users stated that the invasion of crocodiles took place after the construction of the dams. In earlier times, old people testified, there were large numbers of crocodiles in the rivers and ponds and in their experience crocodile numbers had decreased. They related the perceived decrease in the number of crocodiles to the long drought periods in the area, the destruction of the crocodiles' natural habitats because of urbanization, poaching for meat and organs, and the lack of motivation from the stakeholders to undertake dam management for the conservation of ecosystem functions, including the protection of the crocodiles.

Although, respondents framed the presence of the crocodiles as problematic because of their negative effects on local livelihoods and people's tranquillity, in all the three villages people seem to have a particular relationship with the crocodiles.

Both causes and solutions are, however, framed differently in the three communities. Whereas in Nikki and Sakabansi, respondents seek solutions in changing the ecological environment, requiring others (the council, fishermen, and crocodiles) to change their behaviour, Fombawi respondents seek to adapt their own behaviour by respecting and applying traditional and practical rules for sharing their dam. Damage per crocodile is the highest in Nikki and the lowest in Fombawi, suggesting that the crocodiles in Nikki behave more aggressively than those in Fombawi (Fig. 5).

Table 1. Stakeholders' main interests, the main impediments they perceive and the tensions identified.

N°	Stakeholders	Main interests	Main impediments perceived by stakeholders	Tension
1	Herders/UCOPER	Manage conflict between farmers Defence of herders' interests Improve water quality Involvement in the APD management UCOPER participates in the delimitation of national and international transhumance corridors	Recurrent conflict between farmers and herders Water pollution Silting up of dams	Blaming farmers for impeding access to the dams Blaming the Council of Nikki for taking a position favourable to the farmers Herders considered as strangers; their interests are not usually taken into account
2	Vegetable producers	Getting free access to dams	Decrease in vegetable yield Diseases and pests Lack of specific mineral fertilizer Lack of materials and seeds Destruction of garden fences by cattle Harassment in accessing credit Difficultly in accessing potential markets for products Low organization of vegetable producer associations	Tension with the Council of Nikki who want to expel them from their present location The Council of Nikki seen as a potential enemy
3	Non-vegetable farmers	Cropping in the surrounding of the APDs	Destruction of crop by cattle Conflict between farmers and herders Problem of land tenure	Tension with the Council of Nikki who decided to displace farmers farming in the radius of 1 Km from the APDs The Council of Nikki seen as a potential enemy
4	Daily users of the dams	Maintaining free access to the dams	Strong decrease in fish yield Silting of dams Increasing dryness Poor water quality Absence of attention to fish production Aquatic plant invasion Lack of fishing equipment Crocodile predation on valuable fish species	Tension with the Council of Nikki

Table 1. continued.

5	Fishermen	Intensification of fish production	Presence of crocodiles The silting of dams Poor water quality	Tension with the town council
6	Dam Management Committee (CoGes)	Participation in dam management	Not motivated to carry out their functions	Tension with the Council of Nikki
7	Crocodiles	Maintaining access to their habitat and food source in the dams	Poaching	Human-crocodile conflict in Nikki
8	Council of Nikki	Raising more financial resources for local development Maximization of dam income Increasing their power in decision making	Aquatic plant invasion Silting of dams Poor water quality Invasion of the dams by crocodiles	Tension with vegetable producers of Nikki Blamed by the CoGes and users Tension for a prejudiced mismanagement of the dam income Feeling of frustration among all the stakeholders
9	Communal Centre for Agriculture Promotion (CeCPA)	Advise and help farmers and herders in relation to various agricultural practices	Aquatic plant invasion Silting of dams Poor water quality Invasion of the dams by crocodiles	Tension with the Council of Nikki
10	Forests and Natural Resources Management Service (DGFRN)	Assist in choosing valuable trees for the reforestation of the water edge Contribute to ecosystem rehabilitation	Deforestation of the water edge Silting of dams Poaching of crocodiles	-
11	Participative Artisanal Fisheries Development Programme (PADPPA)	Improve fish production Increase local people's income Contribute to the sustainable use and management of the dams	Aquatic plant invasion Silting of dams Invasion of the dams by crocodiles	-
12	Agricultural Engineering Service (DGR)	Maintenance of the dam infrastructures	Silting of dams Destruction of the dam infrastructures	-
13	Netherlands Development Organization (SNV)	Reduce farmer-herder conflict Promote sustainable agriculture	Conflict between herders and farmers	-

Table 2. Problem and cause frames relating to living with crocodiles as constructed by the stakeholders in the agro-pastoral dams.

Village	Problem frames	Cause frames
Nikki	Fear of crocodiles	Illegal hunting of crocodiles
	Aggressiveness of crocodiles	Human population growth
	Attacks on livestock and dogs	Deforestation around the dams
	Predation on valuable fish	Food shortage for crocodiles during the dry season
	Damage to fishing equipment	Crocodiles and dogs are enemies
	Digging holes in the dykes	Water pollution
Sakabansi	Fear of crocodiles	Large specimens
	Predation on valuable fish	People's negative behaviour towards crocodiles
	Attacks on livestock and dogs	Illegal hunting of crocodiles
	Destruction of fishing nets	Fishermen's selfishness
	Damage to fishing equipment	Incompatibility between fish farming and crocodile conservation
	Digging holes in the dykes	
	Injuries to children	
Fombawi	Injuries to children	Non-respect of traditional rules relating to crocodiles
	Predation on fish species	
	Attacks on livestock and dogs	
	Digging holes in the dykes	

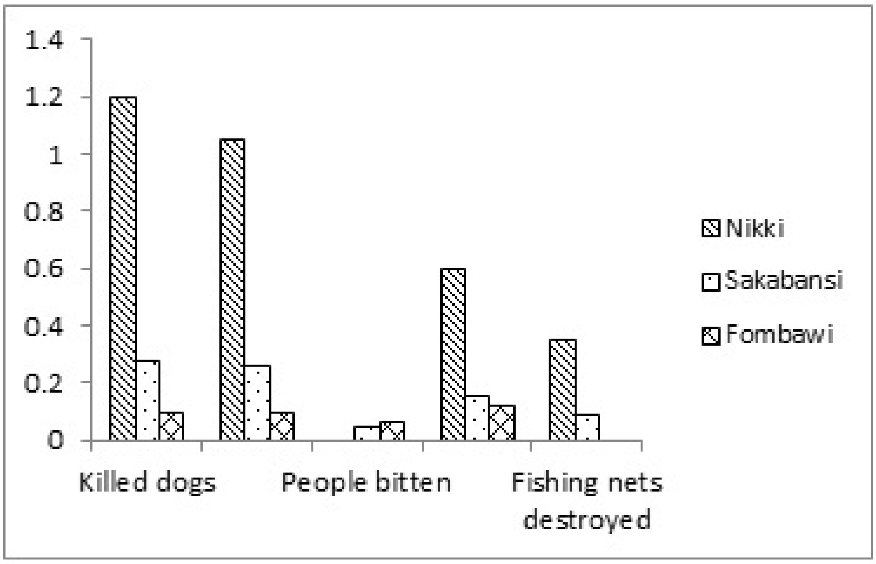

Figure 5. Number of different types of damage caused per crocodile in Nikki, Sakabansi and Fombawi dams from August 2009 to December 2012.

Further investigation is merited to determine whether or not crocodiles behave less aggressively when dealt with according to specific institutions. Intensive communication among stakeholders in the three villages is recommended to exchange experiences and ideas that may support a peaceful human-crocodile relationship inspired by existing institutional solutions. Even though the existence of damage, respondents framed the crocodiles as lying at the heart of the APDs because, according to them, the availability of water depends on the crocodiles, because they maintain the water in the dams by digging holes so that the ground water can be reached. At Nikki and Sakabansi, crocodiles are considered wild animals, but with the particular additional assumption that: It is not good to kill crocodiles because this can bring misfortune (farmer, Nikki, July 2009).

This assumption, however, does not prevent people from hunting the crocodiles, because they are thought to eat too much fish and because they are considered good bush meat. At Fombawi, crocodiles are conserved for their specific role in the local culture: crocodiles are thought to be holy, protecting people from bad luck. Old people interviewed in Fombawi explained: We have a great chance to have some specimens of crocodiles in our village and we thank God for that (CoGes member, Sakabansi, October 2009). Neither killing crocodiles nor collecting them to give them away as pets is allowed in Fombawi. There is considerable local respect for the crocodiles and, as a result, most people have learned to peacefully live together with them by sharing fish and water. The rules and behaviours for sharing are reinforced in celebrations and rituals and handed down to children in folk tales. Many people in Nikki and Sakabansi blame the people from Fombawi because they assume that the crocodiles present in their own dams have come from the Fombawi dam.

Further investigation is merited to determine whether or not crocodiles behave less aggressively when dealt with according to specific institutions. Intensive communication among stakeholders in the three villages is recommended to exchange experiences and ideas that may support a peaceful human-crocodile relationship inspired by existing institutional solutions.

Water quality and fish biodiversity

APD water quality is problematic, because of the significantly high levels of nitrite, nitrate, iron, and chemical oxygen demand. Nitrate and nitrite, probably the result of agricultural runoff, dump runoff, and human and animal wastes, may be responsible for the invasion of aquatic plants in the APDs resulting from eutrophication reducing water quality and altering the ecological structure and function of APDs. The water in APDs is unsuitable for both human and livestock consumption because it is contaminated with harmful bacteria (Coliforms, *Enterococcus faecalis*, *Escherichia coli*, spore of *Clostridium*, *Salmonella typhi*, *Campylobacter jejuni*, and so forth). The total fish catch in numbers is highest in Fombawi (43%), followed by Sakabansi (29%) and Nikki (28%). Four large size marketable species are targeted for fishing: *Clarias anguillaris*, *Clarias gariepinus*, *Oreochromis niloticus* and *Tilapia mariae*, representing 31%, 44%, and 86% respectively of the total fish population in Nikki, Sakabansi, and Fombawi. *Oreochromis niloticus* is the only prominent large species and is not part of the natural population. The almost complete absence of *T. mariae* and *C. gariepinus* in the Nikki APD is imputed to daily fishing. Twenty fish species were identified in the three APDs. This low diversity may be attributed to damage caused by agricultural practices, selective fishing (large size fish), and crocodile predation on fish. The study concludes that one solution for maintaining APD ecosystem health consists of watershed management based on monitoring ecosystem services such as water quality and fish biodiversity.

Setting up of an innovation platform

The three meetings show that discussions among the different stakeholders of the different villages have supported the 'unfreezing' existing frames and the bridging of frames of different stakeholders. Stakeholders discussed the situation and gradually their frames started to enlarge, to connect to other stakeholders' frames. The result of the meetings is that people are more open now to other frames. Thus preconditions for changing the situation start to be developed. As change is a continuous process that need creative adaptations along the way and a sustained thinking to progress, it can be assumed that more meetings, facilitated by professionals, will help people co-construct conditions for developing new realities, and co-construct rules to deal with these new realities. Those rules could be based on different building blocks:

- Respecting formal rules related to dealing with crocodiles;
- Discussing the costs of living with crocodiles and how these costs could be compensated and/or reduced;
- Developing practical rules for dealing with the crocodiles, inspired traditional rules that express respect;
- Respecting practical rules to peacefully live with the crocodiles.

Besides, the complexity of APD management, linked to the multiple uses and the multiplicity of stakeholders, leads to the underlying recurrent and intractable conflicts that people have been experiencing. All stakeholders (daily users of the dams, herders, vegetable farmers, farmers cropping near the dams, and fishermen) want to benefit from the dams. To guarantee sustainability of the services provided by the APD ecosystems and to come up with an appropriate management scheme, a collective learning process that involves the contextual re-ordering of relations in multiple social networks is needed (Leeuwis and Aarts 2011). Given that innovations emerge from interactions among different stakeholders (Leeuwis and van den Ban 2004), frequent communicative exchanges among stakeholders are of critical significance for change. For APD sustainability, experiences from elsewhere support our suggestion about organizing repeated interactions among a relatively small number of stakeholder representatives who are able to develop institutions for monitoring and enforcing a degree of cooperation, and who are regarded as legitimate by all stakeholders (Vollan and Ostrom 2010; Vollan 2008; Ostrom 2011). A stakeholder platform would be a useful vehicle for organizing collective action that can make change happen in APD use and management (Nederlof *et al*. 2011; Struik *et al*. 2014). Such platforms are often emergent institutions which can take many forms, from simple informal 'spaces' where stakeholders meet, to more organized arrangements with particular rules and functions. Regular meetings through a platform may help stakeholders involved in APDs to raise their ability to talk and think together, to share knowledge through conversation, and to enhance their collective wisdom (Isaac 1999). It is a place for social learning that allows stakeholders to interact to solve a problem, through which they acquire new skills (both technical and social), produce knowledge, develop relationships, and share local experiences (about conflicting activities around APDs, water pollution, siltation of APDs, APD ecosystem sustainability, the role of crocodiles in a healthy ecosystem, food security, climate change, and poverty alleviation). As a result, through social mobilization, action planning, and self-evaluation, the platform will search for solutions to problems. Exchanges of knowledge - including both local and scientific knowledge - and experiences may result in the co-creation of conditions to transcend blaming and instead discuss differences and similarities among stakeholders (Pearce and Littlejohn 1997) and develop new realities, including rules to deal with these new realities (Isaac 1999).

Conclusion

Intensively used by local communities, livestock, and crocodiles for diverse purposes, APD ecosystem services contribute to users' livelihoods. This research has resulted in a sense of urgency because all the stakeholders involved experience problems relating to the use or management of the dams. Meanwhile, they interpret the problems differently, including causes and solutions, resulting in a continuation and even deterioration of the situation. It is this collective irresponsibility that contributes to water pollution, loss of fish diversity, and persistent and intractable conflicts among users themselves, with the local government and with crocodiles. This implies that a dialogue among all stakeholders should be organized on a regular basis, making people aware of their interdependence, exchanging experiences, practices, and ideas (to capitalize on existing institutional diversity as the inspiration for change and also to co-create commonly accepted solutions).

Researchers from both biological and social sciences could develop collaborative efforts and use direct observations, measurements and communication tools to assess human-wildlife interactions and to evaluate management actions. By doing so, we will be better equipped to optimize the coexistence between wildlife and people.

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Cave Isolation Sparks Ecological and Evolutionary Divergence in the African Dwarf Crocodile (*Osteolaemus tetraspis*)

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Abstract

We present the first ever observations of African Dwarf crocodiles (*Osteolaemus tetraspis*) selectively utilizing a cave ecosystem. Cave dwelling crocodiles showed, in some instances, extreme phenotypic characterization and many individuals appeared to be entrapped within the cave system. We undertook to better understand the degree to which cave crocodiles are isolated both ecologically and evolutionarily from their surface dwelling conspecifics - which can be found less than 2 km away. We analyzed differences in cave and surface dwelling crocodile diet and then assessed body condition amongst Dwarf crocodile populations to better understand the implications of a semi-hypogean life on this species. To test for evolutionary divergence, we amplified mitochondrial genes and microsatellites in cave and surface dwelling Dwarf crocodiles to test for haplotype and genotype population structure, migration between cave and surface populations, and signs of population bottlenecks. Crocodiles captured in the caves appear to be foraging exclusively in the caves, eating mostly cave crickets and bats, whereas crocodiles captured in the surrounding forest habitat consumed primarily freshwater crustaceans and insects, and were not found to consume cave dwelling prey. Juvenile cave crocodiles had significantly higher body condition compared to juvenile forest crocodiles, which did not vary amongst forest locations. Genetic analyses indicated that the cave crocodiles are genetically isolated from forest populations, containing at least one private mitochondrial haplotype and being identified as a unique genetic cluster. This is supported by migration analysis, which indicate no migration into or out of the caves despite the presence of individuals with surface dwelling genetic profiles, indicating these individuals may not be contributing reproductively. Modeling of past demographic events also detected a 99.5% population decline approximately 3300 ybp - though, this could also correspond to the colonization event. Our results provide a unique insight into facultative cave use by a principally surface dwelling species and reinforce the necessity for further research into this unique system to better understand the evolutionary scale implications of cave habitat.

Adult *Osteolaemus* sp. nov., Côte d'Ivoire. Photograph: Matthew Shirley.

Future Challenges of Nile Crocodile Management in Egypt

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Abstract

The Nile crocodile (*Crocodylus niloticus*) was historically revered for its strength and utilized as guardian to the Pharaohs and priests of ancient Egypt. It was familiar during the ancient Egyptian times, that some creatures were venerated by the ancient Egyptians. In other words, the Pharaohs did not worship the creature itself but the obvious feature in that creature. Crocodile-headed man was venerated and named as Sobek in reference to the God of power. To honor Sobek, a huge temple was constructed in Kom Ombo in 200 BC, where people worshiped him as the soul of strength. However, by the 1950s crocodiles were virtually eradicated from the Nile River. The construction of the High Dam at Aswan and subsequent impoundment of Lake Nasser gave the Nile crocodile a second chance in Egypt and by the 1980s they were again seen irregularly. The vast, remote, highly productive habitat of Lake Nasser accelerated the revival of the crocodile population and by the 1990s crocodiles were a common feature of the biological landscape. Lake Nasser is considered one of the largest man-made lakes in the world with a shoreline of around 6000 km. Soon thereafter, fishermen started to become vocal about their numbers and the impact of crocodiles on their lives and livelihoods, prompting the Nature Conservation Sector to consider management strategies for this wildlife resource.

In 2008 the Nature Conservation Sector, Egyptian Environmental Affairs Agency, Ministry of State for Environmental Affairs, recognized the potential of *C. niloticus* as an economic resource, and in turn, their sustainable utilization as an answer to the demands of the large and influential Lake Nasser fishing community. A crocodile monitoring program was initiated at this time with the aim of instituting some form of crocodile harvest. The first two years of the program met with great success and resulted in trained personnel, preliminary estimates of population size, indicators of abundance for continued monitoring efforts, and collaborations with international specialists.

In 2009, the Egyptian Crocodile Management Unit was established to oversee future crocodile management and related activities. Radio-telemetry studies have been launched to study the spatial ecology of the species in Lake Nasser to better understand the home range, habitat use, and population dynamics of this species. In 2010 we further advanced our efforts by successfully petitioning CITES to downlist the Egyptian Nile crocodile population from Appendix I to Appendix II with a zero export quota. We consider this a critical first step to catalyze sustainable development, including regular monitoring, law enforcement, public awareness and ecotourism of this species in Egypt.

Now is the time to capitalize on the momentum created by this initial program and we therefore propose the initiation of a formal, Government-sponsored Crocodile Management Unit (CMU). The CMU will have the following mission statement: "To protect the crocodiles of Egypt and support the betterment of Egyptians through sustainable utilization of the crocodile natural resource."

When the CMU was created as part of the Nature Conservation Sector benefits were achieved on three levels: conservation, sustainable utilization, and public awareness.

1. Long-term Crocodile Conservation and Sustainable Management
2. Sustainable Utilization of the Crocodile Resource
3. Public Awareness, Education, and Capacity-Building

The value of crocodiles as a sustainable natural resource is widely recognized and can include benefits to both the Egyptian Government, in the form of a self-sustaining management program and international recognition, and the Egyptian people through employment, tourism, and direct harvest. After more than 5 years surveying the wild population, we have covered approximately 50% of the lake. However, conservation and development for crocodile management cannot proceed without an existing infrastructure. Moreover, it is forbidden by law in Egypt to hunt, kill, or catch species of wild birds and animals listed in the executive regulations (including Nile crocodile); or to possess, transport, circulate, sell or offer to sell these species, either dead or alive. It is also forbidden to damage the nests or eggs of listed birds and animals. The CMU acts to enforce this law in cooperation with the police department by confiscating many live and dead crocodile specimens and reintroducing the live ones to their natural habitat in the Lake. At the beginning of 2016, the Egyptian Government indicated its aspiration to launch the first crocodile farm together with the implementation of a long-term conservation strategy. There are many challenges facing such a program concerning the sustainability, overexploitation pressures, high marketing competition and limited community engagement in management/conservation plans. The successful utilization of the species could be a pioneer experiment that could be replicated with other natural resources in Egypt.

Dead or Alive? Factors Affecting the Survival of Victims during Attacks by Saltwater Crocodiles (*Crocodylus porosus*) in Australia

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Abstract

Conflicts between humans and crocodilians are a widespread conservation challenge and the number of crocodile attacks is increasing worldwide. We identified the factors that most effectively decide whether a victim is injured or killed in a crocodile attack by fitting generalized linear models to a 42-year dataset of 87 attacks (27 fatal and 60 non-fatal) by Saltwater crocodiles (*Crocodylus porosus*) in Australia. The models showed that the most influential factors were the difference in body mass between crocodile and victim, and the position of victim in relation to the water at the time of an attack. In-water position (for diving, swimming, and wading) had a higher risk than on-water (boating) or on-land (fishing, and hunting near the water's edge) positions. In the in-water position a 75 kg person would have a relatively high probability of survival (0.81) if attacked by a 300 cm crocodile, but the probability becomes much lower (0.17) with a 400 cm crocodile. If attacked by a crocodile larger than 450 cm, the survival probability would be extremely low (<0.05) regardless of the victim's size. These results indicate that the main cause of death during a crocodile attack is drowning and larger crocodiles can drag a victim more easily into deeper water. A higher risk associated with a larger crocodile in relation to victim's size is highlighted by children's vulnerability to fatal attacks. Since the first recently recorded fatal attack involving a child in 2006, 6 out of 9 fatal attacks (66.7%) involved children, and the average body size of crocodiles responsible for these fatal attacks was considerably smaller (384 cm, 223 kg) than that of crocodiles that killed adults (450 cm, 324 kg) during the same period (2006-2014). These results suggest that culling programs targeting larger crocodiles may not be an effective management option to improve safety for children.

Introduction

Conflicts between humans and wildlife, especially large carnivores such as crocodilians, are becoming a complex conservation challenge worldwide (Treves and Karanth 2003; Treves *et al*. 2006; Dickman 2010). In the case of crocodilians, they pose a threat to people, livestock or pets in local urban and rural communities (Aust *et al*. 2009; Gopi and Pandav 2009). Crocodile attacks result in serious injury or death of a victim in most cases (Mekisic and Wardill 1992; Gruen 2009; Wamisho *et al*. 2009), and even crocodilian species that are considered harmless to humans are often viewed with fear. During the 1950s to 1980s, many crocodilian species were threatened due to overexploitation and habitat loss (Martin 2008), but protection and implementation of effective conservation programs have seen some populations achieve extensive recovery (Platt and Thorbjarnarson 2000; Mazzotti *et al*. 2007; Fukuda *et al*. 2011) while others remain endangered (Thorbjarnarson *et al*. 2002; Thapaliya, Khadka and Kafley 2010; Van Weerd 2010). Conservation actions typically aim to increase depleted crocodilian populations, and the success of such conservation programs invariably leads to an increase in negative interactions between people and crocodilians (human-crocodile conflict; HCC) (Sideleau and Britton 2012). Increased HCC may also be related to increasing human populations (Fukuda, Manolis and Appel 2014), urbanisation and encroachment by humans into crocodile habitats for tourism, recreation, agriculture or other purposes (Elsey and Woodward 2010).

While HCC may be an increasing issue, numerous attacks by crocodilians go unreported or are poorly documented in many countries where crocodilians are distributed (Sideleau and Britton 2012). Attacks by American alligators (*Alligator mississippiensis*) are well documented, although many attacks are provoked (eg while handling animals) (Conover and Dubow 1997; Harding and Wolf 2006; Langley 2010). Fatal attacks by *A. mississippiensis* are uncommon, reflecting their smaller size and more docile nature relative to other crocodilian species (Manolis and Webb 2013). More aggressive species such as Saltwater crocodiles (*Crocodylus porosus*) and Nile crocodiles (*C. niloticus*) (Brien *et al*. 2013) are responsible for a much higher mortality of humans in unprovoked attacks. Between January 2008 and October 2013, 528 attacks by *C. porosus* and 466 by *C. niloticus* were reported worldwide (Charles Darwin University and Big Gecko 2014). A lack of details for increasingly common but poorly documented attacks prevents systematic examination of the incidents, although such evidence-based information is essential for management programs to improve human safety and reduce HCC. While some statistics of crocodilian attacks are reported elsewhere (Caldicott *et al*. 2005; Dunham *et al*. 2010; Langley 2010; Fukuda *et al*. 2014), there have been no studies that specifically examined factors affecting the fate of a victim, whether they would be injured or killed during an attack.

Here we examine a 42-year dataset of attacks by *C. porosus*, the largest (Britton *et al*. 2012), most aggressive (Brien *et al*. 2013) extant crocodilian species in northern Australia where one of the largest *C. porosus* populations in the world exists

(Webb *et al*. 2010). We hypothesize that the outcome of an attack is affected by certain factors associated with a crocodile, victim, and environment, and identify which of these factors most significantly affects the probability of a victim's survival. We use the results to inform the key messages for public safety programs, especially for children, the sector considered most vulnerable to fatal attacks in recent years.

Materials and Methods

We conducted this retrospective study under approval by the Northern Territory Department of Land Resource Management and the Parks and Wildlife Commission of the Northern Territory.

Study area

The study area was the tropical coastal regions of northern Australia, consisting of the Northern Territory, Queensland, and Western Australia. The study area covers the natural distribution of *C. porosus* in Australia (Fig. 1) where it inhabits a range of brackish, freshwater and saline water bodies, including beaches, billabongs, floodplains, lagoons, lakes, mangroves, rivers, swamps, and waterholes (Webb and Manolis 1989; Fukuda *et al*. 2007). The climate is monsoonal with distinct wet (November-April) and dry (May-October) seasons. The annual minimum and maximum temperature typically ranges from 16 to 37°C, and the annual rainfall is 1000-1700 mm (Bureau of Meteorology 2014). The area covers several towns and townships, including many remote indigenous communities.

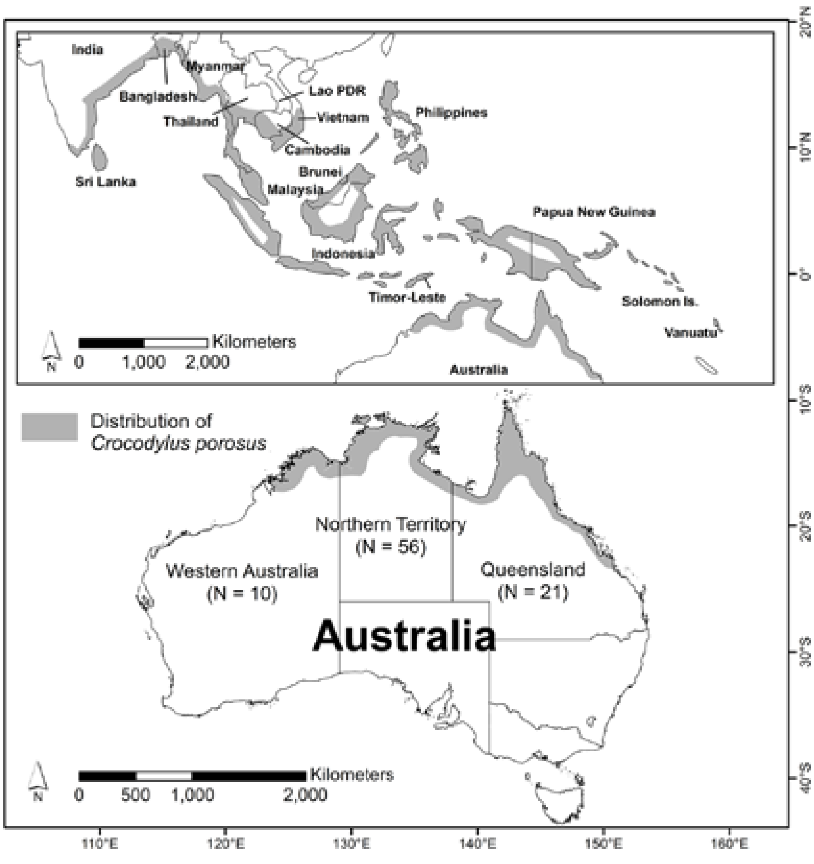

Figure 1. Distribution of *Crocodylus porosus* in the world and Australia. N is the number of crocodile attacks used in the analysis.

Historical data

We compiled historical records of *C. porosus* attacks across northern Australia since the species was protected in Western Australia (1970), the Northern Territory (1971), and Queensland (1974), by 1) collating the internal reports and databases kept within government and police agencies, 2) searching media sources, such as archived newspapers and websites, and 3) consulting with independent databases (Y. Fukuda, C. Manolis, unpublished data). We excluded: provoked attacks resulting from voluntary contact with crocodiles such as when catching crocodiles or collecting their eggs; attacks by crocodiles in captivity or escapees from crocodile farms, even if unprovoked; attacks that did not cause any injury (including death), because these attacks were often not reported; and, suspected incidents such as victims going missing without witness or evidence as a crocodile attack. In two cases two victims were involved in an attack by the same crocodile. We treated such a case as two separate attacks because our focus was whether a victim was injured or killed (result) rather than whether a victim was attacked or not (causation). We collected details of each incident, including 1) the date, time, location, and severity of an attack, 2) the total length of the crocodile either measured or estimated, and 3) the age, origin (local or visitor), and activity of the victim at the time of the incident. All of our compiled data, except for confidential information, are accessible in a publicly available database (Charles Darwin University and Big Gecko 2014) and general trends are summarized by (Fukuda *et al*. 2014). Personal information on the victims was anonymized and de-identified prior to the analyses.

Analysis

To examine the consequence of crocodile attacks and their attributes, we modelled the relationships as a binary logistic regression, using Generalized Linear Models (GLM). We coded the crocodile attacks into fatal and non-fatal as a binary response variable (0= fatal, 1= non-fatal). From the information we collected for each incident, we prepared explanatory variables that we considered biologically and statistically meaningful for the management of HCC. For example, review of a few fatal cases, for which detailed information was available in coroner's reports (Cavanagh 2004), revealed that victims were dragged under the water by a crocodile and the primary cause of their death was drowning. This indicates that the size of a crocodile was an important factor affecting the fate of a victim, but it may also be affected by the size of the victim. To account for this relationship we derived an explanatory variable, the difference in size between a crocodile and victim. We considered the size of both crocodile and human best expressed as body mass (kg) rather than length or height. Although the weight of individual crocodiles involved in incidents is unknown, we estimated it from their length, using a length-weight equation. As a previous conversion equation (Fukuda *et al*. 2007) erroneously overestimates the body mass of a crocodile, we derived a new equation by fitting an exponential function (Y = aXb where Y and X are the body mass and length of a crocodile, respectively) to morphological data of Saltwater crocodiles in different sizes reported in previous studies (Webb and Messel 1978; Britton *et al*. 2012). We estimated the weight of victims from their sex and age, using equations obtained by fitting a quadratic function (Y = aX2 + bX + c where Y and X are the body mass and height of a person, respectively) to the average height and weight of people in Australia (males and females, separately) at the age of 20-80 years (Australian Bureau of Statistics 2012). Because the data for the weight of people under 20 years of age were not available for Australia, we used the World Health Organization (WHO) growth reference data for 5-19 years [average height and Body Mass Index (BMI)] (World Health Organization 2006) to estimate the weight of 5-19 years old (males and females, separately). Although the WHO dataset included countries other than Australia, we assumed that these international estimates would approximate those of Australians. As a result, we derived a continuous explanatory variable, the difference in body mass between a crocodile and the victim (Δ weight).

Other explanatory variables we selected were position of a victim at the time of an incident, alcohol status of a victim (Alcohol), time of an incident (Day/night and Month), presence of a companion directly rescuing a victim from a crocodile (Assistance), age (Age), and origin (Origin) of a victim. Some of these variables were dichotomous variables such as Alcohol (intoxicated or not), Assistance (assisted or not), Day/night (day or night), Origin (local or visitor). Months were grouped into seasons (Season) that consisted of early dry (May-July), late dry (August to October), and wet (November to April) according to (Webb 1991). Age was continuous integers. Position of a victim at the time of an incident was a three-level categorical variable (on land, on water, or in water). On-land position represented fishing from the bank, hunting, and other activities such as camping near the water or collecting the water with a bucket. On-water position was boating, and in-water position included diving, swimming and wading.

We followed standard procedures for data exploration (Zuur, Ieno and Elphick 2010) and ensured that there were no outlying observations in the variables and also no collinearity between the explanatory variables. We fitted a binary logistic regression to the historical data of crocodile attacks, using the logit link function in the binomial family of GLM (Crawley 2005) using R version 3.1.1. We used the information-theoretic approach (Burnham and Anderson 2002) to identify a minimum adequate model from a set of a priori models in which each model labelled M1-M10 was associated with a specific hypothesis (Table 1). Our sample size was relatively small (N= 87) and thus we used Akaike Information Criteria corrected for small samples (AICc). We compared the models using AICc and Akaike weight. We assessed the importance of each explanatory variable within the minimum adequate model, using likelihood ratio test. We then predicted the probability that a victim would survive a crocodile attack using the explanatory variables identified as most significant within the model.

Based on the findings from the analysis, we provided recommendations and management implications to improve public safety and reduce the incidence of HCC, particularly in relation to a risk to children. We highlighted differences between children and adults as a victim of fatal crocodile attacks by comparing the proportion of each group since the first fatal attack on a child (2006), and the average size of crocodiles responsible for these attacks. In this study we defined children as less than 18 years of age.

Results

Between 1970 and 2014, there were 109 unprovoked attacks on humans by *C. porosus* in the wild across northern Australia. However, 22 records did not have the full detail of the crocodile, incident or victim, and were excluded from the analysis, leaving a full sample size of 87 attacks (27 fatal and 60 non-fatal). The number of attacks generally increased over years (Fig. 2).

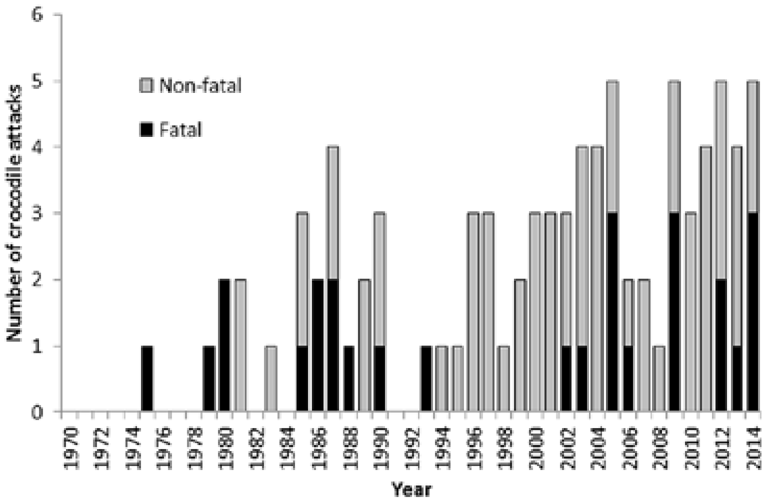

Figure 2. Number of fatal and non-fatal attacks by *Crocodylus porosus* divided into 5-year periods between 1970 and 2014 in Australia.

The exponential function fitted to estimate crocodile weight showed a significant fit (a = 2.552 with SE = 0.011, b = 3.321 with SE= 0.002), as did the quadratic functions fitted to estimate human weight (amale= -0.012 with SE= 0.001, bmale= 1.201 with SE= 0.104, and cmale= 60.346 with SE= 2.346, and afemale= -0.008 with SE= 0.001, bfemale= 0.813 with SE 0.113, and cfemale= 52.378 with SE= 2.570). These fitted equations enabled the continuous estimation of Δ weight to be included in the candidate GLM as one of the explanatory variables.

In the set of a priori models, the smallest AICc (51.04) was achieved by M3 (Table 2). Assistance showed a large standard error in relation to the estimated coefficient (Table 3). Further investigation indicated that we had quasi-complete separation due to the Assistance variable. Quasi-complete separation is a relatively unknown feature of Bernoulli GLMs, and it occurs when a predictor (Assistance) separates the binary response variable (Survival) up to a certain high degree (Allison 2004). In this case we had the 'Yes' level of Assistance (N= 12) exclusively linked to 'Non-fatal'. With quasi-complete separation, the maximum likelihood estimation of the corresponding parameter does not exist. However, the likelihood ratio test is still valid in quasi-complete separation, and results indicated that Assistance was significant (L= 3.59, df= 1, P= 0.02, Table 4).

Table 1. A priori candidate models of binary logistic regression to explain the fate of a Saltwater crocodile attack.

Model	Expression	Hypothesis
M1	Δ weight + Position	Main cause of death is drowning and the risk is affected by Δ weight and Position
M2	Δ weight + Position + Alcohol	Risk of drowning is increased by the consumption of alcohol
M3	Δ weight + Position + Assistance	Risk of drowning is reduced by the assistance from the second person
M4	Season + Day/Night	Crocodiles are more active in certain seasons and day or night
M5	Alcohol + Position	Victim's survival is reduced by their inappropriate behaviour
M6	Alcohol + Position + Age + Alcohol:Age	Alcohol consumption is more common at a certain age
M7	Sex + Age + Sex:Age	Victim's physical strength to fight a crocodile is determined by sex and age
M8	Position + Origin + Position:Origin	Certain activities are more common for local people or visitors
M9	Position + Day/Night + Position:Day/Night	Certain activities are more common in day or night
M10	Null	None of the covariates affect victim's survival

Table 2. Model selection values of the candidate models of binary logistic regression.

Model	df	AICc	Model likelihood	Akaike weight
M1	4	54.15	0.21	15.88
M2	5	55.25	0.12	9.14
M3	5	51.04	>0.99	75.06
M4	4	115.14	<0.01	0.0
M5	4	99.87	<0.01	0.0
M6	6	104.0	<0.01	0.0
M7	4	114.79	<0.01	0.0
M8	6	107.69	<0.01	0.0
M9	6	105.2	<0.01	0.0
M10	1	109.82	<0.01	0.0

Table 3. Estimate and standard error (SE) of the explanation variables in the minimum adequate model (M3) of the binary logistic regression.

Variable	Estimate	SE
Intercept	1.91	0.56
Δ weight	-0.02	<0.01
Position (on-land)	4.91	3.55
Position (on-water)	2.58	1.38
Assistance (Yes)	18.34	2621

Table 4. Likelihood ratio test (LRT) of the minimum adequate model (M3) of the binary logistic regression.

Variable	Df	LRT	P
Δ weight	1	46.80	<0.01
Position	2	9.0	0.01
Assistance	1	5.36	0.02

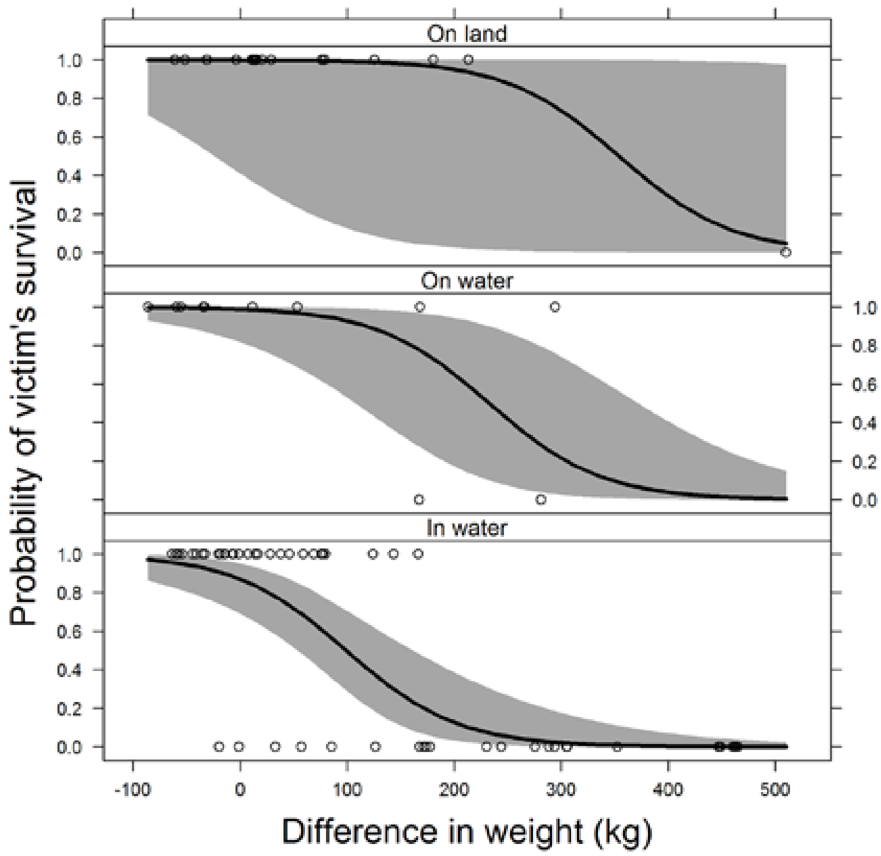

Figure 3. Estimated probability (solid line) that a victim survives a Saltwater crocodile attack over the difference in weight between a crocodile and victim in on-land, on-water, and in-water positions. Grey shade is 95% confidence band and open symbols are the raw binary data of crocodile attacks (N= 87).

As a result we selected the model with Assistance, Δ weight, and Position (M3) as the minimum adequate model. In the selected model, Δ weight showed a much more significant effect on the response than Position while the effect of Assistance was marginal because of the quasi-complete separation (Table 4). Probability of victim's survival decreased over the consistent range of Δ weight more rapidly in water than on water or on land (Fig. 3).

The first fatal attack involving a child in Australia occurred in 2006 in the Northern Territory. Since 2006, 6 out of 9 fatal attacks (66.7%) across Australia have involved children. The average size of crocodile involved in fatal attacks on children and adults was 384 cm (223 kg) and 450 cm (324 kg), respectively.

Discussion

The probability of survival in a crocodile attack may not be decided by a single factor but rather a complex synergy of multiple variables in a unique situation. However, our results indicated that certain variables affected the probability of a victim's survival more strongly than others. The difference in body mass between crocodile and victim was the most significant factor deciding the fate of a victim. In the in-water position (for diving, swimming or wading) an average-sized person weighing 75 kg would have a relatively high probability of survival (0.81) if attacked by a 300 cm crocodile. The probability of survival becomes lower (0.17) with a 400 cm crocodile, and is greatly reduced (0.02) with a 450 cm crocodile. Even a person with very large body mass (120 kg) would have a very low survival probability (0.05) with a 450 cm crocodile. This is consistent with most attacks involving >450 cm *C. porosus* resulting in fatalities in east India (Kar and Bustard 1983) and the Northern Territory of Australia (Fukuda *et al*. 2014).

The minimum size of *C. porosus* reported responsible for a fatal attack in Australia is 235 cm, but the victim was a 13-year-old boy whose body mass was small enough for the crocodile to drag him under the water. The probability of survival of children is lower than adults, even with a smaller crocodile. The average size of crocodiles killing children was noticeably smaller (384 cm, 223 kg) than that of crocodiles killing adults (430 cm, 324 kg). This stark contrast highlights the particular vulnerability of children to a crocodile attack. Nevertheless, it should be noted that the size of a crocodile has a much greater influence than that of a victim because crocodile body mass increases exponentially with size [eg a 617 cm Saltwater crocodile weighed 1075 kg, (Britton *et al*. 2012)].

The position of a victim at the time of an incident highlights risks associated with different depth of water and its influence on the survival of the victim is significant. For a 75 kg person being attacked by a 400 cm crocodile, on-water (boating) and on-land (fishing, hunting or other) positions improves the probability of their survival from 0.17 to 0.73 and 0.97, respectively. There were only two fatal cases associated with boating, although boating is a common leisure activity in the study area. It should be noted that the victims of both fatal cases were in small (<350 cm long) boats, including a canoe. The use of boats smaller than 450 cm in habitats containing *C. porosus* carries a higher risk. If attacked by a 450 cm crocodile, the probability of survival of a victim on water lowers to 0.21, but that of a victim on land remains relatively high (0.73).

Taking together the effect of the body mass and position, our results suggest that the primary cause of death during a crocodile attack is drowning. This is further supported by observations in the Northern Territory that the bodies of some of the victims retrieved within 24 hours after a fatal attack were relatively intact with no major trauma (Cavanagh 2004) (Dani Best, unpublished data). Drowning is also reported as a major cause of death in attacks by *A. mississippiensis* (Harding and Wolf 2006) and *C. porosus* elsewhere (Kar and Bustard 1983). This contrasts with shark attacks where most victims die of excessive loss of blood (Woolgar *et al*. 2001). In none of the fatal post-1970 crocodile attacks was there evidence that the victim escaped the attack and died as a result of wounds later. However, in four attacks by *C. porosus* in Australia before 1873, the victim reportedly escaped from the crocodile, but died later as a result of the wounds received (Charlie Manolis, unpublished data). These historical cases are considered to reflect the lack of medical facilities and transport at that time. Improved communication, access to remote locations and medical care has most likely prevented some non-fatal attacks resulting in fatality due to trauma.

Another variable potentially important as shown by the likelihood ratio test was the presence of a second person proving assistance to a victim. All attacks in Australia by crocodiles larger than 400 cm on un-aided victims in the water were fatal (Manolis and Webb 2013), but the presence of company increased the survival probability of a victim in most cases by preventing them from being dragged into the water and moving them out of range.

These factors identified as significant provide important implications for the mitigation of HCC. Safety awareness programs need to be designed to target the risk to children from crocodile attack. In 5 of the 6 fatal cases involving children, the victim was swimming in deep water, which is the highest risk activity that a person may undertake in crocodile habitat. Given that any waterway within the range of *C. porosus* in northern Australia should be considered occupied habitat due to the recovery of crocodile populations to near pre-unrestricted hunting levels (Fukuda *et al*. 2007, 2011), any swimming activity other than in locations identified "safe" by the authority poses an unacceptable risk. More crocodile attacks tend to happen at the beginning and end of the wet season although fatal cases occur all year around (Fukuda *et al*. 2014). However, the survival of a victim does not depend on seasons or time of a day (daylight or night). This suggests that extreme caution should be exercised whenever entering crocodile habitats.

Inevitably, following any attack there are calls for culling of the crocodile population to reduce the risk of crocodile attack (Webb 2012). If culling were considered a management option, it would most likely target larger animals as these are commonly seen by the public as posing the greatest risk. Intensive culling could reduce the encounter rate with larger individuals. However, the effect of removing dominant individuals on the dynamics of a population is unknown (Campbell and Dwyer 2013). Removal of large crocodiles may lead to a higher number of subordinate individuals. It should be noted that children would remain vulnerable to smaller crocodiles even if larger crocodiles are removed. To assure safety, any culling program would have to remove all crocodiles from a location, which is not a practical option for management given the high mobility and dispersal of the species across a range of habitats (Brien *et al*. 2008; Campbell *et al*. 2010, 2013). Culling programs would not ensure the absence of crocodiles in a targeted area (Webb 2012; Britton and Campbell 2014) and water-related activities in crocodile habitats would remain unsafe to the public. Within a decade after protection four fatal attacks occurred even though crocodile abundance was very low across northern Australia. Continuous public education campaigns to raise the safety awareness may be a more effective management option to reduce HCC. In many cases, crocodiles attacks can be prevented through such education programs (Fukuda *et al*. 2014).

Crocodile attacks are not a major cause of mortality in Australia. In the study area between 2004 and 2013, 0.02 deaths per 100,000 people (12 in total) were associated with crocodile attacks, in contrast to 8.12 deaths per 100,000 (5432) by road accidents (Australian Government Department of Infrastructure and Regional Development 2014). However, the frequency of crocodile attacks has been increasing (Manolis and Webb 2013; Fukuda *et al*. 2014) and management programs should incorporate evidence-based options to mitigate HCC. Our findings and recommendations may apply to other countries where *C. porosus* is distributed, as well as to other species known to commonly attack people (Sideleau and Britton 2012) such as American crocodile (*C. acutus*), Mugger crocodile (*C. palustris*) and Nile crocodile (*C. niloticus*).

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This study was conducted under approval by the Northern Territory Department of Land Resource Management and the Parks and Wildlife Commission of the Northern Territory as part of the Northern Territory Government's crocodile management program (Leach, Delaney and Fukuda 2009). It did not involve any treatment of live animals. The crocodile management program complies with the Animal Welfare Act (Northern Territory of Australia 2013) and the Code of Practice on the Humane Treatment of Wild and Farmed Australian Crocodiles (Natural Resource Management Ministerial Council [NRMMC] 2009). We thank K. Appel, T. Nichols, D. Best and J. Papple at the Parks and Wildlife Commission of the Northern Territory, G. Lindner at Parks Australia, K. Boddington, S. Bradley and M. Casey at the Northern Territory Police, and many others from various organisations for their assistance in compiling historical crocodile attack data. G. Edwards and A. Fisher provided comments on the manuscript.

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Microbial Man-eaters - the Hidden Danger in Working with Crocodiles

Larissa McLeod

Abstract

Crocodilians are typically found in tropical regions. Unfortunately, so are some of the deadliest parasites and infectious diseases that affect humans. This means that the people who work with crocodiles often have far more to be concerned about than simply the crocodiles themselves. This presentation aims to provide some basic information on the worst of the microbial agents encountered by the people who work with crocodiles in the field, including signs and symptoms of infection, as well as some prevention strategies. It is hoped that awareness can lead to a decrease in the severity and incidence of infection.

Preliminary Assessment of the Abundance of Indo-Pacific Crocodile (*Crocodylus porosus*) in Palawan, Philippines

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Abstract

The occurrence of remnant populations of the Indo-Pacific Crocodile on the main island of Palawan and associated small islands was reported by Ross in 1982 and Regoniel *et al.* in 1992. The present study is part of our effort to estimate the abundance of *C. porosus* in the country which could be useful in understanding the problem of potential conflicts between humans and crocodiles. Thirteen rivers in southern Palawan were surveyed from October 2014 to January 2016 using standard ground spotlighting and key informant interviews. Of the 124 crocodiles observed, an overall average corrected relative density of 0.23 individuals/km and 2.15 individuals/km were arrived for the mainland rivers and in rivers in small islands, respectively. The Canipaan River had the highest density of 0.55 individuals/km in the mainland rivers while Tukanigalo River and Bugsuk River in Balabac Islands recorded the highest densities at 1.26 individuals/km and 10.92 individuals./km, respectively, in the small islands. These areas had an estimated crocodile population of 346 crocodiles in intact crocodile habitats and had less dense human populations. However, in all the rivers studied, land conversion, mangrove debarking (resulting in death of mangrove trees), human persecution and occasional hunting by the increasing number of migrants have resulted in inevitable human and crocodile conflicts in all rivers studied. The island of Bugsuk, which has the largest viable breeding population of crocodiles ever recorded in the Philippines, is an exception. The establishment of the Critical Habitats (CH) on Palawan the mainland and associated islands under the Wildlife Resources Conservation and Protection Act of 2001 is strongly recommended to the Palawan Council for the Sustainable Development.

Introduction

The Indo-Pacific Crocodile (*Crocodylus porosus*) is widely distributed in the East and Southeast Asian Region and extends to Oceanian countries. In the Philippines, the populations of this species are still present but are considered as remnant in coastal estuarine areas to upstream rivers in four major island groups, Luzon, Visayas, Mindanao, and Palawan. A drastic and continuous decline of the Philippines indigenous population occurred in early 1940s to late 1980s caused by intense hunting for commercial skin trade.

The works of Ross in 1982 under the Smithsonian Institution/World Wildlife Fund - International (SI/WWF) Project have first recorded the remaining population of *C. porosus* in the Philippines including the island of Palawan. In the field works of Regoniel *et al.* (1992), the remnant populations were known to be distributed in areas with less human population and pristine wetland vegetation. According to Messel *et al.* (1992), *C. porosus* is found in exceedingly low numbers in some wetland habitats especially in Mindanao and Palawan.

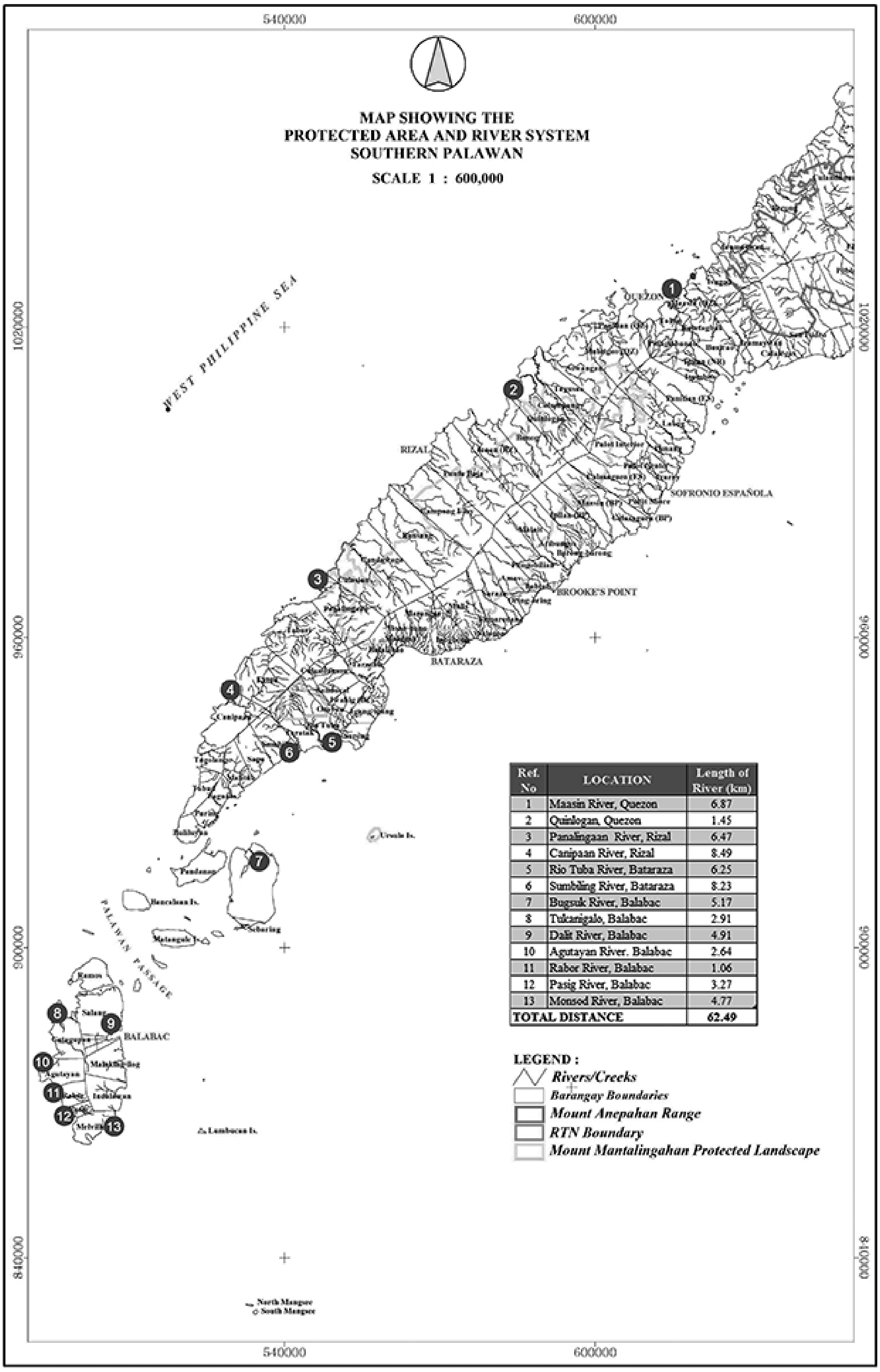
With its slow population recovery aggravated by human persecution and habitat loss, the Philippine Government listed *C. porosus* as Critically Endangered (CR) in its recent iteration of Philippine Red List of Threatened Species for terrestrial fauna in compliance with the Philippine Wildlife Resources Conservation and Protection Act of 2001. However, it is considered Lower Risk (LR) under the global IUCN Red List of Threatened Species being widespread in the Indo-Pacific Region. The Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES) also listed the species in Appendix I wherein the international trade of specimen is prohibited but close cycle farming is allowed in reference to global status.

Earlier studies on the existence of *C. porosus* in the country were focused on the distribution and status. Their results suggested that the population is severely depleted, fragmented and is under continuing threats because of habitat degradation and occasional hunting (Regoniel *et al.* 1992; Manalo 2004; Manalo *et al.* 2012), but there are no current population estimates to contend with. Thus, in order to complement with the other national conservation initiatives, the Crocodylus Porosus Philippines, Incorporated (CPPI) spearheaded this initial effort to determine the population density of *C. porosus* in the various parts of the Philippines including southern Palawan, the known stronghold of this species.

Methods

Study area

This study was conducted in major habitat of *C. porosus* in southern Palawan. The entire Province of Palawan is declared as Mangrove Swamp and Forest Reserve by virtue of the Presidential Proclamation No. 2152 in 1981 and a UNESCO Man and Biosphere Reserve (MAB) in 1991 in recognition of its rich landscapes and biodiversity and their significance to the Palaweños way of life. The mangrove forests of the province represent the few remaining old growth or pristine mangrove, accounting for about 22% (Long and Giri 2011) of mangrove cover in the country. Thirteen estuarine and coastal mainland and rivers in small islands in the municipalities of Quezon, Rizal, Bataraza and Balabac that are known to harbor considerable populations of *C. porosus* were surveyed. The map below lists and illustrates the rivers surveyed (Fig. 1).



Ref. No	LOCATION	Length of River (km)
1	Maasin River, Quezon	6.87
2	Quinlogan, Quezon	1.45
3	Panalingaan River, Rizal	6.47
4	Canipaan River, Rizal	8.49
5	Rio Tuba River, Bataraza	6.25
6	Sumbiling River, Bataraza	8.23
7	Bugsuk River, Balabac	5.17
8	Tukanigalo, Balabac	2.91
9	Dalit River, Balabac	4.91
10	Agutayan River. Balabac	2.64
11	Rabor River, Balabac	1.06
12	Pasig River, Balabac	3.27
13	Monsod River, Balabac	4.77
TOTAL DISTANCE		62.49

Figure 1. Locations of rivers surveyed in southern Palawan, Philippines, 2016.

Data collection and analysis

Standard spotlighting survey methods in a river transect cruise were employed to estimate the abundance of *C. porosus* in four municipalities of southern Palawan in October 2014, October 2015 and January 2016. Night surveys using a medium-size motorized boat proceeded either one-way downstream or upstream depending on tidal conditions. Each river and its tributaries were checked to spot-count crocodilian tapetal reflections according to the methods described by Magnusson (1980) and Graham (1988). Sighted crocodiles were approached closely and marked its actual positions in the waterways marked using a handheld Garmin E-trex Summit HC Global Positioning System (GPS) device. Distance of the rivers were measured based on the GPS navigation track logs calculated using the Garmin MapSource ver. 6.13.7 and verified with the Google Earth ver.7.1.2. Sizes of recorded crocodiles were estimated based on the 7 standard size classes (Table 1) adopted from Bayliss (1987). The abundance of crocodiles in terms of relative density was estimated and corrected using the correction factors adopted from Bayliss (1987).

Environmental issues and concerns in response to human-crocodile coexistence were noted, verified and recorded. The obtained data on human and crocodile conflicts (HCC) were compared to the Southeast Asia and Oceanian countries crocodile attacks reported in the CrocBITE Worldwide Crocodilian Attack Database, 2015.

Results and Discussion

There were 124 *C. porosus* observed in the 62.49 km of accumulated distance surveyed in 13 rivers, 6 of which are mainland rivers and 7 are rivers in small islands. A total of 7 hatchlings, 91 non-hatchlings were recorded while the rest were sighted as Eyes Only (EO). Non-hatchlings with size ranging from 1.0<3.0 m were observed frequently while those >3.0 were seen evenly distributed in all rivers. There were no crocodiles of about 0.5<1.0 m sighted during the surveys (Table 2). Spotlight counted individuals in all rivers surveyed accounted for only 37.38% of the obtained estimated population. This result was comparable with the finding of Bayliss *et al*. (1986) having 34% of the same species recorded in winding channels with thick vegetation.

Table 1. Size class classification of *C. porosus* and correction factor adopted from Bayliss (1987).

Class	Total Length (m)	Correction Factor
Hatchling	<0.5	1.44
1	0.5<1.0	1.34
2	1.0<1.5	1.30
3	1.5< 2.0	1.34
4	2.0<3.0	1.78
5	>3.0	3.08
6	EO (Eyes only)	6.54

Population densities

Survey results suggested that the observed density values ranged from 0.03 to 3.99 with an average density of 0.45 (±0.54) crocodiles/km. An overall average relative density of 0.09 individuals/km was recorded in mainland rivers covering a total distance of 37.76 km. This density calculation obtained a corrected value of 0.23 individuals/km, resulting in an estimated population of 51 individuals. Among these mainland rivers, Canipaan River recorded the highest corrected density of 0.55 individuals/km.

Table 2. Population densities and detection probability of *C. porosus* in southern Palawan based on spotlight count data.

Ref.	Location	Distance (km)	Size Class (m) (Bayliss 1987) <0.5	1.0>1.5	1.5<2.0	2.0<3.0	>3.0	EO	No. of Sightings	Relative Density (ind./km)	Corrected Density (ind./km)	Estimated Population	Detection Probability (%)
Mainland Rivers													
1	Maasin R., Quezon	6.87	-	-	1	-	1	-	2	0.05	0.12	4	50.00
2	Quinlogan, Quezon	1.45	-	-	-	-	1	-	1	0.03	0.08	3	33.33
3	Panalingaan R., Rizal	6.47	-	1	-	-	1	-	2	0.05	0.12	4	50.00
4	Canipaan R, Rizal	8.49	3	1	4	-	1	1	10	0.26	0.55	21	47.62
5	Rio Tuba R., Bataraza	6.25	-	-	-	-	2	-	2	0.05	0.16	6	33.33
6	Sumbiling R., Bataraza	8.23	-	1	-	1	1	1	4	0.11	0.34	13	30.77
Sub-total		37.76	3	3	5	1	7	2	21	0.09	0.23	51	40.84
Rivers on Small Islands													
7	Bugsuk R., Balabac	5.17	4	26	14	9	7	18	78	3.99	10.92	214	36.45
8	Tukanigalo, Balabac	2.91	-	-	-	3	2	2	7	0.36	1.26	25	28.00
9	Dalit R., Balabac	4.91	-	-	-	3	1	1	5	0.26	0.76	15	33.33
10	Agutayan R., Balabac	2.64	-	-	-	2	1	1	4	0.20	0.67	13	30.77
11	Rabor R., Balabac	1.06	-	-	-	1	2	-	3	0.15	0.41	8	37.50
12	Pasig R., Balabac	3.27	-	2	-	-	1	-	3	0.15	0.29	6	50.00
13	Monsod R., Balabac	4.77	-	1	-	-	-	2	3	0.15	0.74	14	21.43
Sub-total		24.73	4	29	14	18	14	24	103	0.75	2.15	294	33.93
Total		62.49	7	32	19	19	21	26	124	0.47	1.30	346	37.38

In Balabac Group of Islands, *C. porosus* populations were present mostly in the rivers of the islands of Balabac and Bugsuk. The Balabac area logged a comparably higher average density of 0.75 individuals/km in the 24.73 km distance covered. Their presence in Tukanigalo River in Balabac Island recorded a relative density of 0.36 individuals/km which resulted into a corrected density of 1.26 individuals/km. The occurrence of *C. porosus* in Bugsuk was mentioned in the study of Ross (1982) but no data was recorded in the island at that time except for a 2.8 m TL male *C. porosus* delivered to the Crocodile Farming Institute (CPI) in 1988 (Regoniel *et al*. 1992). In this present study, an exceptionally large population was noted in Bugsuk River, Bugsuk Island, Balabac. This single and integral population gave an average relative density of 3.99 individuals/km in 5.17 km of interconnected waterways. In this river, the obtained corrected density of 10.92 individuals/km thus provides an estimated population of 214 individuals. This value was higher than the recorded average densities of 2.40 and 2.73 individuals/km in the 4.4 km river obtained in the studies of Norazmi (2008) and Jet *et al*. (2011) in Kawang River, Sabah. Moreover, the overall corrected density for the rivers in the islands of Balabac was 2.15 individuals/km.

The study of Campbell *et al*. (2010), stated that *C. porosus* has the ability to cross significant marine barriers and colonised new habitats by riding surface currents. Thus, it has been inferred that the population of *C. porosus* in southern Palawan was connected with the population in Kudat District, Sabah, off the northeast coast of Borneo and probably in most of Sabah's rivers. The reported densities of Sabah rivers at 1.10 non-hatchlings/km (Stuebing *et al*. 2002) and of Brunei Bay at 0.33 individual/km (Cox 2006) were lower than the overall southern Palawan density (1.30 individual/km). Yet the Sabah Wildlife Department in 2002 reported that the number of crocodiles in some of their rivers have increased 10-fold in the last 20 years. It is thus highly probable given the close proximity of the islands and the unexplained increases in population density in the northeast coast of Borneo do share the crocodile population.

Environmental issues and concerns

The municipalities in southern Palawan, specifically Quezon, Rizal, Bataraza and Balabac, have been known to harbour viable population of *C. porosus* since well before this study. Regoniel *et al*. (1992) suggested that southern Palawan was the best area for remaining crocodiles, although it may not contain a large population and are likely to migrate if food resources were limited or if greatly disturbed. It was likewise observed that crocodiles thrived in areas with less dense human populations and with intact habitats. We also observed nests in secured areas but these were threatened with poaching.

It was noted in this study that all surveyed rivers were noteworthy habitats for *C. porosus*. These river systems, except Quinlogan River in Quezon and Bugsuk River in Balabac are currently classified as Core Zones or areas with strict protection under the Environmentally Critical Areas Network (ECAN) zoning scheme (Table 3). ECAN is a graded system of protection and development control implemented in the entire province of Palawan by virtue of Republic Act 7611 of the Strategic Environmental Plan (SEP) for Palawan Act in 1992. However, habitats of threatened species, as well as threatened habitats, including mangrove areas are categorized under coastal/marine Core Zone of PCSD Resolution 05-250 or the "PCSD resolution providing guidelines for the terrestrial and coastal/marine zoning."

With the exception to Bugsuk River, all of the surveyed rivers were not spared from habitat destruction and crocodile conflicts caused by human induced disturbances. Mangrove destruction such as debarking, timber poaching, and fuelwood gathering, has been of primary concern. Sopsop *et al*. (2004) indicated that the mangrove forests of the municipalities of Bataraza and Balabac have undergone extensive clearing for debarking activities which extends to mainland Palawan. It was likewise observed that the influx of migrants have tremendously contributed to the conversion of the crocodile habitats into agricultural use which eventually resulted to wildlife poaching. It was concluded by Manalo (2004), that the increasing human pressures in favorable habitats is a major threat for the probable presence of crocodiles.

Recorded direct conflicts with crocodiles were associated with human persecution and wildlife poaching. Our results showed that areas with high human induced pressure impart stress on both habitat and crocodiles, and resulted in human-crocodile conflict (HCC). However, it was noted that the HCC was absent only when human pressures on crocodile were also absent. CrocBite (2016) have recorded 26 cases of crocodile related incidents in the Philippines in 2000-2015, of which 20 incidents occurred in southern Palawan.

In the case of Bugsuk River, the presence of a highly dense (10.92 individuals/km) crocodile population in the marshland area that was free from human intervention was extremely remarkable. Rapid assessment of associated wildlife in the adjacent vegetation in water logged areas showed an abundance of rare faunal species such as the Philippine Giant Golden-crowned Flying Fox (*Acerodon jubatus*), Philippine Cockatoo (*Cacatua haematuropygia*), Palawan Hornbill (*Anthacoceros marchei*) and other species like Philippine Long-tailed Macaque (*Macaca facicularis*), Palawan porcupine (*Hystrix pumila*), Wandering whistling duck (*Dendrocygna arcuata*), Blue-naped parrot (*Tanygnathus lucionensis*), among other wild fauna. This converted marshland served as a ready source of prey items for the *C. porosus* population. Among these species are the large number of roosting *Acerodon jubatus* and *Dendrocygna arcuata* together with freshwater fishes like milkfish, tilapia, carps and mudfish. The presence of these species of wildlife indicates that the entire habitat is intact but could be vulnerable to natural degradation if good management is not in place.

Table 3. Issues and concerns in response to human-crocodile conflict (HCC).

Ref.	Location	Protection Status	Issues and Concerns	HCC
Mainland Rivers				
1	Maasin R., Quezon	ECAN Core Zone	Habitat: continued arrival of immigrant Crocodile: human persecution	Yes
2	Quinlogan, Quezon	ECAN Sustainable Use Zone	Habitat: conversion into agriculture Crocodile: human persecution	Yes
3	Panalingaan R., Rizal	ECAN Core Zone; within MMPL	Habitat: mangrove conversion, fuelwood gathering Crocodile: human persecution	Yes
4	Canipaan R., Rizal	ECAN Core Zone	Habitat: timber poaching, mangrove debarking Crocodile: human persecution	Yes
5	Rio Tuba R., Bataraza	ECAN Core Zone	Habitat: mangrove debarking, fuelwood gathering Crocodiles: human persecution	Yes
6	Sumbiling R., Bataraza	ECAN Core Zone	Habitat: conversion into agriculture Crocodiles: wildlife trade	Yes
Rivers in Small Islands				
7	Bugsuk R., Balabac	ECAN Sustainable Use; Privately Managed	Habitat: none Crocodiles: none	No
8	Tukanigalo, Balabac	ECAN Core Zone	Habitat: mangrove debarking, dynamite fishing, fuelwood gathering Crocodiles: wildlife trade	Yes
9	Dalit R., Balabac	ECAN Core Zone	Habitat: human encroachment Crocodiles: None	No
10	Agutayan R., Balabac	ECAN Core Zone	Habitat: Continued arrival of immigrant Crocodiles: None	No
11	Rabor R., Balabac	ECAN Core Zone	Habitat: mangrove conversion, fuelwood gathering Crocodiles: none	No
12	Pasig R., Balabac	ECAN Core Zone	Habitat: timber poaching, mangrove destruction Crocodiles: human persecution	Yes
13	Monsod R., Balabac	ECAN Core Zone	Habitat: conversion into agriculture Crocodiles: none	No

Conclusions and Recommendations

A stable wild population of *C. porosus* is distributed in four municipalities within the southern Palawan region. Rivers in small islands have recorded a higher density compared to those in mainland Palawan. Several large numbers of crocodiles with densities exceeding 0.50 individuals/km were recorded in areas with less human populations. However, viable breeding populations were observed in less dense rivers, as shown by the presence of nests in these areas. The identification and proclamation of crocodile habitats are appropriate at this point but needs immediate conservation management actions to support them. Human pressures on habitat (mangrove destruction and land conversion) and on crocodiles (human persecution and wildlife trade) remain to be the main drivers of *C. porosus* HCC. But the presence of HCC is related on the occurrence of human pressures on crocodiles. Therefore, the absence of these human pressures (both on habitat and crocodiles) in the privately managed Bugsuk Island favors crocodile populations to have the highest density in the entire Philippines.

Thus it is recommended that: 1. a study on the population dynamics in crocodile habitats must be conducted to further understand the drivers of biological and ecological processes involved; 2. there is a need for the Palawan local government to establish protocols to address and mitigate HCC in order to promote human benefits from crocodiles such as enhancement of fisheries; and, 3. the establishment of support mechanisms for protection such as declaration of areas as Critical Habitat

(CH) under the Wildlife Resources Conservation and Protection Act of 2001 is strongly recommended for areas with relatively high *C. porosus* density. The Palawan Council for Sustainable Development, which has the mandate for this conservation, is strongly urged to act positively on these recommendations.

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Educating the Public and Promoting Community Participation in Crocodile Conservation: An Overview of Efforts around the World

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Abstract

Crocodilian conservation starts with communication. A Public Education and Community Participation (PECP) campaign aims to mobilize, motivate and sustain individual and collective crocodile conservation action. In the case of potentially dangerous animals such as crocodiles, communication campaigns are also of great importance to minimize risks of crocodile attacks on people and livestock. In various countries and targeting various species, individual and group efforts are being exerted to educate the public, develop positive attitudes and mobilize community participation in crocodile conservation. This paper aims to present an overview of the outputs, cognitive and affective outcomes, and impacts of different crocodilian PECP campaigns reported from the different parts of the world. The importance of documenting, monitoring and evaluating the impact of different PECP activities is discussed. A theory of change is presented as a basis for PECP campaigns to increase community support for and participation in crocodilian conservation. Learning from lessons generated from different situations and experiences will help improve the effectiveness of PECP campaigns.

Public Education and Community Participation (PECP) in Philippine Crocodile Conservation in Northern Philippines

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Abstract

The Philippine crocodile (*Crocodylus mindorensis*) is one of the rarest species on the planet with an estimated population of less than 250 mature individuals in the wild. Endemic to the Philippines, this freshwater species only survives in isolated wetlands in Mindanao and northern Luzon. Public opinion about crocodiles has been influenced by popular media and most people now regard crocodiles as dangerous animals and a pest. Traditionally Filipinos had high regard for crocodiles and crocodiles played an important role in Philippine culture, and still do in several indigenous belief systems. But generally, although nationally protected, Philippine crocodiles are killed without repercussions and crocodile habitat is converted. In 1999, a remnant wild population of Philippine crocodiles was discovered in San Mariano, Isabela Province. A Communication, Education and Public Awareness (CEPA) Campaign aimed to increase the support for, and participation in, Philippine crocodile conservation here. This campaign has led to the establishment of eight locally declared Philippine crocodile sanctuaries and an increase of the Philippine crocodile population of less than 20 non-hatchlings in 2000 to more than 80 in 2015 in an area that also showed a strong increase of the human population in the same time period. Evaluations of the CEPA campaign in 2008 and 2013 showed that a majority of people in crocodile areas supported Philippine crocodile conservation (80%), compared to only 20% in control areas. CEPA campaigns and efforts to increase community participation in crocodile conservation remain very important to sustain the community-based Philippine crocodile conservation program in northern Luzon.

How Important is Spatial Information to Preserve Crocodylians? The Crocs Geo-Visor Initiative as a Conservation Tool

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Abstract

As apex predators in their respective ecosystems, crocodylians (alligators, caimans, crocodiles, and Gharials) are facing on increased risk of extinction due to habitat encroachment by human settlements and a general lack of appreciation for their value to the ecosystem. In combination, these two major problems are reducing and shrinking crocodylian ranges across the world. Currently, the majority of crocodylian species are protected at some level (local and/or regional scales), however, to date we have ten out of 23 listed species that are vulnerable, endangered or critically endangered. Two important aspects used to assess risk of extinction are range (its extension and variation through time) and habitat status (quality) of each species. Unfortunately, precise range information (both current and historical) is lacking for the majority of species, making it impossible to accurately measure the actual risk of extinction. One set of factors that contributes to problems with these analyses is the lack of spatial and temporal information that will be required to characterize the species. Based on these requirements, we have created the Crocs Geo-Visor Initiative as a way to collect spatial data under established protocols with a primary goal of developing a curated spatial database for crocodylians around the world. We also created a mobile application for data collection (Crocodylians of the World) in order to encourage participation of citizen-scientists, and to be able to readily easy test data for uncertainty. During the three months since releasing the Crocs Geo-Visor Initiative we have 5510 geolocations from 6 species, representing 16 countries. These have been gathered and curated from current online resources, external authors, and metadata analyses. Data entries range from 1970 to 2015 with a significant concentration of geolocations coming from the period 2000-2010. We identify and discuss some common errors in how researchers handle geographic information, which increases the uncertainty of the data and makes further analyses and conclusive results problematic. Data collected to date can be used to estimate two species ranges (the American and Orinoco crocodiles) in ways we have not done before, which will help improving the accuracy of data used to estimate the risk of extinctions.

Introduction

Crocodylians, as well as many other top predators, are facing a greater risk of extinction due to habitat encroachment by human settlements. In addition, there is an overall cultural de-attachment by so-called civilized peoples regarding the animal populations with which they share the planet (Thorbjarnarson 2010; Balaguera-Reina and Gonzalez-Maya 2010; Balaguera-Reina 2012). These societal ideologies manifest as a general lack of appreciation for the importance of other species, particularly large, charismatic species like crocodylians. Indifferent attitudes towards conservation are inadvertently reducing population numbers and shrinking crocodylian ranges around the world. Exploitation has been the status quo throughout the historical interaction of humans and crocodylians since European colonies settled around the world (Medem 1981). Nevertheless, actions have been taken in the last five decades to shift the vision to more sustainable models (Web 2014). Currently, the majority of crocodilian species are afforded some level of protection (eg at the local and/or regional scales) (Thorbjarnarson 2010), however, to date only some species are listed under the seriously threatened categories (six as critical endangered, one as endangered and three as vulnerable; IUCN 2016).

Two important aspects that have been used to assess risk of species extinction are range (its extension and variation through time) and habitat status (ie quality) (IUCN 2016). The measurements of either one of these variables are totally dependent on the other (ie habitat from range in terms of land-cover areas as well as the amount of preserved vs not preserved habitat). Unfortunately, precise range information (both actual and historical) is missing for the majority of species, precluding accurate estimation of the actual risk of extinction. On the other hand, current spatial analyses about range dynamics derived from stochastic and anthropogenic impacts are almost non-existent for the Crocodylia, increasing the uncertainty about how effective existing conservation efforts are. One serious impediment to developing these kinds of analyses is the lack of continuous spatial and temporal information about the species. This implies that there is a paucity of historical data available for comparison to determine whether crocodylians' ranges have been and are being impacted by, for example, stochastic climatic or habitat changes or man-made infrastructure (eg settlements or land-use). Both impacts require different approaches if we want to pursue sound and effective conservation plans for those species.

Collections and museums have served as the primary repositories of biological information for centuries, keeping track of knowledge that has been previously discovered and continuing to add new information as science advances and methodologies evolve (AAM 2008). Today museums are getting more actively involved in the use of new technologies to collect data (AAM 2008). Technologies such as GPS, 'smart phones', and iPads among others have become a valuable means to collect data since they are used all over the world as gadgets that we use in our daily lives, generating large amounts of information about people and their surroundings (Bowen and Pistilli 2012). The use of these gadgets to collect relevant information has been shown to be useful in various natural science fields, essentially using citizen-scientists as recruits for common and easily performed observations and tasks (Dickinson *et al*. 2010). However, concerns have been raised about the quality of these data (Silvertown 2009; Mackechnie *et al*. 2011). It is here where modern data management techniques for collecting and preserving information in a scientific manner must be employed. Because of the need to have such resources, we created the Crocs Geo-Visor Initiative as a way to collect spatial data under established protocols with the ultimate goal to be able to offer a curated spatial database for crocodylians around the world and thus, preserving these data for future generations.

We also created a mobile application for data collection (Crocodylians of the World) in order to encourage participation of citizen-scientists and generate readily testable data for uncertainty. Therefore, the aim of this manuscript was to describe the initiative as well as the methods used to curate the spatial data being contributed by the general public (as citizen-scientists) and researchers. We also present a preliminary compilation of the data collected over the first three months since the release of both the initiative and the mobile application, highlighting how these resources can be used for conservation purposes.
The Crocs-Geo-Visor Initiative, what is it all about?

The Crocs Geo-Visor Initiative is a geographical/user-friendly database which is focused on gathering continuous spatial information about crocodylian sightings, nests, and conflicts as an effort to share easily collected, yet crucial data for use in the formulation of conservation and management plans. This platform allows researchers, decision makers, and even "croc-lovers" to visualize and analyze spatial data. This geodatabase is completely free-access, with no charge to use or submit the information.

The collected data are submitted directly by authors, reviews of metadata, and papers published with geospatial information. Each geolocation is treated as an individual specimen included in a collection, with the submission being credited to an author (or authors) (Pastore 2009). Other types of information are also collected such as: type of data (sightings, nests, or conflict), species name, publication year, site, date when data were collected, specific locality, collection method, country, habitat, and general comments.

This interactive platform was created using ArcGIS Online services and server (ESRI 2016) using the Texas Tech University (TTU) license as well as the server of the Department of Biological Sciences at TTU. The spatial database was divided by species as feature services (the way it is named in ESRI) in order to keep data collection consistent. All spatial data were hosted on ESRI servers under coding protection, following the ESRI code of conduct (ESRI 2016). The landing page to visualize this information on the web was created using Microsoft Expression Web (Microsoft 2010) and hosted in the server of the Department of Biological Sciences at TTU.

Crocodylians of the world mobile application

Under the slogan "Take a nice picture of the crocodylian in front of you and help us to preserve them! Be a citizen-scientist and share your knowledge with the world!", this mobile application is a strategy to get people closer to these animals in a more scientific-manner, while including them in the collection of vital information for crocodylian conservation assessments. This quick report allows the general public's submission to include 7 variables: picture of the specimen, geolocation, author, year, species name (as either the scientific or common name), location, date, and general comments. Of these, the photograph of the specimen, its geolocation, and species name, are critical to insuring the value of the data. The picture is assessed using diagnostic features per species that allow us to verify that the identification (scientific or common name) was accurate. Any photographs that do not allow us to make a clear diagnosis of the species will be not included in the database. Geolocation information is taken directly from the mobile device used to make the report and is compared with the location reported by the user. This information is also collected using the ArcGIS Online services and server, as well as the server of the Department of Biological Sciences at TTU. The native application was created using app studio for GIS (ESRI 2016) and is currently available for Android Technology.

Results and Discussion

During the three months since releasing the Crocs Geo-Visor Initiative we have recorded 5510 geolocations (see Table 1) from 6 species, representing 16 countries. These have been gathered and curated from current online resources, external authors, and metadata analyses. The six species are: American crocodile (*Crocodylus acutus*), Orinoco crocodile (*C. intermedius*), Spectacled caiman (*Caiman crocodilus*), Yacare caiman (*C. yacare*), Black caiman (*Melanosuchus niger*), and Schneider's Smooth-fronted Caiman (*Paleosuchus trigonatus*); all of which inhabit the Neotropics. The 16 countries include the USA, Mexico, Belize, Honduras, Nicaragua, Costa Rica, Panama, Colombia, Venezuela, Ecuador, Peru, and Bolivia on the mainland and Cuba, Jamaica. Haiti, and Dominican Republic on insular areas covering North, Central and South America.

Table 1. Number of observation hosted to date per species on the Crocs Geo-Visor Initiative.

Species	Sightings	Nests	Conflicts	Total
American crocodile (*Crocodylus acutus*)	1342	0	7	1349
Orinoco crocodile (*Crocodylus intermedius*)	1234	100	0	1334
Spectacled caiman (*Caiman crocodilus*)	1000	0	0	1000
Yacare caiman (*Caiman yacare*)	337	1228	0	1565
Black caiman (*Melanosuchus niger*)	120	140	1	261
Schneider's Smooth-fronted Caiman (*Paleosuchus trigonatus*)	1	0	0	1
Total	4034	1468	8	5510

The majority of data hosted come from sightings with a small number of nests and conflicts geolocations (Table 1). The Yacare caiman and the American crocodile have the largest number of geo-references, followed by the Orinoco crocodile and the Spectacled caiman. Data range from 1970 to 2015 with a largest number of geolocations coming from the 2000-2010 period.

A total of 15 authors and two institutions from three countries have submitted information to date. One database derived from the regional habitat conservation priorities analyzed for the American crocodile by Thorbjarnarson *et al*. (2006) hosted in databasin (www.databasin.org) was also curated, included, and referenced in this initiative. We also curated and included information from 13 manuscripts and technical reports. All these data can be reviewed and analysed using the Crocs Geo-Visor Initiative portal (http://www.faculty.biol.ttu.edu/densmore/CrocsGeoVisorInitiative.html).

Concerning the mobile application, we have had 27 downloads of the software in three countries and one record included coming from Venezuela, which was verified and curated. We presume that the small number of reports is due mainly to the lack of publicity we have given the app, as we are still de-bugging software malfunctions. We are anticipating to have the app completely functional by July 2016 and available for both, Apple and Android, increasing the number of reports from both professional scientists and the general public.

Overall, after three months of hard work the initiative has proven to be a valuable way to preserve and keep track of spatial information under standardized protocols, thus increasing our confidence in the data. One common error that commonly popped up during curation was the lack of standardized procedures for the GPS collected spatial information by different researchers. Defining the datum (geolocation) uncertainty is an important part of spatial data collection, which in general, is not included. It is also important to collect the datum as close as safely possible from the animal to avoid misleading habitat analyses. If the field method does not allow that (ie tracking crocodiles from land), the geolocation should be collected including both the azimuth and distance to the animal georeferenced. This method allows spatial corrections to be defined with an uncertainty value, to insure that the animal was accurately located. Nevertheless, data collected until now might be used to estimate species ranges (mainly for American and Orinoco crocodiles) in ways we have not done before, which can increase the accuracy of data included in estimating the risk of extinction. This database is also an easy way to create synergies among researchers and citizen-scientists, to better provide insights about where the animals are and about their range dynamics.

Scientists cannot possibly monitor the presence/absence of crocodiles all the time, all around the world, however, people who live within the probable ranges can do so. These are the circumstances when a citizen-scientist's report and interpretation will be valuable (Battersby and Greenwood 2004). We expect that this initiative will serve as a bridge between the scientific community and people that are living and working around crocodylians sharing one common goal, to guarantee the survival of the animals, as well as the ecosystems where they are living in. Further analyses will be conducted in collaboration with all authors who provide information to the initiative in order to better approximate the current and historical ranges of both the American and Orinoco crocodiles.

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Using Citizen Scientists to Monitor Mugger Crocodile Population and Threats: Implications for Conservation of Crocodiles

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Abstract

Long-term monitoring studies designed to survey large areas often face the challenge of large scale data collection due to limited resources, both funds and man power. Researchers can overcome this hurdle by utilizing the skills of volunteers, or citizen scientists. Citizen science - public participation of non-scientific personnel in scientific research - has become an important tool for monitoring and evaluating environmental change. Citizen scientists currently play active roles in a wide range of environmental monitoring projects, and their contributions have enabled the researchers to collect large scale information at minimal cost. One such citizen science program, the 'Charotar Crocodile Count' initiative by Voluntary Nature Conservancy, has successfully utilized volunteer to collect information on the distribution and abundance of Mugger crocodiles (*Crocodylus palustris*) and human-crocodile relations across the agricultural landscapes of Charotar region in Gujarat, India. We assessed the reliability of the population count done by the citizen scientists with the data collected by the research team of Voluntary Nature Conservancy. We also examined the individual-level motivations and experiences of citizen scientists participating in Charotar Crocodile Count and impacts of their participation on the conservation of crocodiles. Our assessments suggest that population data collected by citizen scientists were very much reliable and could be used for management purpose as well. The major motivations for joining such program were to gain skills in wildlife monitoring through participation, an opportunity to observe crocodiles in wild and contributing to crocodile conservation. Our assessment suggests that citizen science initiatives while increasing the scientific literacy among the general public, enable large-scale data collection, help monitor the status of wildlife populations and could lead to positive impacts on biodiversity.

Next Gen Croc: Cultivating the Next Generation of Crocodilian Biologists

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Abstract

Citizen science is an emerging field harnessing the power of the public or amateur scientists to assist in the conservation of biodiversity. Projects incorporating citizen scientists has substantially contributed to the fields of ecology and conservation in addition to positively increasing the awareness and support of the community in regards to wildlife conservation. In an effort to develop wildlife awareness and foster the new generation of conservationists and crocodilian biologists in Belize, we have developed the Next Gen Croc Tours on Caye Caulker, Belize, in which local high school students will lead nocturnal mangrove and croc tours. In addition to educating tourists and locals about crocodiles and their environment, these tours will include performing official monthly, nocturnal eyeshine surveys to assist in the monitoring of the local population of *Crocodylus acutus* (American crocodile). We anticipate the creation of this eco-tourism project with student involvement will provide the young participants a new appreciation for their local wildlife and environment in concomitance with improving their scientific, leadership and communication skills, while simultaneously assisting them financially in their education. Moreover, the continual monitoring of the crocodile population will assist the Belize Forest Department in management efforts of the local *C. acutus* population, as well as provide new insight in the regional dispersal, habitat preference, and ontogenetic habitat shifts of *C. acutus*.

Beauty is Skin Deep: Use of Histology to Study Crocodile Skin Blemishes

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Abstract

In order to meet increasingly stringent standards for premium quality crocodile skins, for the past several years, we have been conducting research aimed at determining which blemishes in the raw skin will result in blemishes in leather, tracking healing rates for blemishes, and investigating the microscopic (histological) characteristics of blemishes. For histology, we used the standard stain combination of haematoxylin and eosin, as well as trialed several special histochemical stains aimed at enhancing visualisation of the various features of blemishes. Haematoxylin and eosin (HE) was essential as the basic stain, to show the architectural and cellular detail as it is usually represented to pathologists. In addition, with HE stain, chromatophores in the superficial dermis remained intact, and their visualisation could be enhanced using polarized light. This resulted in the discovery that lucencies visible on the light table were due to a relative paucity of chromatophores in raw skin blemishes.

Various methods were investigated to enhance the staining of collagen, which was of interest since it is all that remains of the raw skin after processing to leather. Picrosirius red (PSR) only stains collagen, and with polarised light, the dermal pattern of collagen bundles and any alterations in them in blemishes, were strikingly displayed. However, Masson's trichrome stain was found to stain collagen sufficiently delicately to rival PSR, and had the advantage of also staining the other tissue and cellular elements with good differentiation. Using these stains, obvious alterations in collagen bundle density and organization were appreciable in blemishes, providing a structural reason for the visibility of the blemishes in leather. The corollary of staining collagen, the substance remaining in processed leather, is staining ground substance, the substance between collagen bundles that is removed in processing. For this, we found alcian blue stain for acidic mucins of glycosaminoglycans with periodic acid-Schiff (PAS) counterstain to be very useful. The amount of ground substance in blemishes frequently appeared increased compared to normal dermis, which, since ground substance is removed in processing, likely explains why many blemishes are slightly depressed in processed leather. Use of PAS had the advantage of also demonstrating any alterations in the basement membrane, since it stains the neutral mucins that are a component of this structure. We investigated possible changes in blemishes of other connective tissue elements such as elastin and reticulin with Verhoeff's and Gordon and Sweet's stains, respectively, but did not find significant alterations in these substances in blemishes versus normal dermis.

The Code Size: Behavioural Response of Crocodile Mothers to Offspring Calls Depends on the Emitter's Size, Not on its Species Identity

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Abstract

Maternal care is widespread in crocodilians with mothers sometimes protecting juveniles from predation and cannibalism. Yet, our knowledge on this behaviour remains scant, and we still know very little about the factors that can modulate the mother's behavioural reaction to offspring calls. Here we compare the acoustic structure of distress calls emitted by juvenile crocodilians from different species of both Crocodylidae and Alligatoridae and demonstrate that these calls bear information about the emitter's size. Additionally, playback experiments show that Nile crocodile *Crocodylus niloticus* mothers react more to vocalizations of smaller juveniles. Finally, playback experiments on Orinoco crocodiles *Crocodylus intermedius* and spectacled caimans *Caiman crocodilus* reveal that females react more to calls of smaller hatchlings than to the calls of larger juveniles even if they belong to a different species. Crocodilian females thus pay more attention to the size code than to the species identity code carried by the juvenile's calls. These results suggest that size information outweighs species identity information in crocodilian mother-offspring communication. Larger juveniles need less maternal care, and could even represent a risk of cannibalism for younger offspring. Also, the probability of providing allo-specific maternal care is very low as different crocodilian species rarely breed in the same micro-habitat, thus relaxing the evolutionary constraint leading on maternal ability to recognize juveniles from another species.

Introduction

Investigations on mother-offspring acoustic communication in Crocodilians have demonstrated that newborn and juvenile calls support information that is relevant to both the mother and siblings (Young *et al*. 2014). Thus, females guarding their nest react to the calls of near-term embryos by providing assistance during the hatching process (Pooley 1974, 1977; Vergne and Mathevon 2008; Somaweera *et al*. 2013). Later, vocalizations continue to mediate mother-juvenile interactions: when young are seized by a predator, they emit "distress" calls that attract the mother (Staton 1978; Vergne *et al*. 2011). Similarly, newborn vocalizations mediate hatching coordination (Vergne and Mathevon 2009) and later, group cohesion (Vergne *et al*. 2011). Crocodilians and birds are the modern representatives of archosaurs, a monophyletic group that also includes the extinct dinosaurs and pterosaurs (Janke and Arnasson 1997; Grigg and Kirshner 2015). Parental care appears as a shared characteristics among archosaurs. However, our knowledge on mother-offspring communication in Crocodilians remains scant compared to what we know in birds. Specifically, there is a gap of knowledge related to how juvenile acoustic signals modulate maternal behaviour.

The aim of the present study is to characterize the growth-induced modifications of juvenile vocalizations in crocodilians, and to investigate the potential effect of these changes on maternal response. Indeed, we hypothetized that the acoustic structure of juvenile calls should change with juvenile's growth, and that these modifications might provide information modulating the mother's behaviour. We recorded individuals of several species belonging to Alligatoridae and Crocodylidae, and assessed the effect of body size on the call acoustic structure. Using playback experiments, we further tested the reaction of Nile crocodile females to calls from juveniles of different sizes. Finally, we challenged female Orinoco crocodile and spectacled caimans with calls from juveniles of the other species and of different sizes. Here we report that size-related information embedded in the calls of juvenile crocodilians modulates maternal care and that this information can be more relevant to mothers than species identity.

Methods

<u>Acoustic analysis</u>

We recorded calls of juvenile American alligator *Alligator mississipiensis*, Nile crocodile *Crocodylus niloticus*, spectacled caiman *Caiman crocodilus*, and Orinoco crocodile *C. intermedius* (between 1 and 20 calls/individual). Calls were emitted spontaneously when animals were hand captured (recording material: SCHOEPS MK4 cardioid microphone; NAGRA-LB recorder). Acoustic analyses were performed with Seewave R package (Sueur *et al.* 2008) and PRAAT software (Boersma and Weenink 2012). The calls' acoustic structure was characterized using a set of 13 acoustic features describing both the distribution of energy among the frequency spectrum and the call pitch. We calculated the following spectrum properties within a 0-5 kHz bandwidth: the mode (maximal frequency) of the frequency spectrum, the first quartile of energy (Q25), ie the frequency value corresponding to 25% of the total energy spectrum, the third quartile of energy (Q75), ie the frequency value corresponding to 75% of the total energy spectrum, the interquartile range (IQR), ie the difference between Q75 and Q25, the centroid of the frequency spectrum (cent), the skewness, a measure of spectrum asymetry, the kurtosis, a measure of spectrum peakedness, the spectral flatness (sfm), ie the ratio between the geometric mean and the arithmetic mean of the spectrum (this ratio can vary between 0 and 1, with sfm of a noisy sound tending towards 1, and sfm of a pure tone signal tending towards 0). The F0 contour was then extracted under PRAAT and used to derive the following parameters: the F0 value at the beginning of the call (start pitch), the maximum and minimum F0 values (max pitch and min pitch), the F0 mean value (mean pitch), and the F0 value at the end of the call (end pitch). We used a multivariate principal component analysis to reduce the 13 non-independent acoustic parameters described above into two independent Acoustic Dimensions (AD1 and AD2). We further used linear mixed effect models with AD1 and AD2 as dependent measures (fixed effects: individual size, crocodilian species, and interaction between size and species; random effect: individual identity; package lme4, R version 3.1.2).

Playback experiments on female Nile crocodiles

Female Nile crocodiles (N= 9; Okavango Delta, Botswana) were challenged by playing calls emitted by small (total body length 28-36 cm) and large (63.5-98 cm) juvenile Nile crocodiles. Playbacks were conducted during the peak hatching period, on wild animals. Eight out of the 9 females were tested at night. Prior to playback, the position of the female and of the nest were assessed and a remote-controlled amplified loudspeaker (FoxPro Fury©) was positioned on the river bank close to the nest. The observers remained in a boat at 30-50 m from the loudspeaker and the female. One female was tested during the day, from land, since her nest was inaccessible by boat. In this case, we positioned the loudspeaker on the water edge, near the nest, and a single observer remained 15 m away hidden by vegetation. All females were tested while they were in the water, at 10-30 m from the nest and thus from the loudspeaker. After 10-30 minutes of silence, we played the experimental signals. Each female was tested with calls from different juveniles. Calls were played back at a natural intensity (intensity level: 63 ± 5 dBSPL at 1 m from the loudspeaker). The following ethological intensity scale was used to quantify the females' responses: +2 = approach towards the loudspeaker (more than half the distance between the loudspeaker and the female's initial position); +1 = partial approach (less than half the distance between the loudspeaker and the female's initial position); 0 = no response; -1 = small retreat (less than 5 m); -2 = strong retreat (more than 5 meters). To test for a significant effect of the category of stimulus ("small" juvenile versus "large" juvenile), a linear mixed effect model was used with the reaction intensity as the dependent measure (fixed effect: category of stimulus ie calls from "small" or "large" individuals; random effects: nest identity, playback order).

Playback experiments on female Orinoco crocodiles and spectacled caimans

Female Orinoco crocodiles (captive individuals; Hato Masaguaral, Venezuela) and female spectacled caimans (wild, free-ranging individuals; Hato Masaguaral Venezuela) were tested by playing back calls emitted by juvenile Orinoco crocodiles (large individuals, total length between 40-60 cm), juvenile spectacled caimans (small individuals, total length between 20-30 cm) and juvenile black caimans *Melanosuchus niger* (small individuals, total length between 20-30 cm). Each playback series was composed of 30-35 successive calls (total duration = 1 minute). Playbacks were conducted during the day, in February 2013. The Orinoco females had made their nest around 1 month prior to experimental testing, while the spectacled caiman females were either still attending their young hatchlings or had abandoned them and had gathered with some other adults (during the dry season at Hato Masaguaral, spectacled caimans gather together on the remaining ponds). For the experiments with the captive Orinoco females, we positioned the loudspeaker on the nest at a moment when the female was in the water. Each female (N= 7) was successively tested with three experimental signals, on different days (calls from a juvenile Orinoco crocodile, a juvenile spectacled caiman, and a juvenile black caiman; each female was tested with recordings from different individuals; signals emitted in a balanced order between females). The following ethological intensity scale was used to quantify the females' responses: +3 = approach towards the loudspeaker (at less than 1 m, meaning that the female climbed on her nest); +2 = partial approach towards the loudspeaker (remaining at more than 2 m from the loudspeaker, meaning that the female approached the nest by swimming but stayed in the water); +1 = orientation towards the loudspeaker (no approach); 0 = no reaction. To test for a significant effect of the category of stimulus, a linear mixed effect model was used with the reaction intensity as the dependent measure (fixed effect: category of stimulus; random effects: female identity, playback order).

For the experiments with the free-ranging spectacled caimans, we positioned the loudspeaker on the water edge of ponds where at least one female was present (N= 18 locations on different ponds; between 1 and >10 individuals on each pond; closest individual at more than 20 m from the loudspeaker). At each location we played back the three experimental signals during three separated playback sessions, on different days (calls from a juvenile Orinoco crocodile, a juvenile spectacled caiman, and a juvenile black caiman; each location was tested with recordings from different individuals; signals emitted in a balanced order between locations). As it was not possible to assess the behaviour of a single particular female, a simple measurement of behavioural response was used by counting how many females approached towards the loudpspeaker during the playback and one minute after the playback. To test for a significant effect of the category of stimulus, a linear mixed effect model was performed with the number of females approaching the loudspeaker as the dependent measure (fixed effect: category of stimulus; random effects: location, playback order).

Ethical note

The experimental protocol in the Okavango was approved by the Ministry of Environment, Wildlife and Tourism of Botswana (Permit no. EWT 8/36/4 XXVI). The experimental protocol in Venezuela was approved by the Hato Masaguaral. The experimental protocol in captivity was approved by the Institutional Animal Ethical Committee of the University of Saint-Etienne (Authorization no. 42-218-0901SV09).

Results

Body size influences call acoustic structure

The acoustic structure of juvenile calls depended on the size of recorded individuals (Fig. 1; LME on AD1 scores with individual size as a fixed effect: χ^2= 52.2, df= 1, P<0.0001; AD2 scores: χ^2= 83.60, df= 1, P<0.0001). Calls of larger juveniles showed more energy in the lower part of their frequency spectrum than calls from smaller individuals while calls from smaller individuals were higher pitched. In addition to demonstrating the importance of individual size as a factor explaining call structure, the comparison between juvenile calls underlined differences between crocodilian species (LME on AD1 scores with species identity as a fixed effect: χ^2= 322, df= 4, P<0.0001; AD2 scores: χ^2 = 61.6, df= 4, P<0.0001).

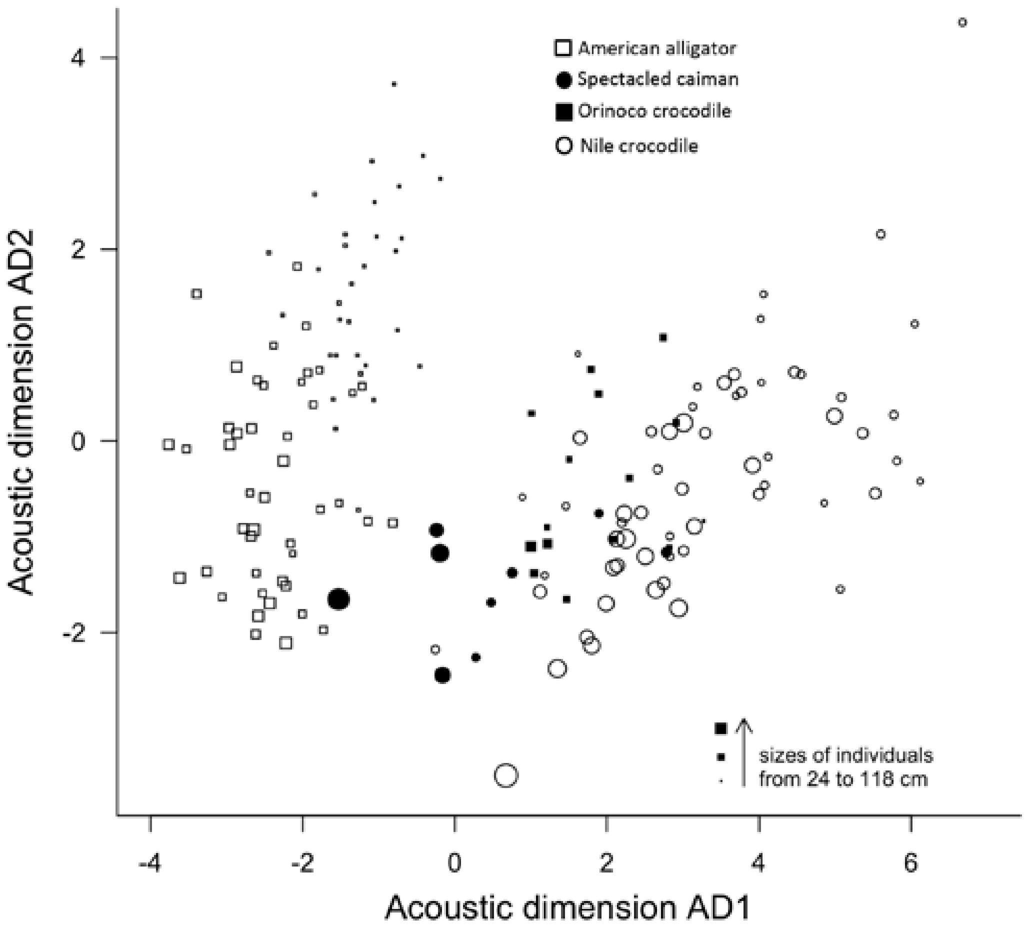

Figure 1. Distribution of juvenile calls in the acoustic space.

Calls from smaller juveniles are more attractive whatever their species

When tested with calls from juveniles of their own species, female Nile crocodiles reacted differently depending on the call's size category (GLM: χ^2= 5.53, df= 1, P= 0.019, N= 9). In response to calls from hatchlings, females moved more towards the loudspeaker. Conversely, calls from large juveniles hardly elicited an approach. These results demonstrate that female Nile crocodiles pay attention to the size information embedded in the juvenile calls and react more to smaller individuals. When tested with calls from juvenile Orinoco crocodiles, juvenile spectacled caimans, and juvenile black caimans, female Orinoco crocodiles reacted differently depending on the experimental signal (GLM: χ^2= 8.79, d = 2, P= 0.012, N= 7; Figure 2). Specifically, they reacted more to calls from spectacled caimans or black caimans than to calls from Orinoco crocodiles (post-hoc test multiple comparison tests: reaction to spectacled caiman calls versus reaction to Orinoco crocodile calls: Z= -2.54, P= 0.030, N= 7; reaction to black caiman calls versus reaction to Orinoco crocodile calls: Z= -3.26, P= 0.003, N= 7; Fig. 2). Conversely, their behavioural reactions towards spectacled caiman calls and black caiman calls were not significantly different (Z= -0.725, P= 0.749, N= 7; Fig. 2).

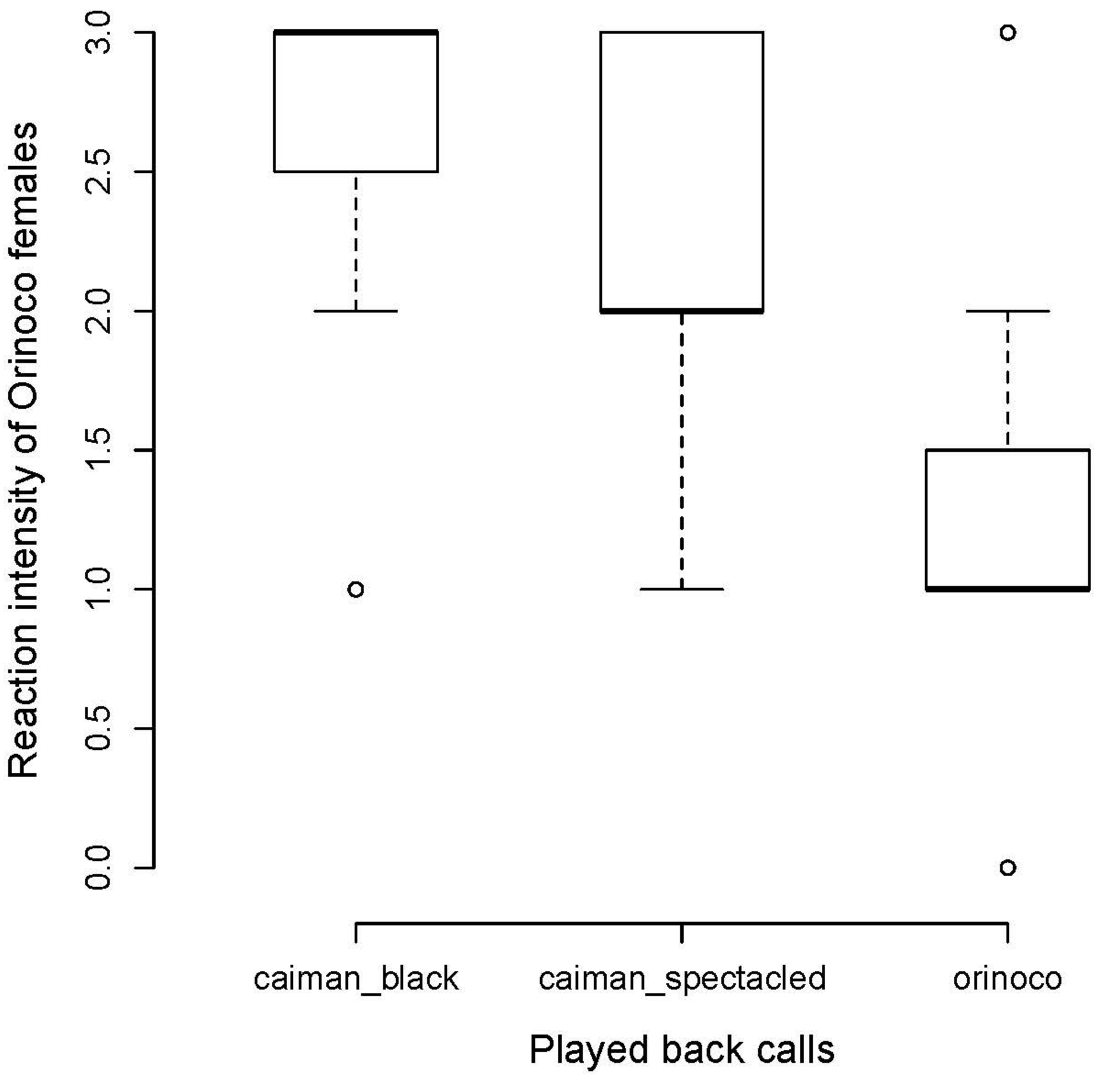

Figure 2. Relative intensity to the behavioural reaction of Orinoco crocodile females to calls from juveniles of different species.

Similarly, the number of female spectacled caimans that were attracted by playback was different depending on the experimental signal (GLM: χ^2= 14.0, df= 2, P<0.001, N= 17; Fig. 3). Specifically, calls from spectacled caimans and black caimans attracted more females than calls from Orinoco crocodiles (post-hoc test multiple comparison tests: spectacled caimans versus Orinoco crocodiles: Z= -3.69, P<0.001, N= 17; black caimans versus Orinoco crocodiles: Z= -3.23, P= 0.003). Conversely, calls from spectacled and black caimans were similarly attractive (Z= 0.45, P= 0.896).

These two experiments with Orinoco crocodiles and spectacled caimans show that adult females seem to pay more attention to the size information than to the species identity coded by the juvenile calls.

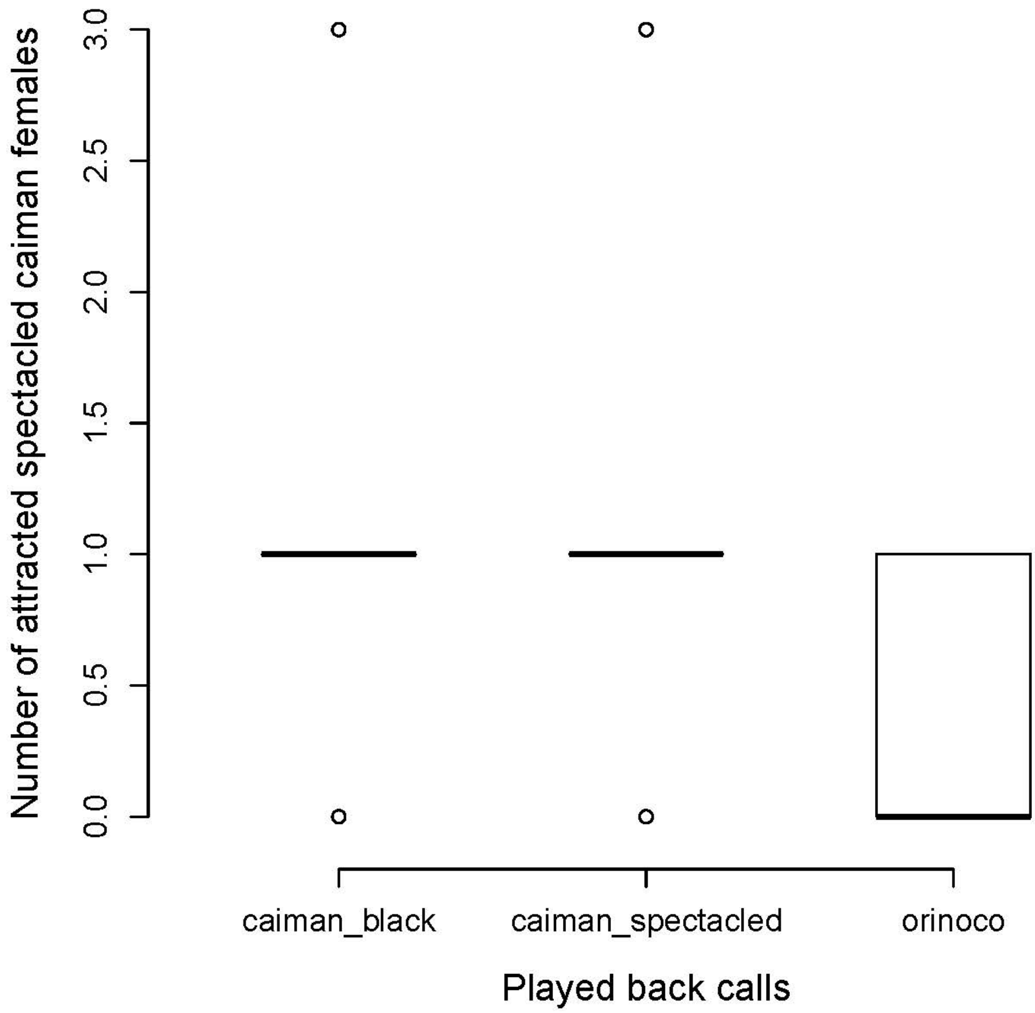

Figure 3. Behavioural reaction of spectacled caiman females to juvenile calls from different species.

Discussion

The acoustic analysis shows that vocalizations emitted by juvenile crocodilians contain reliable information about the emitter's size, with smaller individuals uttering higher pitched calls with a wider frequency bandwidth. Furthermore, playback experiments on Nile crocodiles in the wild demonstrate that this information is accessible to adult breeding females, which approached the loudspeaker more when hearing calls of smaller individuals. Finally, playback experiments on Orinoco crocodiles and spectacled caimans reveal that females react more to calls of smaller hatchlings than to the calls of larger juveniles even if they belong to a different species. Crocodylian females thus pay more attention to the "size code" than to the "species identity code" carried by the juvenile's calls.

The influence of growth on vocalizations is probably linked to the growth-induced modifications of the vocal vibratory membranes and the vocal tract. The palatal valve, which is used for sound production in crocodilians (Vergne *et al*. 2007; Vergne *et al*. 2009; Riede *et al*. 2011; Riede *et al*. 2015), grows with the animal, as do the resonators constituted by the nasal and buccal cavities, and this could explain modifications of the calls' acoustic properties (Taylor and Reby 2010). The playback experiments showed that Nile crocodile adult females respond less to calls of larger juveniles. This result suggests that crocodilian mothers pay selective attention to calls emitted by smaller individuals (hatchlings). This behaviour could suggest maternal care is more vigilant to offspring highly susceptible to predation (Somaweera *et al*. 2013).

Addditionally, the results from the experiments on the Orinoco crocodile and the spectacled caiman females suggest that crocodilian mothers care less about species identity which is yet embedded in juvenile calls (Vergne *et al*. 2012; Chabert *et al*. 2015), paying preferential attention to the "size" information. This is a puzzling situation since the absence of acoustic species-specific recognition may have fitness consequences: it seems that there is no barrier impairing a mother from a given Crocodylian species to provide care to a young from another species. To our best knowledge such a behaviour (cross-specific parental care) has however never been observed in the wild (although anecdotical observations has been reported in captivity, Brueggen pers. com.). The main reason may rather come from the fact that different crocodilian species rarely breed in the same micro-habitat than from a developed ability of recognizing young from its own species - although more studies are needed to investigate the potential roles of other communication channels [eg olfaction, as well as behavioural cues (Brien *et al*. 2013a,b)]. By maintaining the probability of providing allo-specific maternal care as very low, geographical segregation between species may have relaxed the evolutionary constraint leading on maternal ability to recognize juveniles from another species.

In conclusion, our study shows that vocalizations of juvenile crocodilians bring reliable information about size that is of importance to the mother. These sound signals may intervene in the dispersal of the crocodile family by modulating maternal reaction.

N.B. Part of this study has been published elsewhere (Chabert *et al*. 2015. Scientific Reports, 5:15547, DOI: 10.1038/srep15547). See this paper (available for free on the website of Scientific Reports) for a more thorough discussion.

Acknowledgements

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Croc Talk: American Crocodile Acoustics in Belize

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Abstract

Bioacoustics is an emerging field that plays a significant role in understanding animal ecology and behaviour, and provides new means for applied conservation. Despite evidence regarding the importance of acoustic signals to animal ecology, and the impact of human activity on animal communication, there are a lack of data regarding crocodilian acoustics. The investigation of American crocodile bioacoustics provides further knowledge regarding behaviour and ecology, and may be key in the development of new management techniques. The objective of the project is to record crocodile acoustic signals and develop a foundation of data regarding call structure in addition to behavioural significance in Belize. These data are imperative to assess the implementation of acoustics in direct conservation measures, and as a means to assess the impact of human noise on crocodile health and behaviour. In order to assess this, behavioural and acoustic sampling has been conducted at two sites (Ambergris Caye and Caye Caulker) with further data collection slated for the summer of 2016. Autonomous recording units and digital recording equipment were used to collect acoustic signals from 17 crocodiles. Of the 5 types of calls recorded, the most frequently produced was the juvenile distress call. Analyses of distress calls indicated that the frequency at 95% duration was greater for hatchlings (mean= 4425.2; SE= 154.4) than for juveniles (mean= 2689.6; SE= 175.9); peak frequency also was higher for hatchlings ($P<0.001$). Peak power and delta frequency were higher for juveniles ($P<0.001$). Additionally instances of distress call production were noted if recording was unsuccessful. The proportion of crocodiles that produced distress calls during capture varied between size classes by location; for juveniles at Ambergris Caye (100%) and Caye Caulker (30%) ($P<0.001$), and adults and sub-adults combined at Ambergris Caye (50%) and Caye Caulker (11%) ($P<0.05$). Initial interpretation indicates that there may be a link to human disturbance in relation to the production of distress calls, specifically distress calls produced by adult crocodiles. Acoustic production shifts may be the result of environmental stress and has the potential to alter interpretation of sound and behaviour. Modified sound production and interpretation is increasingly showing deleterious effects to breeding success, offspring survival, and social interaction in multiple amphibian, bird, and mammal species. This research is a vital first step in determining the large-scale effect of human environmental and soundscape disturbance to crocodile acoustics and behaviour.

50 Shades of Gray: Ventral Darkening of Tomistoma in Response to Exposure to Visible Light

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Abstract

Physiological colour change is a rapid process observed in many ectothermic taxa in response to environmental changes, as part of the animal's circadian rhythm or used for communication with conspecifics. Sunda Gharial (*Tomistoma schlegelii*) is the first crocodilian species in which physiological ventral colour change has been observed in response to changes in ambient light. Here we examined the effects of three factors on the ventral colour change in juvenile *T. schlegelii*: length of time exposed to light, light intensity and light frequency. We measured greyscale values of the ventral scales of 19 captive juvenile *T. schlegelii* (50.6 ± 4 cm body length) held under different light conditions using a chromameter, and used ImageJ to measure greyscale values of calibrated ventral scale photographs of the animals. In order to identify the location of photosensitive body parts we also exposed *T. schlegelii* with either their eyes, head, flanks or no part of the body covered to either darkness or a light environment. All tested *T. schlegelii* had significantly darker ventral scales after 75 minutes of exposure to light (R^2= 0.67, $F_{1,158}$= 324, P<0.001) and significantly lighter ventral scales after 70 minutes in the dark (R^2= 0.45, $F_{1,43}$= 36.66, P<0.001). Animals kept in lower light intensity (2.5 Lux) were also significantly darker than animals kept in brighter light (4200 Lux, $F_{16,2}$= 5.838, P<0.05). Exposure to light through different filters led to ventral colour darkening in all animals with significant variation among filters ($F_{10,5}$= 9.286, P= 0.001), and animals exposed to light through mid- and high-frequency yellow and pink filters were darker than animals exposed to low-frequency red-filtered light (Tukey, P<0.01). Eyes were the only body part found to detect the ambient light that triggers ventral colour change, with significant change between the ventral colour of *T. schlegelii* that had covered and uncovered eyes ($F_{7,1}$= 81.92, P<0.001). While further research is needed on the ecological functions of light-induced ventral skin darkening in *T. schlegelii*, our results suggest it could be linked to camouflage in the shaded environment of a peat swamp forest.

Crocodilian Adaptation to Environment: A Croc of a Different Color

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Abstract

Philippine crocodiles (*Crocodylus mindorensis*) maintained in black environments exhibited dark coloration of skin. A shift to a white tanks resulted in noticeable lightening of the skin within 10 min, and maximal response in 90 min. Covering of the eyes of *C. mindorensis* resulted in conversion to a dark skin tone, indicating that visual light stimulation was responsible for the maintenance of light skin color. Furthermore, shifting light-colored crocodiles from a white tank to a dark tank heavily illuminated with artificial light resulted in minimal darkening of the skin, reinforcing the idea that exposure to visible light was responsible for light skin tone. Experimentation with 11 other members of the Family Crocodylidae showed that these animals exhibited similar responses to light, while members of Alligatoridae showed limited ability to change, indicating that this phenomenon may have developed after the split of these clades. Within the Family Crocodylidae, some members respond to light much stronger than other species, and there was a strong correlation of the skin lightening response and serum a-MSH levels. Examination of the ultrastructure of crocodile skin revealed that, in crocodile exposed to high levels of light, the melanocytes contained centrally-located melanosomes, making the skin appear lighter in color. In contrast, the melanocytes in animals housed in dark-colored tanks were distributed throughout the dendritic arms of the cells, and stretched toward the surface of the skin, make the surface appear darker in color. We conclude that members of the family Crocodylidae can rapidly change color in response to background, potentially for crypsis to evade predators and to aid in predation.

Diving in a Warming World: Environmental and Physiological Determinants of Dive Duration in Crocodiles with Increasing Temperatures

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Abstract

Temperature has a pervasive effect on physiological function and organismal performance especially in ectothermic animals like crocodiles. Dive duration of ectotherms is influenced by physiological responses and capacities and has been shown to be highly thermally dependent, where elevated temperatures can significantly reduce dive times. Given that diving plays a significant ecological role in crocodiles, including foraging and predator avoidance, understanding the potential effects of predicted rises in global temperatures on this critical behaviour is of interest. Using field data and taking an experimental approach we examined the thermal sensitivity of diving in estuarine crocodiles and explored the flexibility and plasticity of physiological systems to buffer the negative impacts of elevated temperatures on dive duration. Crocodiles were exposed to one of three long-term thermal treatments, designed to emulate water temperatures under different climate change scenarios (ie current summer, 28°C; 'moderate' climate warming, 31.5°C; 'high' climate warming, 35°C), and physiological responses and dive capacity was subsequently tested. The capacity of crocodiles to mitigate the effects of temperature on diving ability via thermal phenotypic plasticity and compensation was investigated.

Food Habits of the Yacare Caiman (*Caiman yacare*) in Corrientes Province, Argentina

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Abstract

Caiman yacare (Yacare caiman) and *Caiman latirostris* (Broad-snouted caiman) are the Crocodylia species present in Argentina. There are studies about the diet of *C. latirostris* in Brazil and Uruguay, those studies detail the different types of prey that were found in stomach contents, mostly insects, crustaceans, and mollusks. In Argentina, little is known the trophic ecology of these animals, and the only record was a study that describes the type of prey for both species. The aim of our study was to examine stomach contents of *C. yacare* to know prey items and they sizes, and if there was any ontogenetic change. Data were collected in 6 lakes in Corrientes Province (Argentina). We obtained 47 stomach contents of *C. yacare* (individuals between 15 to 119 cm snout-vent length), samplings were conducted at the beginning of spring. The most important preys were Insects (Coleoptera, Orthoptera, Odonata and Hemiptera), mollusks (Pomacea), freshwater crustaceans (*Pseudopalaemon* and *Trichodactylus*). Vertebrates (reptiles, fish and birds) were found less frequently. We found no evidence that prey items of the caiman change substantially as they get bigger, but we found that as caiman get bigger, insects consumption decreases. Caiman that consumed more mollusks also consumed more crustaceans. Class I caiman did not consume vertebrates, Class II consumed squamata and other caiman, and once they get bigger consume birds. We conclude that as caiman get bigger they keep using previous food items, but amplify the trophic niche.

In Memorium: Louis J. Guillette, Jr.

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Abstract

Louis J. Guillette, Jr., one of the world's most influential research scientists in the field of environmental health, died on 6 August 2015 at the age of 60. His death creates a gaping hole in the lives of many and in the fields of environmental health, crocodilian reproductive biology, and ecotoxicology. His life, however, provides a wonderful legacy - the people he trained and the passion he instilled in them to continue his quest for harmonizing human activity with environmental, wildlife and human health. In this presentation, we briefly touch on Lou's life, his work with crocodilians, and our relationships with this great mentor and friend.

Semen Collection in the American Crocodile (*Crocodylus acutus*): Our Training Experiences in Costa Rica

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Abstract

As part of the development of an artificial insemination program in the Saltwater crocodile, we have established a highly successful non-invasive and repeatable method for semen collection. However, the question remained, could our technique also be applied to other species, especially those with higher conservation needs? As part of a crocodile reproduction workshop conducted at the Costa Rican Institute of Technology in February 2016 we were able to train our colleagues in the successful collection of semen from 3 American Crocodiles (*Crocodylus acutus*). Although initial attempts to collect semen from the Common Caiman (*Caiman crocodilus*) were unsuccessful during the workshop, a second attempt in late April by our Costa Rican colleagues proved to be successful - this finding highlights the importance of understanding the role of seasonality in crocodilian semen production. The success of our non-invasive semen collection technique in both species in Costa Rica, gives us confidence that our method is not only transferrable to other species but that it is also a skill that can be taught. We have now commenced an international research collaboration with our Costa Rican colleagues to explore the seasonality of reproductive physiology and sperm production in the American Crocodile and Common Caiman. This research, in association with our ongoing reproductive studies into the farmed Australian Saltwater crocodile, will not only be the basis of new reproductive and genetic management technology for crocodile production and conservation but also allow us to establish assessment of breeding soundness in male crocodiles throughout the world.

Variation in Nest Temperatures of the American Alligator found on the Kennedy Space Center/Merritt Island National Wildlife Refuge

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Abstract

Information on nest temperatures of the American alligator (*Alligator mississippiensis*) constructed in the wild is limited. Nesting temperatures during a critical thermal sensitive period determine the sex of alligators and are therefore critical in establishing the sex biases in recruitment efforts of alligators within a given community. Nest components, varying environmental conditions, and global warming could have a significant impact on nest temperatures, thus affecting future generations of a given population. One hundred and seventy-four programmable thermistors were inserted into 58 nests from 2010 through 2015 nesting cycles. Three thermistors were placed inside each nest cavity (one on top of the eggs, one in the middle of the eggs, and one at the bottom of the clutch of the eggs) to collect temperature profiles in the incubation chamber and throughout the entire incubation period. One thermistor was also placed near or above these nests to obtain an "ambient" air temperature profile. Once retrieved, data from these thermistors were downloaded to examine temperature profiles throughout the incubation period as well as during the period of sexual determination. These data would help establish survival rates related to nest temperature and predict sex ratio of recruited neonates at the Kennedy Space Center. Over three million temperatures have been recorded since 2010 for the alligator thermistor study giving us insight to the recruitment efforts found here. Precipitation was the largest influence on nesting temperatures outside of daily photoperiod, with immediate changes of up to 8^{o}C.

Adaptive and Disruptive Epigenome-by-Environment Dynamics: Linking Molecular Mechanisms to Ecological Impacts

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Abstract

Unlike traditional models for reproductive health, the American alligator is a long-lived apex predator that undergoes temperature-dependent sex determination (TSD). Further, these animals are oviparous and field collected eggs allow for the investigation of environmental effects on developmental processes. Here, we use the alligator as a model to (1) examine the role of DNA methylation patterning during TSD and (2) investigate how exposures to endocrine-disrupting contaminants (EDCs) during this period may alter the epigenetic landscape in a manner that influences subsequent reproductive function. When compared to their counterparts living in relatively pristine environments, alligators undergoing natural exposures to EDCs display a severely abated ovarian transcriptional response to gonadotropin stimulation. Here, we employ reduced-representation bisulfite sequencing to explore the sexually dimorphic DNA methylome in gonads from embryos exposed to either male- or female-promoting temperatures. We identify numerous temperature-dependent methylated regions within the alligator genome. In addition, we use targeted bisulfite sequencing on the Illumina platform to examine how DNA methylation status of the CYP19A1 promoter, a gene displaying sexually dimorphic expression and DNA methylation patterns, varies in field-collected embryos originating from contaminated environments. We find that the robustness of sexually dimorphic CYP19A1 promoter methylation is reduced in embryos originating from a site contaminated with EDCs. We next probe the consequences of developmentally inappropriate Estrogen Receptor activity on subsequent CYP19A1 expression and find that treatment with a selective ESR1 agonist prior to gonadal differentiation results in down regulation of CYP19A1 in stage 27 ovaries. Results presented here suggest that DNA methylation patterning may play an integral role in mediating the effects of incubation temperature on sex determination and that EDCs may exert their effects by compromising sexually dimorphic epigenetic patterns acquired during development.

Nitrate Induces a Type 1 Diabetic Profile in Alligator Hatchlings

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Abstract

Crocodilians are long-lived predators, making them interesting subjects to study how changes in water quality affect health. As human populations grow globally, the demand for agricultural production intensifies and sewage inputs increase. Fertilizers, manure, and sewage are the main sources of rising nitrate levels in surface and ground waters, and their effects on human and wildlife health are only partially known. Several published studies suggest that nitrate in drinking water may promote development of type 1 diabetes (T1D), a chronic autoimmune disease that affects 1 in 300 children by the age of 18. T1D is caused by a loss of insulin-producing pancreatic beta cells, leading to poor blood glucose regulation and a host of downstream complications. To investigate the potential role of aquatic nitrate in T1D etiology, we exposed female hatchling alligators to environmentally relevant concentrations of nitrate in their tank water (reference, 10 mg/L, or 100 mg/L NO_3-N) from hatch through 5 weeks or 5 months of age. At each time point, we investigated several markers of T1D: plasma levels of glucose, triglycerides, testosterone, estradiol, and thyroid hormones; weight gain or loss; pancreas, fat body, and thyroid weights; presence of immune cells in the pancreas; and beta cell number. To confirm internal dosing, we measured nitrate levels in plasma and urine, along with whole blood methemoglobin levels. Using cluster analysis, we observed that 100 mg/L NO_3-N, and to a lesser extent, 10 mg/L NO_3-N, induced a profile of signs consistent with early T1D.

Surgical Removal of the Abdominal Fat Body (Steatotheca) of the American Alligator with Observations on the Effect of Insulin Injections

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Abstract

Crocodilians possess an unusual fat body situated just below the liver on the right side of the abdominal cavity. This structure, often referred to as the lateral abdominal fat body, is well vascularized, and fed by a small branch from the mesenteric artery. The first description of this organ was by a group of Jesuit astronomers/mathematicians in Siam, probably with little experience in anatomy, who dissected three crocodiles.They thought the fat body was the pancreas, “with a consistency of firm congealed fat and colored white with a slight tint of red” (my translation) Gouye 1688. John Hunter (1728-1793) described it as a “fatty pancreas” or “pancreas asselli”, a lymphatic gland (Owen 1861). Owen (1831) in his description of the anatomy of an American crocodile, *Crocodylus acutus*, mentioned it: “there is a lacteal gland at the root of the mesentery as large as the spleen “, but gave no further information. Eberth 1864 was the first to make a histological study of the tissue. He described one from an alligator as having “a net of fibrous connective tissue filled with numerous fat cells.” The abdominal fat body (or steatotheca) of the Crocodylia has no homologue in other vertebrates. To investigate its possible function, fat bodies were surgically removed (FBX) from juvenile alligators and growth monitored for three months. Initial body mass was 494 g $\pm$ 18 (N= 24) FBX; 490 g $\pm$ 21 (N= 24), sham. Final body mass 955 g $\pm$ 43 FBX and 1105 g $\pm$ 69, sham. Initial total length 56.7 cm $\pm$ 0.53, FBX, sham, final total length 72.7 $\pm$ 0.93, FBX, 73.7 $\pm$ 1.04, sham. FBX had no significant effect on growth or body mass in the three months following surgery. Bovine and alligator insulin were injected and blood samples were taken prior to, and at 6, 12, 24 and 72 h post injection and analyzed for plasma lipid profiles using thin layer chromatography-flame ionization detection. Total plasma triglycerides showed a significant increase at 24 h in response to insulin in intact alligators but not in the FBX group. In contrast total plasma cholesterol esters decreased in FBX at 24 h, but not in sham. There were no significant differences between the two groups in plasma total fatty acids, phospholipids, cholesterol or total lipids.

Is Fish or Chicken-based Feeds Good for the Commercial Production of Nile Crocodiles and can Crocodile Meat be used in Animal Feeds?

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Abstract

The demand for high quality crocodile skin products is high and continually increasing globally. Commercial crocodile farmers strive to optimise skin quality and growth rates to improve profitability of their operations. The feeds used in crocodile production are known to influence the growth rates of crocodiles, and may possibly affect the quality of skins. In this study we propose to investigate the nutritional value of chicken and fish based feeds in crocodile production. In addition we aim to evaluate the net effect of these alternative feeds on the growth rates of the crocodiles and the potential effect of temperature, water quality and stocking densities on growth rates. The study will be undertaken at the Albert Falls Crocodile Farm in collaboration with the University of KwaZulu-Natal. Multivariate statistical procedures will be used to evaluate the effect of the feeds and other determinants on the wellbeing of the crocodiles. Thereafter crocodile meat will be used to formulate five fish feeds that will be tested to raise various size classes of commercial aquaculture fishes. This study will not only result in the improvement in feed technologies for the commercial production of crocodiles but allow for the commercial diversification of crocodile products.

Danish Crocodile Zoo and Hato Masaguaral: Combining Forces to Conserve Orinoco Crocodiles

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Abstract

The critically endangered Orinoco crocodile, *Crocodylus intermedius*, is found in low numbers in Colombia and Venezuela. Hato Masaguaral is a ranch in Venezuela that is breeding the species for release into the wild. Since 2012, the Krokodille Zoo in Denmark has been proudly supporting the efforts of the ranch to raise the crocodiles, and to date the ranch is releasing between 150 and 300 crocodiles each year. Over the past two years we have travelled to Venezuela to support and join in the releasing of Orinoco crocodiles. In this presentation, we will detail the problems faced by the ranch, and how we are trying to help them overcome these challenges. We will look at the success of the project and our *ex-situ* plans for Orinoco crocodiles. Being a part of this project is important to the Krokodille Zoo, and has been our main focus for crocodile conservation the last years.

Mating Dynamics and Population Genetics in a Coastal Population of *Alligator mississippiensis* at the Tom Yawkey Wildlife Center

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Abstract

The American alligator (*Alligator mississippiensis*) represents a key sentinel species in the coastal Southeast as well as a valuable natural resource. Yet, little is known regarding the mating and nesting dynamics of this species in terms of both maternal and paternal size, mating ranges, multiple paternity and mate fidelity. The Yawkey Wildlife Center (YWC) near Georgetown, S.C. represents both a unique and ideal opportunity to investigate these dynamics. The population of alligators inhabiting the YWC is relatively small, isolated, and has historically remained free of hunting pressures. In addition, long-term capture data (>30 years) characterizing the locations, growth, size, and sex of individuals comprising this population have been steadfastly recorded. We initially used existing microsatellite markers to examine parentage in 10 nests from 2011. Those markers had been developed and screened for polymorphism from alligators originating in Louisiana, USA. In a sample of 98 adults from YWC the expected and observed heterozygosities were 0.538 and 0.535 respectively with an average allelic diversity of 5.2. With such low variability our ability to exclude parents was too weak. In an attempt to increase our power we developed new loci using DNA from a YWC adult and a next generation sequencing approach. We identified more than 3000 loci and screened 48 across a subset of YWC adults. We identified 10 to use for subsequent analyses with ~100 adults from YWC. Our variation improved with expected and observed heterozyosities increasing to 0.792 and 0.795 respectively. In addition the allelic diversity more than doubled to 11.1. We then screened hatchlings from 38 nests sampled from 2011-2013. A female was caught at or near 17 of those nests, but maternity could only be assigned in 8 cases. For the 21 nests without a putative mother, we were able to assign maternity for three nests. Within the 11 nests with a known maternal genotype we estimated that a minimum of 11% had multiple paternity. The population at YWC may be larger than originally thought given the low number of adults in our genetic database.

Taxonomic Status of the Rio Apaporis Caiman

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Abstract

In February 1952, Prof. Federico Medem conducted an exploration to the Apaporis River, between the falls of Jirijirimo and Puerto Yaviya (0º 7' N, 71º 0' W, approximately). During field expedition, Medem collected 21 individuals of *Caiman crocodilus* that showed a noticeable difference from specimens from other localities in the region, especially in the elongated and comparatively narrow snout and in certain external characteristics. All specimens were deposited at the Field Museum of Natural History, Chicago, Illinois. In 1955 Medem described officially *Caiman crocodilus apaporiensis*. However, Rio Apaporis Caiman has been controversial in the literature. Unfortunately, the life-history information of the Rio Apaporis Caiman is limited and anecdotal, and morphological changes in particular, has remained somewhat opaque. Therefore, we examined the holotype and 17 paratypes of *C. c. apaporiensi*s Medem 1955 deposited at the Field Museum of Natural History. Exploratory multivariate analyses (principal component and discriminant function) based on 21 morphological characteristics of *C. c. apaporiensis*, *C. yacare* and the *C. crocodilus* complex (*C. c. chiapasius*, *C. c. fuscus* and *C. c. crocodilus*) were performed. Our results showed a clear separation of *C. c. apaporiensis* from *C. yacare* and *C. crocodilus* complex, which could suggest that the environmental heterogeneity and geographic isolation drives the existence of evolutionary independent lineages within the *C. crocodilus* complex. However, there are many missing pieces that need to be in place before it will be possible, in particular both molecular and life-history data, to clarify the taxonomic and conservation status.

Backing up the Efforts of Medem, Blohm, Thorbjarnarson, Seijas, Ardila and Ayarzagüena: Preserving the Orinoco Crocodile *Crocodylus intermedius* at the Dallas World Aquarium, Texas, USA

Luis Sigler and Daryl Richardson

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Abstract

The conservation of wildlife species has an important trend; for the case of the Orinoco crocodile (*Crocodylus intermedius*), it is important to recognize the pioneering work of Federico Medem in Colombia and Tomas Blohm in Venezuela, followed by the line of inquiry established by Thorbjarnarson and Ayarzagüena, the coworkers and successors of each of these groups, plus the new working groups that have joined. This species was considered critically endangered in the 1970s due to the intense irrational exploitation that occurred in the first half of the twentieth century. At that time, 3 million Orinoco crocodile were estimated to occur in their natural range; today, only 1500 are estimated to occur in Venezuela and no more than 200 in Colombia. Despite conservation efforts that were established in both countries, political and economic situations have not yet been able to consolidate the intense effort that has been made. In 1997, The Dallas World Aquarium built the biome "Orinoco - Secrets of the River" with the idea of highlighting the biodiversity of this important river basin in South America; and in 1998, imported a couple of adult Orinoco crocodiles in an indefinite loan from the government of Venezuela. The first egg laying in 1999 was lost. Some modifications were made to the exhibit and since 2003 healthy offspring have been obtained by artificial incubation. In 2008, 54 juveniles were sent to Venezuela to be released in three Natural Protected Areas. The rest of the offspring produced were sent to 14 different zoos in the United States and even six were sent to Denmark. To avoid inbreeding, most of the produced hatchlings were females. We hope to acquire sub-adult male specimens in the near future to form breeding pairs and help the propagation of the species with the goal of releasing the hatchlings in their historical distribution area. The Orinoco crocodile is still considered one of the 10 vertebrate species that may disappear in the near future due to the limited number of their wild populations and the interaction of human activities that in some way impact their eggs, their food and on specimens of different sizes because they are considered as dangerous.

Siamese Crocodile Conservation in Cambodia: An Update on Recent Activities and Progress

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Abstract

The Cambodian Ministry of Forestry and Fisheries has been collaborating with Fauna & Flora International (FFI) and the Wildlife Conservation Society (WCS) to help save the critically endangered Siamese crocodile, *Crocodylus siamensis*, from extinction since its rediscovery in the wild in 1999. The main focus over the past few years has been to empower and support communities in protecting crocodiles and their habitats, monitor wild populations, captive breeding, release of captive bred animals, and establish national conservation strategic and action plans. Here, we present an update of the recent conservation activities. Surveys indicate that the population, although low, appears to be stable, with some evidence of breeding in two locations in the past two years. There has been considerable effort in improving the captive breeding program (FFI and the Forestry Administration (FA)) and are in the process of expanding the number of breeding pairs. In January FFI and FA conducted its third release of captive-bred individuals into a new remote sanctuary site. There has been progress engaging crocodile farmers in support of captive breeding and conservation as well. Development of a National Conservation Strategic Plan has progressed as well but has faced delays from government restructuring.

American Alligator Home Range and Movement Patterns in Coastal South Carolina

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Abstract

Movement patterns have profound implications for many ecological processes like species abundance and distribution, predator-prey interactions, and habitat selection. In the southeastern USA, American alligators (*Alligator mississippiensis*) are a keystone species, capable of altering habitat structure and function. Therefore, alligator movement patterns are likely to have consequences for both the structure and function of alligator populations, and other wetland species. We captured and marked 24 adult male alligators with Telonics TGM 4310 GPS satellite transmitters (± 10 m accuracy) from April to May, 2015 in the Santee Delta and ACE Basin regions of coastal South Carolina. Each transmitter was scheduled to acquire a location fix every three hours from deployment through September. We estimated daily movement rates and used kernel density estimation (KDE) methods to derive home range size estimates (50, 90 and 95% isopleths), and evaluated the effects of total body length and region on each parameter. On average, we obtained 749 ± 220 SD fixes per individual and a fix rate of 70% ± 13%. Total body length had a weak negative effect (β: 0.05 ± 0.02 SE) on 50% KDE only (0.25 km^2 ± 0.318 SD); but was not supported for the 90% (2.10 km^2 ± 2.75) or 95% KDE (2.48 km^2 ± 3.89). In contrast, body length positively influenced daily movement rates (β: 0.52 ± 0.003 SE), which averaged 0.203 ± 0.14 km/day. South Carolina alligator home range sizes and movement rates are similar to previous studies in Florida and Louisiana, despite distinct habitat differences.

Spatial Ecology of the American Crocodile in a Tropical Pacific Island in Central America

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Abstract

The limited information on the ecology of the American crocodile (*Crocodylus acutus*) has had a major impact on the recovery of this species over time, even though most of the 18 countries in which it is found have banned hunting. The last decade has made it clear that to implement sound conservation and management programs, we must understand crocodile spatial ecology. Telemetry has been the method of choice; however, in only two countries where the species occurs have telemetry studies been published. We characterized the spatial ecology of *C. acutus* by tracking 24 individuals at the southeastern tip of Coiba Island, Panama, between 2010 and 2013 to determine movement patterns, home range, and habitat use, and compared our findings to existing data to create a comprehensive assessment of American crocodile spatial ecology. Females showed a higher average movement distance (AMD) than males; similarly, adults showed a higher AMD than sub-adults and juveniles. However, males exhibited larger home ranges than females, and sub-adults had larger home ranges than juveniles, hatchlings, and adults. There was a relationship between precipitation seasons and AMD, with increased AMD in the dry and "low-wet" seasons, and a reduced AMD during the actual wet season. We found disaggregate distributions according to age groups throughout the 9 habitat types in the study area; adults and hatchlings inhabited fewer habitat types than juveniles and sub-adults. These sex- and age-group discrepancies in movement are likely due to the influences of reproductive events and Coiba's precipitation cycle. Juveniles also showed distinct movement patterns and home ranges; however, with sexual maturation and development, these behaviours were becoming more characteristic of adults and sub-adults. Our findings are amongst a few that support management and conservation planning based on a comprehensive integration of the spatial ecology of the American crocodile in the Neotropics.

Relative Abundance and Current Situation of *Crocodylus moreletii* in Priority Regions of Peten based on the Morelet's Crocodile Surveying Manual

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Abstract

Morelet's crocodile (*Crocodylus moreletii*) is distributed from Tamaulipas and the Gulf of Mexico to the northern part of Guatemala and Belize. This species is highly hunted for its skin and meat. In Guatemala, due to unregulated and excessive exploitation of local wild populations, *C. moreletii* is listed in Appendix I of CITES in order to promote its conservation and protect it from illegal international trade. The main objective of this study was to generate and update basic information on wild *C. moreletii* populations to inform their conservation, management and sustainable use at local, national and regional levels. To fulfill the main objective, the following specific objectives were established: 1. estimate the relative abundance and age structure of *C. moreletii* in the priority areas identified in the Morelet's Crocodile Surveying Manual (Sánchez *et al*. 2011); 2. identify the direct threats to wild *C. moreletii* populations; and, 3. contribute to the implementation of the Manual Trinacional de Monitoreo del Cocodrilo de Pantano.

Sites were sampled between July and November (rainy season), in the San Pedro River, Sacluc River, El Peru Lagoon, Usumacinta River, La Pasion River, Petexbatún River, Petexbatún Lagoon, Sacnab Lagoon, Yaxhá Lagoon and Petén Itzá Lake - which were classified as "open aquatic systems" (rivers) and "closed aquatic systems" (lagoons). A total of 390 km of river and lagoon shore was surveyed using standardized techniques described in Sánchez *et al*. (2011) (see Fig. 1). Among the main results, the encounter rate (TE for its acronym in Spanish) was 1.023 crocodile/km for open aquatic systems, while for closed aquatic systems the TE was 0.4337 croc/km. Sites with the highest TE were: Sacluc River with 2.41 croc/km; San Pedro River with 2.09 croc/km and Yaxhá Lagoon with 2.14 croc/km.

Regarding the age structure, the site with the highest proportion of hatchlings was the Usumacinta River, while Yaxhá Lagoon showed higher proportion of juveniles, and San Pedro River scored higher proportion of sub-adults, adults"and "older adults". On the other hand, a direct correlation was found between hatchlings, juveniles and sub-adults with "forest habitat", and meanwhile adults showed a direct correlation with "high-aquatic vegetation".

Finally, this study shows that *C. moreletii* populations are stable, but also highly threatened at all studied sites. Among the threats, the most important ones were: hunting, human settlements, high presence of trash and trammel nets.

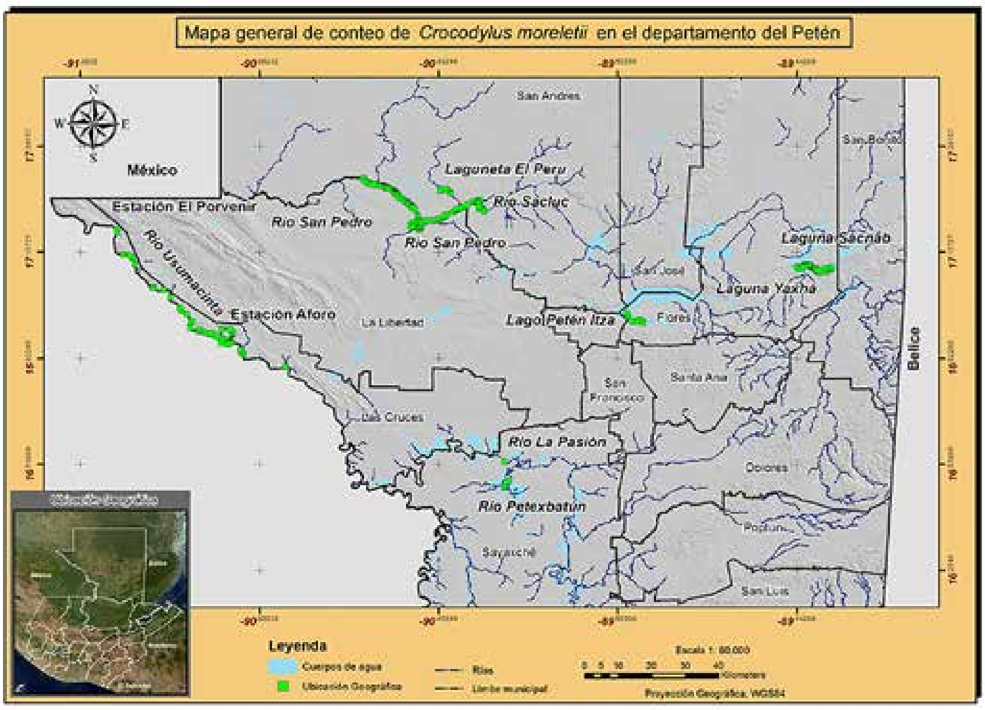

Figure 1. Map of overall counts of *C. moreletii* in the 10 sampling sites.

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The Knowledge of the Morelet's Crocodile *Crocodylus moreletii* in Mexico, Belize and Guatemala

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Abstract

In 2001 there was an initiative in Mexico to determine the current situation of the Morelet's crocodile *Crocodylus moreletii* populations and its conservation status. This project was named "CoPan" as an acronym for "Cocodrilo de Pantano", and was presented at a Tri National Meeting in Laguna del Tigre National Park, Peten, Guatemala in June 2001. A product of CoPan project obtained in 2002 was a manuscript entitled "Analysis of existing information on the Morelet's crocodile (Duméril and Duméril 1851) in Mexico", where 187 publications on the species were reviewed. The results of the population surveys were presented at the CSG meeting in Gainesville 2002; the information analysis was not adequately disseminated and was on hold expecting a better time, which peaked at the CSG meeting in Manaus 2010, when Hesiquio Benitez from the Biodiversity National Commission of Mexico (CONABIO) requested an update of the document, extending its coverage to 2010. Due to the lengthy review process that this book has had, the analysis of information generated on the species had to be extended until 2014. More than 154 additional papers were included in this analysis, which indicated that in less than 12 years, 82% more data was gathered to analyze, in comparison with the documents from the 2002 revision, which meant that in the last 12 years, 45% of the Morelet's crocodile knowledge was written. This significant increase also shows the ease to access information electronically and that more human resources have formed that publish their research. We believe that this book, rather than representing the enormous effort in reviewing the information, is a vast compendium that shows the knowledge about the Morelet's crocodile is large and has existed for some time but had not always reached the scientific community and often was published in meetings and symposia with limited diffusion. This book is divided into 8 chapters covering various aspects of the Morelet's crocodile as the description, natural history in the wild and in captivity, conservation and management in the three countries of its natural range. We want to thank Hesiquio Benitez, the reviewing and editing team of CONABIO and Daryl Richardson, Director of the Dallas World Aquarium for their instrumental help in the conclusion of this book.

Trophic Ecology of American Crocodile (*Crocodylus acutus*) in Coiba Island Marine-Coastal Habitats

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Abstract

This is the first attempt to model trophic networks for marine-coastal ecosystems to support American crocodiles (*Crocodylus acutus*) as biodiversity indicators. From March to June 2013, we collected data on primary productivity from coral reefs and mangroves forests at El Maria and Playa Blanca on Coiba Island, Eastern tropical pacific of Panama. For coral reefs we estimate primary productivity using the Winkler method. For the mangrove forest we used leaf letter production to estimate the amount of carbon. Carbon values for each ecosystem were also transformed into energy values in Kcal/m^2/year. We looked for published trophic information for the species found in both ecosystems and designed two predator-prey matrixes which were analyzed with Ghephi 0.8.2 software to generate two trophic networks. We found that for Coiba marine-coastal ecosystems productivity was low, comparing with other systems at the same latitude, but supports four trophic levels where American crocodile was the top predator. We estimate the ecological efficiency and energy flow for both ecosystems based on net productivity, using reports from the literature on the biomass and energy content of trophic levels of similar sites. Although more information is required, trophic networks and ecological efficiency showed the complexity and vulnerability of this tropical system where the American crocodile acts as trophic regulator of the population density of first and second order consumers, links multiple ecosystem components by foraging on heterogeneous sites, and promotes resource facilitation. Our results support the hypothesis that this species can be use as biodiversity indicator for Eastern Tropical Pacific marine-coastal ecosystems.

Fifteen Years of Philippine Crocodile Research and Conservation in Northeast Luzon: What Do We Know and Where Do We Go?

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Abstract

In 1999, by coincidence, a small remnant wild population of the critically endangered Philippine crocodile *Crocodylus mindorensis* was found in the foothills of the Sierra Madre Mountains in the municipality of San Mariano in northeast Luzon. Previously overlooked as a result of an adverse security situation and a lack of specimen in historical museum collections, northern Luzon was never included in descriptions of the distribution of this endemic freshwater species. At present, the wild Philippine crocodiles of San Mariano are the best studied of their species. Their population is one of only two remaining viable populations in the country, the other being in southwestern Mindanao, an area where the civil insurgency makes research and conservation very difficult. In 1999, all information about Philippine crocodile ecology came from the study of captive animals. In 2016 we know that wild mature individuals of this species prefer large rivers with deep pools and clear water while juveniles prefer stagnant pools and small creeks with slow currents. Nests are either mounds or holes with a maximum clutch of 26 eggs. There is some maternal care but not enough to protect nests from raids by rats, monitor lizards and people. Hatchlings run a high risk of being killed by the stings of ants that are attracted by yolk remnants. Hatchlings stay together in small ponds but eventually become territorial and fight, in captivity to the point where they kill each other. Larger Philippine crocodiles are mostly solitary and seem to disperse from the site where they were born. Marked crocodiles have been found more than 10 km from their nest site, sometimes in unconnected wetlands. Home-ranges of radio-tagged crocodiles increase from several hundreds of metres of river stretch for juveniles to more than 6 km for adults. The crocodiles in San Mariano however survive in an increasingly human-dominated landscape with serious conservation challenges. How do we use our present knowledge of Philippine crocodile ecology to work towards a future in the wild of one of the world's most severely threatened species?

Estimation of Population Size of Crocodile *Crocodylus palustris* (Lesson, 1831) from Two Incomplete Survey Methods - Daylight Ground Counts and Night Counts

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Abstract

Population count of crocodile was undertaken from Warana basin of Maharashtra, India for successive two years 2013-14 and 2014-15. Two main survey methods Daylight Ground Counts and Night Counts were used to access the population. As the crocodile is a shy animal and the study area is so big it was impossible to locate each and every crocodile from study area as well as to cover whole study area. Hence population statistics was applied to calculate the population size of crocodile. The statistical approach explained by Magnusson (1978) was used for present work. The calculation of probabilities for two survey methods estimated that the study area has about 182. It has an approximate variance 806 and hence a standard error of ± 28.39.

Introduction

Crocodiles as known to human being are the largest reptiles present on the earth. They are known since remote past. The Indian mythology represents crocodiles as a ride of Maa Ganga (the Ganga River goddess). Also it is said that the god of rain - Varuna - rides on monster of Makara. Still then world represents only 24 species of crocodiles, of which only 3 are found in India. These are Gharial (*Gavialis gangeticus*), Mugger (*Crocodylus palustris*) and Saltwater crocodile (*Crocodylus porosus*).

In India, Madras Crocodile Bank Trust, Chennai, TN, is the private trust involved in protection and conservation of crocodiles along with the crocodile conservation project of Government of India (GOI). H.R. Bustard, a consultant from Food and Agricultural Organization (FAO) of the United Nations Development Programme (UNDP), was invited to look at the crocodile situation in India GOI in 1974. Before the launching of crocodile conservation project by GOI, Biswas and Whitaker have paid attention on the status of crocodiles in early 1970s. Currently Gharial is found only in restricted parts of Ganga basin, like areas near by Lucknow in UP, Patna in Bihar and parts of Mahanadi at Bhubaneshwar, Orissa. In past they were abundant all over in Ganga, Brahmaputra and Mahanadi basin of northeast India. The Mugger was pre-dominant all over the central and south India from Rajasthan, UP, WB to the Tamilnadu south end of India, but presently they are restricted in very limited parts of same region. In Maharashtra they were present all over the state, but presently they are observed only in the Krishna basin and its all tributaries and the northern Maharashtra at the boundary of MP. The Saltwater crocodiles were dominant all along the east coast of India and Kerala coast, but presently it is found only at the WB and Orissa coast.

In recent 8-10 years, crocodile *Crocodylus palustris* (Lesson, 1831) was reported from Warana basin by peoples from various fields like farmers, news paper reporters and biology teachers. Hence author has tried to estimate its population size by incomplete counts of Daylight Ground Counts and Night Counts and applying statistical approach as explained by Magnusson (1978).

Review of Literature

Many workers have used daylight ground counts method for crocodile survey. According to Graham (1968) daylight ground survey of crocodilians generally reveal only a small percentage of population. Chapman (1970) estimated that percentage as 20-50% for *C. niloticus*. Modha (1967) used daylight ground counts for *C. niloticus* and found them together on few beaches of Lake Rudolf. Pooley (1969) observed that all mature crocodiles of Lake St. Lucia (45 miles long) are came together on 1.5 miles nesting beach. Night Counts is the best method ever used for crocodilians survey. Chabrek (1976) used this method to monitor hunted population. Graham (1968), Campbell (1972), Parker and Watson (1970) and Pernetta and Burgin (1980) have used this method for survey of status of crocodiles. Messel reported about 60-70% of population in tidal rivers of northern Australia with this method. Woodward and Marion (1979) evaluated the factors affecting night counts of *Alligator mississippiensis* in lakes in Florida USA. All of them found that, this method is a superior one than others as the results are more accurate for different workers in same area. Eltringham (1972) and Magnusson (1978, 2008) have used statistical approach to estimate the population size from incomplete counts of elephants (*Loxodonta africana*) and crocodiles (*C. porosus*) respectively. Magnusson (1978, 2008) has used the probability and variance to the data of incomplete nest counts from swamps of the Liverpool River system, northern Australia.

Study Area

The Warana River is a major tributary of Krishna River. It begins its course close to the western crest of Sahyadri at a height of about 987 m above msl at Patherpunj in Patan Taluka of Satara District. Warana River runs north to south direction on the hilltop (Sada) in the Sahyadri ranges. Further it takes eastward turn and runs about 148 km between 16° 33' and 17° 16' N and 73° 33' and 74° 41' E on the famous Deccan Plateau in Maharashtra, just east of the Western Ghats till it joins the river Krishna at Haripur near Sangli City of Maharashtra. The major tributaries of the Warana River on the left bank are Zolambi and Morana and on its right bank are Tanali, Kansa and Kadavi. Zolambi and Tanali Rivers merge in Warana River behind this dam in the Vasantsagar water reservoir.

The study was carried out by dividing the entire study area into four different study regions. The study regions were made by considering ecological conditions, topographical settings of Warana River and previous information about crocodiles sighting in the study area. The study regions made, are as follows:

1. Study Region A - **Chandoli Dam to Sagaon with River** - This is the first and uppermost study region with first part of river Warana and river Kanasa. The length of this study region of river Warana is 48 km while that of river Kanasa is 14 km.
2. Study Region B - **River Kadavi** - River Kadavi is a major tributary of river Warana. It begins its course in the Sahyadri ranges at the border of Kolhapur and Sangli district, and merges in River Warana near village Thergaon. Total length of river Kadavi is about 46 km and covered study area from a dam up to where it merges in river Warana is about 40.8 km.
3. Study Region C - **Sagaon to Shigaon with River Morana** - This part of river Warana runs in the east direction on the famous Deccan plateau and it includes part of river Warana and river Morana. The length of this study region of river Warana is 46 km while that of river Morana is 14 km.
4. Study Region D - **Shigaon to Haripur** - This is the last study region. The length of this study region of river Warana is 36 km.

Methods

The survey methods that are used in present study on nesting biology of crocodiles are described below.

1. Daylight Ground Counts: Ground counts on foot or by boat in river or by a vehicle along the river in daylight is more effective method. When the crocodiles come out of water for basking or for any other purpose they can be sighted easily. This method is easy during breeding season as all adults congregate on a small area. But it is not a suitable method as one can cover a small area for survey. Author have tried to cover maximum study area in a day period and collected maximum data.

2. Night Counts: Counting at night, usually from a boat, with the aid of a spotlight is the most widely used method of censusing crocodiles. For this method a spotlight of 400,000 candlepower Q-beam and 12-volt headlights is used. This spotlight causes the shining of eyes of crocodiles. The tapetum of eyes glows red in spotlight and can be seen from a considerable distance. Usually the spotlight is used by poachers for hunting in night. The method may be biased as the younger animals avoid the spotlight in the night. All crocodiles sighted were classified by total length (TL) as hatchlings (TL<30 cm), juveniles (TL= 30-90 cm), sub-adults (TL= 90-180 cm) or adults (TL>180 cm). Crocodiles that submerged before TL could be determined were classified as 'eyeshine only' (EO). Encounter rates were calculated as the number of crocodiles observed per kilometre of survey route (Platt and Thorbjarnarson 2000a).

Statistical Approach:

Eltringham (1972) and Magnusson (1978, 2008) have used statistical approach to estimate the population size from incomplete counts of elephants (Loxodonta africana) and crocodiles (*C. porosus*) respectively. Magnusson (1978, 2008) has used the probability and variance to the data of incomplete nest counts from swamps of the Liverpool River system, northern Australia. The data about crocodile population obtained by two methods from Warana basin is used statistically in the following way. Let us assume,

Number of crocodiles, seen by both surveys = B
Number of crocodiles, seen by survey 1 but not by survey 2 = S1
And Number of crocodiles, seen by survey 2 but not by survey 1 = S2

If M is the unknown number missed by both surveys and N is the total number of crocodiles, also unknown, then the exhaustive frequencies and the probabilities associated with them are:

$B + S1 + S2 + M = N$

$P1P2 + P1 (1-P2) + P2 (1-P1) + (1-P1) (1-P2) = 1$; P1 being the probability of crocodiles being seen by the first survey and P2 the probability of crocodiles being seen by the second. Hence the unknown parameters can be estimated from the known frequencies B, S1 and S2, by:

$P1 = B/(B + S2)$
$P2 = B/(B + S1)$
$M = S1S2/B$
$N = (B + S1)(B + S2)/B$ -- eq. 1

The model is logically equivalent to that of the Petersen estimate. On the first survey a sample is mapped (marked), the sample of the second survey comprising some entities previously mapped (recaptures), others unmapped. The difference lies in the symmetry of the present model: the first and second surveys are interchangeable. Nonetheless, the well-explored mathematics of the Petersen estimate can be adapted easily to this model. Chapman (1951) has given a correction for the Petersen estimate. Applying this to equation 1 our estimate becomes:

$$N = \frac{(S_1 + B + 1)(S_2 + B + 1)}{(B + 1)} - 1 \quad \text{-----------------------eq. 2}$$

This is, in contrast to the estimate of eqn. (1), exactly unbiased when:

$$S_1 + S_2 + 2B \geq N$$

Its variance can be estimated by a translation of Seber’s (1973:60) formula which is also exactly unbiased when:

$$S_1 + S_2 + 2B \geq N$$

$$Var(N) = \frac{S_1 S_2 (S_1 + B + 1)(S_2 + B + 1)}{(B + 1)^2 (B + 2)} \quad \text{------------------------eq. 3}$$

The use of this method assumes that the counts of the 2 surveys are independent and that there is a constant probability of seeing each crocodile by a given method of survey.

Observationas and Results

Number of crocodiles counted by two survey methods Daylight Ground Counts and Night Counts in different study regions are summarised in Tables 1 and 2 respectively and comparative chart for both survey methods is given in Table 3.

Table 1. Summary of Daylight Ground Counts for *Crocodylus palustris* performed in Warana basin during 2014-15.

Sr. No.	Study Region	Hatchlings	Juveniles	Sub-adults	Adults	Total
1	A	0	0	0	0	0
2	B	2	0	0	1	3
3	C	5	3	0	3	11
4	D	6	3	2	4	15

Table 2. Summary of Night Counts for *Crocodylus palustris* performed in Warana basin in 2014-15.

Sr. No.	Study Region	Eye-shine	Sub-adult	Adult	Total
1	A	0	0	0	0
2	B	7	0	2	9
3	C	14	2	3	19
4	D	18	3	3	24

Table 3. Observations of crocodile population from Warana basin with two survey methods.

Sr. No.	Survey Methods	A	B	C	D	Total
1	Daylight Ground	0	3	11	15	29
2	Night	0	9	19	24	52
3	Both surveys	0	2	5	10	17

Discussion

By applying the probability and variance to the data following population size estimate is obtained.

Total number of crocodiles observed along the river Warana and Kadavi by first survey method - daylight ground counts are 29 (S1= 29), crocodiles observed by second survey method - night counts are 52 (S2= 52) and crocodiles observed by both surveys are 17 (B= 17). Hence the probability of seeing a crocodile by daylight ground count is estimated as P1= 17/(17 + 52) = 0.25, and from the night count is estimated as P2= 17/(17 + 29)= 0.37. The number missed by both surveys is estimated as M= 29 x 52/17= 88.71 (89 and the total number, both counted and uncounted, is estimated by eqn. (2) as N = 181.78 ie 182). It has an approximate variance (eqn. 3) of 805.93 (ie 806 and hence a standard error of $\sqrt{806} = \pm 28.39$). Above statistics about crocodile population size from Warana basin by two survey methods Daylight Ground Counts and Night Counts for all study regions and total study area is summarised in Table 4.

Table 4. Summary of probability and variance of observations about crocodile by two survey methods from Warana basin. S1- Daylight ground counts survey; S2- Night counts survey method; B- Observations by both surveys; P1- Probability of first survey; P2- Probability of second survey; M- Crocodiles missed by both surveys; N- Total number of crocodiles.

Sr. No.	Study region	S1	S2	B	P1	P2	M	N	Variance	SE
1	A	0	0	0	0	0	0	0	0	0
2	B	3	9	2	0.8	0.4	14	23	54	7.35
3	C	11	19	5	0.21	0.31	42	71	353	18.79
4	D	15	24	10	0.4	0.29	36	83	226	15.03
5	Total	29	52	17	0.25	0.37	89	182	806	28.39

The use of this method assumes that the counts of the two surveys are independent and that there is a constant probability of seeing each crocodile by a given method of survey. The first assumption is critical. The second assumption is not critical. The pairs of surveys have been simulated in which the probability of seeing a crocodile, rather than being a constant for a survey, was a random draw from a beta distribution of fixed mean and variance, different distributions being used for the 2 surveys. These produced estimates similar to those of control simulations in which probabilities were set at the means of the beta distributions.

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Management of Estuarine Crocodiles (*Crocodylus porosus*) in Queensland, Australia: A Review of Historical Sightings, Captures and Attacks

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Abstract

We reviewed historical estuarine crocodile sighting (2003-2015), removal (1985-2015) and attack (1971-2015) data for Queensland. The majority of estuarine crocodiles have been sighted and captured for management purposes along the populated east coast of Queensland between Townsville and Cooktown, mostly in and around Cairns. A mean of 226 estuarine crocodiles have been sighted per year (2003-2014; mean TL 2.6 m; range: 0.30-6.0 m), most were sighted during the day (0600-1800 h), with the lowest numbers sighted from June-August (non-breeding/dry season). However, the number of sightings has been increasing over time with a mean of 340 per year since 2011. A total of 565 estuarine crocodiles (mean male: 2.87 m, max: 4.8 m; mean female: 2.12 m, max: 3.4 m) have been captured and either removed (N= 529) or relocated (N= 36) for management purposes since 1985. The number of captures was positively related to the number of sightings in each location. The number of crocodiles captured has fluctuated each year depending upon the management program in place at the time (minimum of <10/year: 1992-1997; maximum of 58: 2014). The highest number of crocodiles were captured November-January (breeding/wet season), with a significant decline July-August (non-breeding/dry season) similar to that observed for sightings. The majority of crocodiles captured were male (70%). However, this ratio has changed over time. A large number of females (37.7%) were captured in October-November (breeding/nesting season). There have been 34 crocodile attacks in Queensland since 1971 (35% fatal), with the mean size of crocodiles responsible for fatal attacks (4.0 m) larger than that for non-fatal (2.5 m). The majority of attacks have involved local people (89.2%), specifically adult males (mean age: 44), with only 9 attacks on females (25%). The highest number of attacks has occurred in the Cairns and Port Douglas-Daintree areas, involving people in the water or on the edge (94.7%). There has been an increase in the rate of non-fatal crocodile attacks over time with an average of 1/year since 1996. The highest number of attacks occurred December-February, with few occurring between May and August (non-breeding/dry season), again reflecting a pattern similar to sightings and removals.

Long-term Monitoring Reveals Declines in Endemic Australian Freshwater Crocodiles following Invasion by Exotic Cane Toads

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Abstract

Invasive predators can cause population declines in native prey species, but empirical evidence linking declines of native predators to invasive prey is relatively rare. Here we document declines in an Australian freshwater crocodile (*Crocodylus johnstoni*) population following invasion of a toxic prey species, the cane toad (*Rhinella marina*). Thirty-five years of standardized spotlight surveys of four segments of a large river in northern Australia revealed that the density of freshwater crocodiles decreased following toad invasion, and continued to decline thereafter. Overall, intermediate-sized freshwater crocodiles (0.6-1.2 m) were most severely impacted. Densities of Saltwater crocodiles (*C. porosus*) increased over time and were generally less affected by toad arrival, although toad impacts were inconsistent across survey sections and size classes. Across the entire river, total freshwater crocodile densities declined by 69.5% between 1997 and 2013. Assessments of this species' status within other large river systems in northern Australia, where baseline data are available from before the toads arrived, should be prioritised. Our findings highlight the importance of long-term monitoring programs for quantifying the impacts of novel and unforseen threats.

Introduction

Biological invasions are a major threat to global biodiversity (Lövei 1997; Chornesky and Randall 2003). Introduced predators, in particular, have caused declines and extirpations of many vertebrate species (Fritts and Rodda 1998; Wiles *et al.* 2003; Johnson 2006). Similarly, competitive interactions between invasive and native species have caused extinctions or extirpations in various vertebrate groups (island birds, (Sax, Gaines and Brown 2002); freshwater trout, (Allendorf and Lundquist 2003); freshwater turtles (Cadi and Joly 2004). However, empirical evidence linking invasive prey species to declines in native predator populations is relatively rare.

One notable exception involves the invasion of cane toads (*Rhinella marina*) throughout tropical Australia (Phillips *et al.* 2007). Cane toads were introduced to eastern Queensland from 1935-1937 to control two beetle pests in sugarcane crops (Tyler 1976; Easteal 1981). Since their introduction, the toads have colonised more than 1.2 million km^2 of Australia (Urban *et al.* 2007). The invasion has had deleterious effects on a range of native Australian fauna because the toads possess a cardiac glycoside to which much of the native fauna has no prior evolutionary history (Gowda, Cohen and Khan 2003; Shine 2010). Ingestion of the toxin causes mortality in many frog-eating predators (Burnett 1997), including quolls, lizards, and snakes (Lever 2001; Phillips *et al.* 2003; Pearson *et al.* 2013). However, with few exceptions (Brown *et al.* 2011), there remains a paucity of monitoring data before and after the arrival of toads with which to assess their longer-term impact on native fauna at the population level.

The short-term impact of cane toads on freshwater crocodiles (*Crocodylus johnstoni*) has been the subject of several studies in Australia, but results remain equivocal, with reported impacts varying from very significant (Letnic, Webb and Shine 2008; Britton, Britton and McMahon 2013) to negligible (Catling *et al.* 1999; Doody *et al.* 2009; Somaweera and Shine 2012). Interestingly, where negative impacts have been reported, declines have been biased toward intermediate-size classes (Letnic *et al.* 2008; Britton *et al.* 2013). Nonetheless, previous studies have mostly involved single surveys before and after the arrival of toads, and have only considered impacts two to three years post-invasion. The longer-term effects of cane toads on freshwater crocodile population size and structure remain unknown. Because effects of invasive species can be temporary or amplified over time (Strayer *et al.* 2006; Strayer *et al.* 2011; Willis and Birks 2006), understanding the impacts of cane toads requires consideration of both short-term and long-term perspectives. Laboratory trials suggest that the other Australian crocodile species, the Saltwater crocodile (*C. porosus*), is less vulnerable to the toad's toxin than is the freshwater crocodile (Smith and Phillips 2006), which is generally supported by a lack of reports of dead Saltwater crocodiles following toad invasion. Here, we use standardized monitoring data gathered over 35 years (Webb *et al.* 1994; Fukuda *et al.* 2013), from a large river in the Northern Territory of Australia with tidal and non-tidal segments, to document changes in density and population structure of freshwater and Saltwater crocodiles, before and after the invasion of cane toads.

Materials and Methods

Study species

Two crocodilian species occur in northern Australia, where they are generally considered apex predators, feeding on insects, crustaceans, fish, frogs, turtles, mammals, and waterfowl (Webb and Manolis 1989, 2010). The endemic Australian Freshwater crocodile, *C. johnstoni*, is usually restricted to freshwater habitats. The Saltwater crocodile, *C. porosus*, occurs throughout the Indo-Pacific region and inhabits freshwater, brackish water, and saline water (Webb and Manolis 1989). Both *C. johnstoni* and *C. porosus* are known to prey on *R. marina* (Covacevich and Archer 1975; Letnic and Ward 2005).

Freshwater and Saltwater crocodiles in Australia were extensively hunted for their skins until they were protected in 1964 and 1971, respectively. The population of Saltwater crocodiles in the Northern Territory is now thought to be similar to the population size before extensive commercial hunting began (Fukuda *et al.* 2011). The post-hunting increase in the abundance of freshwater crocodiles is assumed to be similar to the recovery recorded for Saltwater crocodiles (Webb *et al.* 1994).

Study Area

Crocodile surveys were conducted on the Daly River in the Northern Territory, Australia (Fig. 1). Climate in the study area is tropical monsoonal with a distinct dry season (May-October) and wet season (November-April). The Daly River is tidal and seasonally saline for approximately 100 km upstream from the mouth, where the banks are generally lined with mangroves. The upstream reaches of the Daly River extend for 200 km and are freshwater, non-tidal, and contain a mix of sandy and rocky banks dominated by riparian trees (eg *Pandanus* and *Melaleuca* species).

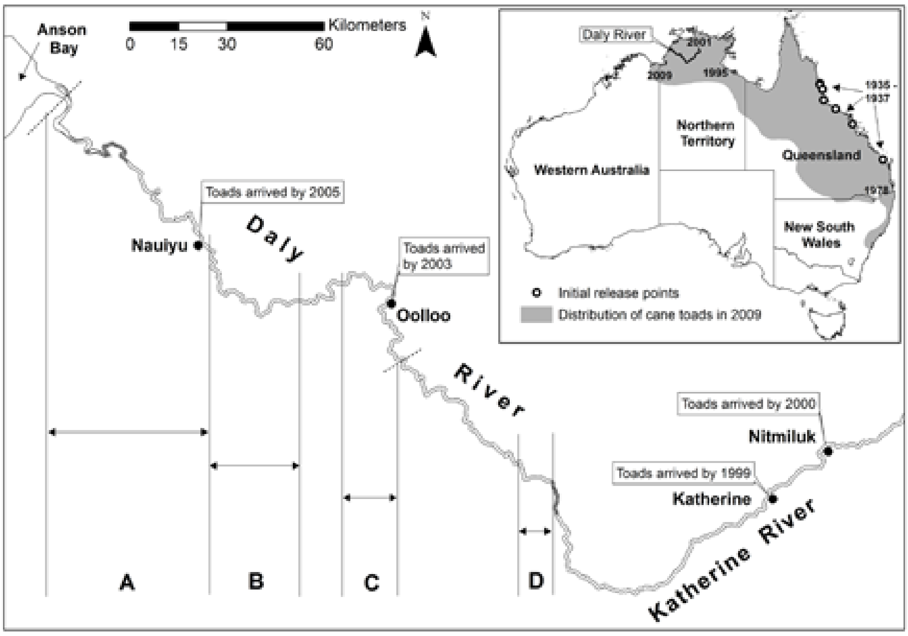

Figure 1. Distribution of cane toads (*Rhinella marina*) in Australia, and the survey sections (A-D) where freshwater crocodiles (*Crocodylus johnstoni*) were monitored in the Daly River of the Northern Territory, Australia. Cane toad distribution data were taken from (Tingley *et al.* 2014).

Cane toads invaded from the east and were established in the upper reaches of the Daly River drainage at Katherine and in Nitmiluk National Park by 1999-2000 (I. Morris, J. Burke, personal communication), although some earlier sightings were reported (FrogWatch 2013). Standardized nocturnal road surveys revealed that the toads then spread downstream along the Katherine River and were established in the Daly River at Oolloo by 2003 and Nauiyu by 2005 (Doody *et al.* 2009); G. Wightman, personal communication). Adult cane toads can apparently survive in 40% seawater (Liggins and Grigg 1985), allowing them to inhabit the lower reaches of the Daly River.

Monitoring data

Crocodile surveys were carried out once a year from 1978-2013 in four sections (A-D in Fig. 1). Section A (87.6 km) is the most downstream, tidally-influenced section, with a salt wedge moving progressively upstream during the dry season.

Section B extends upstream from a concrete road crossing that stops tidal influences and the upstream penetration of saline water. Sections B (51.4 km), C (57.8 km) and D (16.5 km) are freshwater, with sandy and rocky banks. Survey frequency differed between survey sections, with section A having the most frequent surveys (23/35 years), followed by sections B and C (18 surveys each) and section D (17 surveys) (Table A1 in Supplementary Material).

Crocodile monitoring in each section followed standardized spotlight survey protocols (Messel *et al.* 1981; Fukuda *et al.* 2013). Surveys were conducted during the dry season, at night, mostly during the cooler period of the year (July to September), and at low tide in tidal areas, when mudbanks were exposed below the mangroves during the surveys. Under these conditions, spotlight river surveys are precise and repeatable for detecting long-term population trends of crocodilians (Webb *et al.* 1987; Fujisaki *et al.* 2011; Fukuda *et al.* 2011). Crocodiles were located by experienced observers with a spotlight (100W or 200 000 candlepower) from a boat travelling at 15-20 km/h along the river. Each crocodile sighted was approached as closely as possible to determine species (based on their distinctive head morphology) and to estimate total length in contiguous 0.3 m size classes: the smallest animals were grouped as a <0.6 m class. Saltwater crocodiles <0.6 m are usually hatchlings from the preceding (current calendar year) nesting season, while freshwater crocodiles in this size class include individuals <3 years of age.

During these surveys, animals which could not be approached closely enough to confirm species and/or size were noted as "eyes only". The proportion of "eyes only" animals varies with observer confidence in making species and size determinations (Webb *et al.* 1987), crocodile density, water depth (affecting the ability to approach), wariness, and other factors. On average, 55.4% (SE= 1.7, N= 77 surveys from 1978-2013) of crocodiles sighted were "eyes only". These observations were excluded from the analysis here, which required both species and size to be known, which added randomly to the variance around our relative abundance estimates, but not to the trends in those estimates over time.

Statistical Analyses

To determine whether cane toad establishment influenced relative densities of Saltwater and Freshwater crocodiles (number of crocodiles per linear km), we conducted time-series intervention analyses (Huitema and McKean 2000). This approach is used to examine changes in a dependent variable before and after an intervention and is increasingly used in ecology [eg hurricane effects on snail abundance: (Prates *et al.* 2011); algal blooms on cod populations: (Chan *et al.* 2003); canopy disturbance events: (Druckenbrod *et al.* 2013); cane toad impacts: (Brown *et al.* 2011)]. Specifically, we used segmented regression to examine changes in the level and slope of the relationship between relative crocodile density and time, before and after toads became common on the Daly River. For section A of the river, where we had the most complete crocodile survey data, we used 2005 as the date of cane toad arrival (based on consistent reporting of toads at Nauiyu). No crocodile surveys were conducted in sections B-D from 1998-2008, which covers the period of toad arrival. Specifying a precise date of toad arrival was therefore unnecessary for sections B-D.

Relative densities of freshwater and Saltwater crocodiles in each survey section were modelled separately according to the following multiple regression equation: $Y_i=\alpha_0 + \beta_1 T_i + \beta_2 P_i + \beta_3 S_i + \varepsilon$ (eq. 1); where Y_i is the untransformed relative density of crocodiles in year i; α_0 is the pre-toad intercept, the relative density of crocodiles at time zero; β_n are regression coefficients describing the effects of independent variables T, P, and S; and ε represents an error term. For year i, T represents the number of years since that survey section was first surveyed, P is the presence/absence of toads (coded as 0 before 2005, and 1 thereafter), and S is a variable that contains zeroes up to 2007 and the number of years since toads arrived thereafter [see Huitema and McKean (2000) for further details].

Specifying the segmented regression in this way produces three intuitive regression coefficients, which can be interpreted as follows: β_1 is the pre-toad slope, which describes the average increase in relative density per year; β_2 is the post-toad level change, which measures the change in elevation of the time-series associated with the arrival of toads (ie the difference between the predicted values of Y_i before and after toad arrival), and β_3 is the post-toad slope change, the difference between the post-toad and pre-toad slopes. Regression coefficients were estimated via maximum likelihood using generalized least squares in R 3.0.1 [*gls* routine in library *nlme*, (Barton 2013; R Development Core Team 2013)].

The errors in eq. 1, ε, are assumed to be independently and identically distributed with constant variance. To determine whether accounting for temporal autocorrelation and heterogeneous variances could improve model fit, we also built models that included first-order autocorrelation structures and constant variances within the two levels of variable T (toad presence). First-order autocorrelation structures model the residuals at time t as a function of the residuals at time t-1 (Zuur *et al.*

2009). Different variances were estimated for each level of T because preliminary analyses suggested heterogenous spread in the residuals before vs. after toad arrival in some of the time-series, violating the homogeneity of variance assumption.

The appropriate level of model complexity for each time series was selected in two steps. First, four models with and without first-order autocorrelation and different variance structures were compared using Akaike's Information Criterion corrected for small sample sizes (AICc) (Zuur *et al*. 2009), and the model structure with the lowest AICc was retained for subsequent analyses. These initial models contained all of the fixed effects in eq. 1. Second, we used AICc to rank 5 candidate models containing different combinations of fixed effects (ranging from an intercept-only model to a saturated model with all fixed effects). To account for model selection uncertainty, we calculated weighted averages of parameter estimates across the models comprising ~95% of the Akaike weights (Barton 2013; R Development Core Team 2013).

Effects of cane toads on freshwater crocodiles may be size-specific (Letnic *et al*. 2008), and so we conducted the above analyses on all size classes of freshwater crocodiles combined, as well as on each size class separately. For Saltwater crocodiles, we only fit models to data from sections A and B, as this species was rare in the upper freshwater reaches. Furthermore, size-specific models were only fit to data from section A for Saltwater crocodiles, due to low numbers of observations in many size classes in the other sections. To examine whether the cane toad establishment influenced the size structure of freshwater crocodile populations, we repeated all of the aforementioned analyses using average total length as the response variable. These size structure analyses were only conducted on Saltwater crocodile data from sections A and B.

Results

Relative freshwater crocodile densities on the Daly River declined following the colonisation of toads in all four river segments (Fig. 2). Relative density declined by 75.3% between 1997 and 2013 in Section A, but by 66.1% between 2004 and 2013 after the toads arrived. Relative densities declined by 68.6%, 67.5%, and 56.6% from 1997 to 2013 in sections B, C, and D, respectively. Across all four sections combined the relative density of freshwater crocodiles declined by 69.5% between 1997 and 2013.

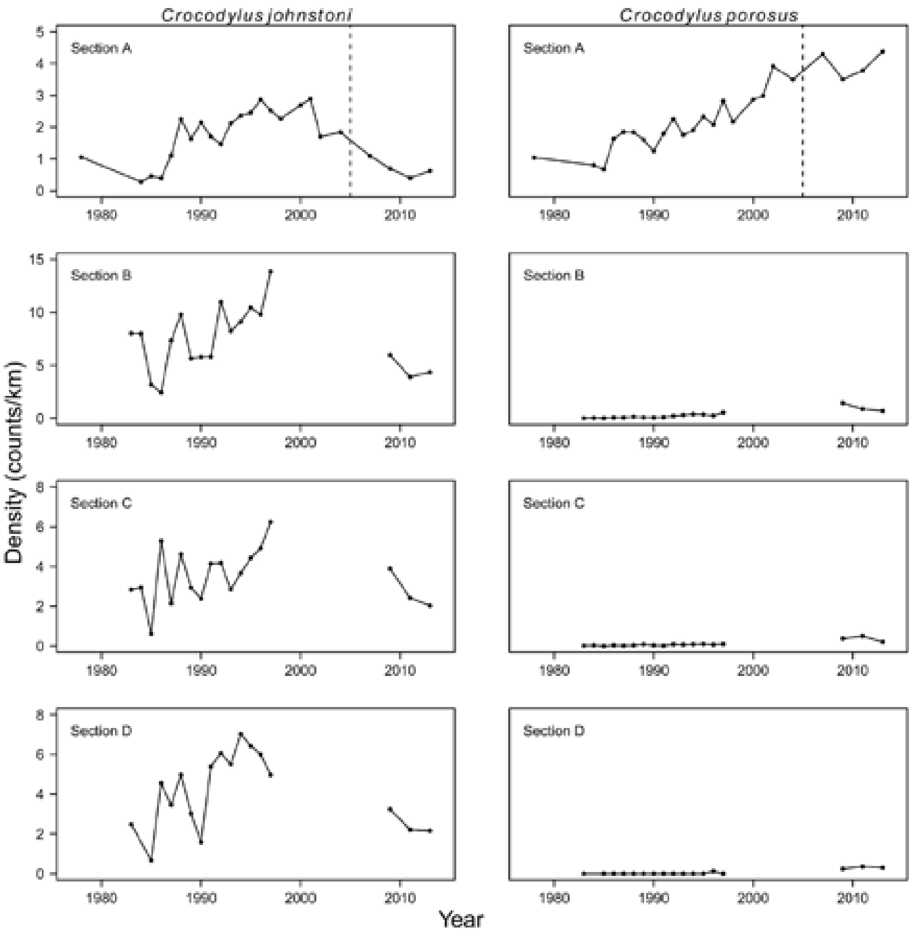

Figure 2. Trends in the relative densities of freshwater crocodiles (*Crocodylus johnstoni*) and Saltwater crocodiles (*C. porosus*) at four sites (rows) in the Daly River, NT, Australia. The dotted line demarcates the point at which invasive cane toads became common in section A (first row). Note that the scale on the y-axis is consistent within sites, but differs between sites.

Time-series intervention analyses revealed decreases in the level and slope of the relationship between freshwater crocodile densities and time coincident with the arrival of toads in all four survey sections (Table 1; Fig. 2). When each size class was analysed separately, intermediate-sized freshwater crocodiles were generally found to undergo the most dramatic declines (Fig. 3). Specifically, there was a strong decrease in the predicted relative densities of 0.6-0.9 m and 0.9-1.2 m freshwater crocodiles across all four survey sections (post-toad level change: Fig. 4). There was also evidence that intermediate size classes started to decline following toad establishment (post-toad slope change: Fig. 4), although confidence intervals for slope-change estimates in some survey sections overlapped zero, and both smaller and larger size classes declined in section D relative to the other survey sections (Fig. 4). Across the entire river, freshwater crocodiles that were 0.6-0.9 m and 0.9-1.2 m declined by 90.9% and 89.6%, respectively, following toad arrival (1997-2013). These declines in intermediate size classes resulted in increases in the mean lengths of freshwater crocodiles sighted following toad arrival in all four sections (post-toad level change: Table 2; Fig. 5).

Table 1. Model-averaged coefficients and 95% confidence intevals showing the effect of cane toad establishment on the relative densities of freshwater crocodiles (*Crocodylus johnstoni*) and Saltwater crocodiles (*C. porosus*) in all size classes combined in the Daly River, NT, Australia. Bold 95% confidence intervals do not overlap 0. Absence of a coefficient estimate indicates that a variable was not selected in the highest ranked models according to AIC. Models were not fitted to the *C. porosus* data from sections C and D due to low numbers of observations at these sites.

		Pre-toad slope		Post-toad level change		Post-toad slope change	
Species	Section	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
C. johnstoni	A	0.016	(-0.085, 0.117)	-1.696	(-2.746, -0.645)	-0.154	(-0.265, -0.042)
C. johnstoni	B	0.374	(-0.022, 0.769)	-10.675	(-17.293, -4.058)	-0.832	(-1.437, -0.227)
C. johnstoni	C	0.112	(-0.093, 0.317)	-3.351	(-6.609, -0.093)	-0.586	(-0.880, -0.291)
C. johnstoni	D	0.311	(0.105, 0.516)	-7.285	(-11.287, -3.282)	-0.585	(-0.870, -0.300)
C. porosus	A	0.111	(0.090, 0.132)	-	-	-0.052	(-0.203, 0.100)
C. porosus	B	0.031	(0.022, 0.041)	0.598	(0.363, 0.833)	-0.217	(-0.274, -0.161)

Table 2. Model-averaged coefficients and 95% confidence intevals showing the effect of cane toad establishment on mean total length of freshwater crocodiles (*Crocodylus johnstoni*) and Saltwater crocodiles (*C. porosus*) in the Daly River, NT, Australia. Bold 95% confidence intervals do not overlap 0. Absence of a parameter estimate for a given variable indicates that the variable was not selected in the highest ranked models. Models were not fitted to the *C. porosus* data from sections C and D due to low numbers of observations at these sites.

		Pre-toad slope		Post-toad level change		Post-toad slope change	
Species	Section	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
C. johnstoni	A	-0.002	(-0.012, 0.008)	0.624	(0.363, 0.884)	0.015	-0.055, 0.086)
C. johnstoni	B	-0.009	(-0.027, 0.009)	0.597	(0.172, 1.022)	-0.038	(-0.148, 0.071)
C. johnstoni	C	-0.006	(-0.022, 0.010)	0.509	(0.132, 0.886)	0.024	(-0.075, 0.123)
C. johnstoni	D	-0.011	(-0.026, 0.004)	0.793	(0.440, 1.147)	0.049	(-0.036, 0.134)
C. porosus	A	0.023	(0.009, 0.038)	0.250	(-0.128, 0.628)	0.043	(-0.044, 0.131)
C. porosus	B	0.004	(-0.005, 0.014)	-	-	-0.029	(-0.051, -0.008)

Across the entire river, relative Saltwater crocodile densities increased by 74.2% between 1997 and 2013 (Fig. 2). In section A, relative densities increased by 25.1% between 2004 and 2013, whereas relative densities increased by 26.4%, 104.8%, and 300.0% in sections B, C, and D, respectively, between 1997 and 2013. Overall densities of Saltwater crocodiles declined with distance upstream.

There was a positive relationship between Saltwater crocodile densities and time in sections A and B, but the effect of toad arrival differed between survey sections. In section A, there was no evidence of a change in the level or slope of the relationship between total Saltwater crocodile density and time coincident with the arrival of toads (Table 1). However, in section B, the relative density of Saltwater crocodiles increased following toad arrival, but decreased thereafter (Table 1). Relative densities of 0.6-0.9 m and 1.5-1.8 m Saltwater crocodiles decreased following toad invasion in section A (post-toad intercept change: Fig. 6), and there was a decrease in the slope of the relationship between density and time in the 0.9-1.2 m and 1.8-2.1 m size classes (post-toad slope change: Fig. 6). Although small sample sizes preclude a formal statistical analysis, these patterns appeared to be generally inconsistent across the other three river sections (Figs. A1-A4 in Appendix). Trends in the mean length of Saltwater crocodiles also varied across river sections (Fig. 5). In section A, sizes increased over time, but intercept and slope-change estimates were highly uncertain, with wide 95% confidence intervals (Table 2). In contrast, in section B, sizes declined following toad arrival.

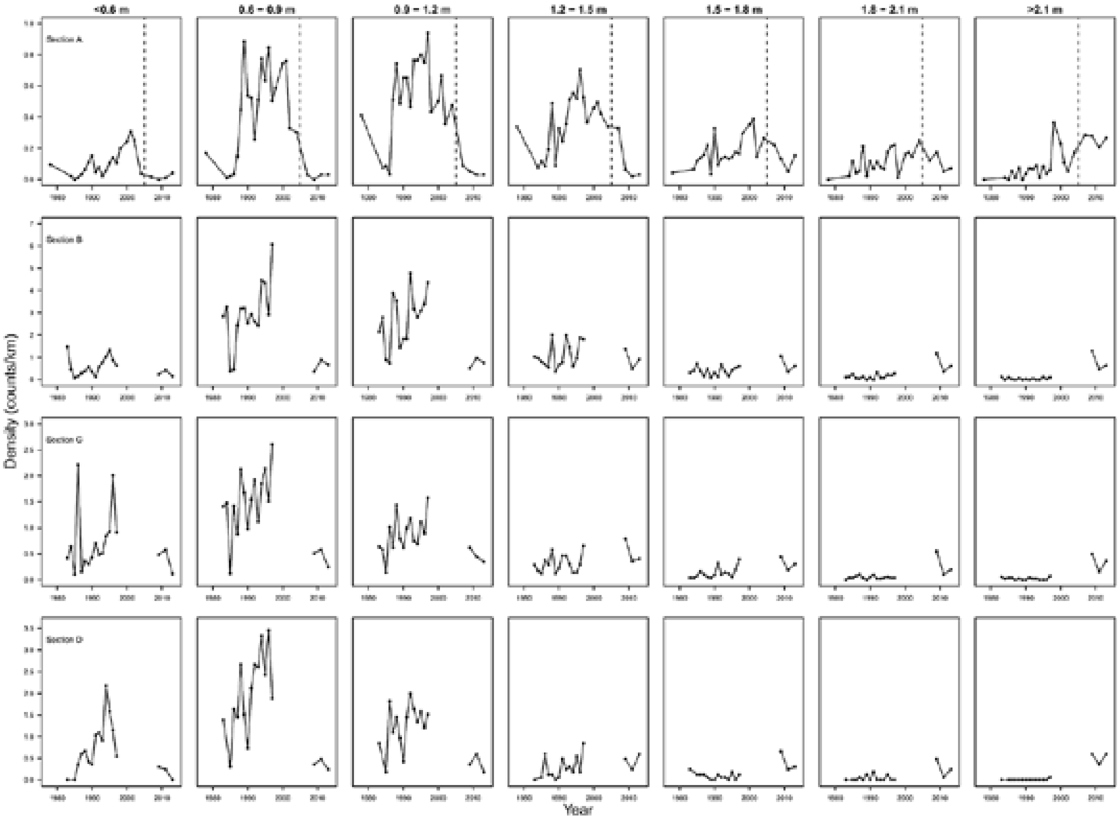

Figure 3. Trends in the relative densities of seven different size classes (columns) of freshwater crocodiles (*Crocodylus johnstoni*) at four sites (rows) in the Daly River, NT, Australia. The dotted line demarcates the point at which invasive cane toads became common in section A (first row). Note that the scale on the y-axis is consitent within sites, but differs between sites.

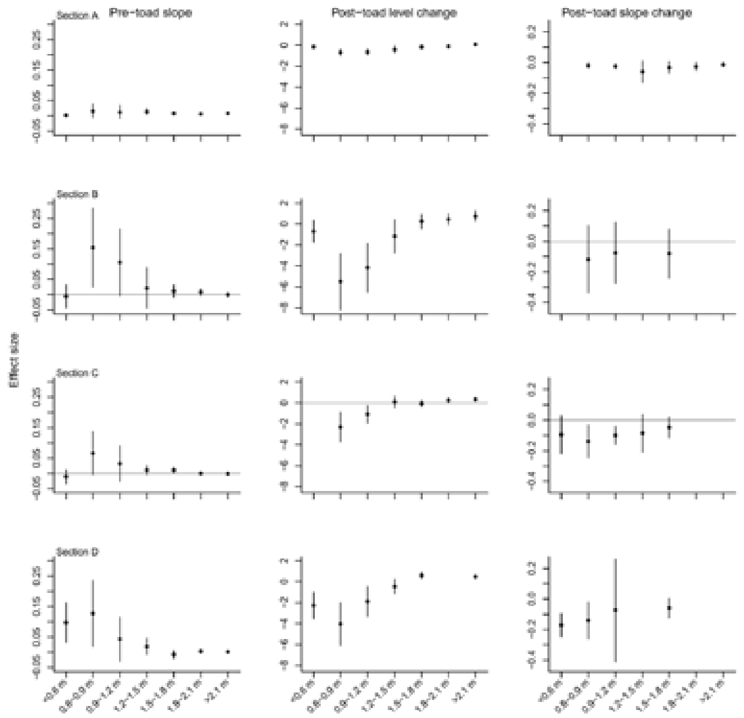

Figure 4. Model-averaged parameter estimates and 95% confidence intervals showing the effect of cane toad establishment on the relative densities of 7 different size classes of freshwater crocodiles (*Crocodylus johnstoni*) at four sites (rows) in the Daly River, NT, Australia. Absence of a parameter estimate for a given variable and size class indicates that the variable was not selected in the highest ranked models. The grey horizontal line in each plot demarcates 0.

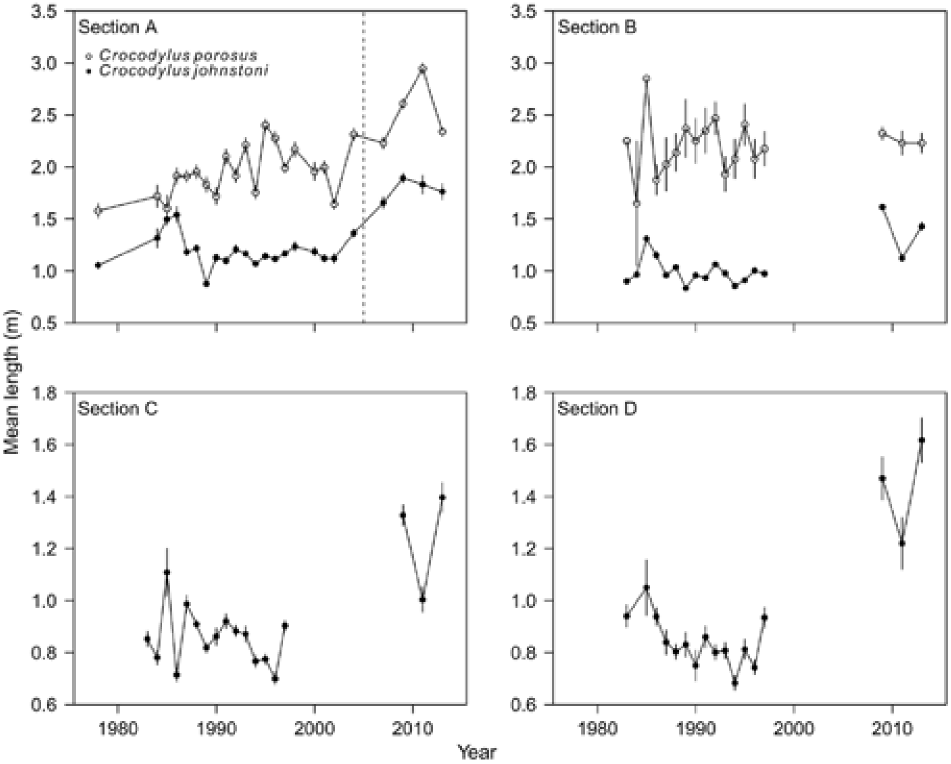

Figure 5. Trends in the average total lengths of freshwater crocodiles (*Crocodylus johnstoni*) and Saltwater crocodiles (*C. porosus*) in four sections (A-D) of the Daly River, NT, Australia. The dotted line demarcates the point at which invasive cane toads became common in section A.

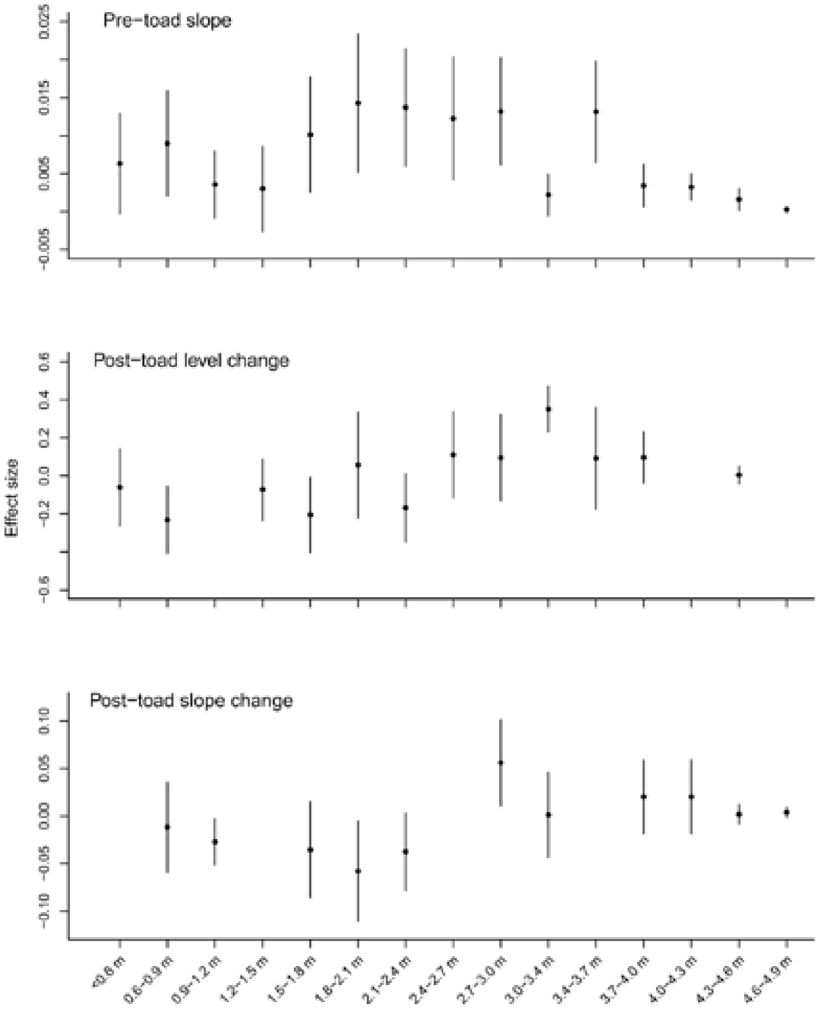

Figure 6. Model-averaged parameter estimates and 95% confidence intervals showing the effect of cane toad establishment on the relative densities of seven different size classes of saltwater crocodiles (*Crocodylus porosus*) in section A of the Daly River, NT, Australia (models were not fitted to the data from sections B-D due to low numbers of observations at these sites). Absence of a parameter estimate for a given variable and size class indicates that the variable was not selected in the highest ranked models. The grey horizontal line in each plot demarcates 0.

During surveys in 2009, 2011, and 2013, we observed freshwater crocodiles feeding on cane toads, and occasionally found dead freshwater crocodiles with no obvious signs of trauma in the 0.9-1.5 m size range. No such observations were made on Saltwater crocodiles.

Discussion

Colonisation of the Daly River by cane toads coincided with declines in the densities of freshwater crocodiles. In all four survey sections, we detected declines in the elevation and slope of the density time-series concurrent with toad arrival. Declines in density were particularly pronounced in the 0.6-0.9 m and 0.9-1.2 m size classes. Across the entire river, these declines in intermediate size classes resulted in an increase in the average length of the surviving freshwater crocodiles, from 1.17 m in 1997 to 1.90 m in 2013. In contrast, there was less evidence that small (<0.6 m) crocodiles declined following toad arrival. The only exception was survey section D, where there were decreases in the level and slope of the density time-series. However, in all four survey sections, small crocodile declines appear to have begun before the arrival of toads. Freshwater crocodiles of different sizes feed on different prey items (Webb and Manolis 2010) and (Somaweera *et al*. 2011) found that free-ranging hatchlings were more likely to consume native frogs than cane toads, even though both prey items were common. Furthermore, cane toads that are small enough to be consumed by hatchling freshwater crocodiles typically possess very low levels of toxin due to strong allometry in toxin content (Shine 2010). Despite this allometry, we also found that large freshwater crocodiles were less affected by toad establishment than were intermediate size classes. This finding accords with the results of (Smith and Phillips 2006) which suggested that even a large cane toad may not possess a sufficient amount of toxin to kill a large freshwater crocodile. Taken together, these results indicate that the impact of cane toads on freshwater crocodiles is size-specific, and that intermediate size classes are at greater risk.

Our surveys paint a more uncertain picture of toad impacts on Saltwater crocodiles. In section A, the total density of Saltwater crocodiles continued to increase unabated following toad invasion. In section B, Saltwater crocodile densities increased immediately following toad arrival but then began to decline. Furthermore, when each size class within section A was analysed separately, there were decreases in either the elevation or slope of the relative density time-series in the 0.6-0.9 m, 0.9-1.2 m, 1.5-1.8 m and 1.8-2.1 m size classes, suggesting that 0.6-1.2 m individuals may be at greater risk in both crocodilian species. However, Saltwater crocodile declines in these intermediate size classes appear to be less consistent across different river sections compared to the results for freshwater crocodiles. Thus, overall, our results suggest that Saltwater crocodiles were less affected by toad invasion than were freshwater crocodiles. This observation supports the results of laboratory trials, which demonstrated that Saltwater crocodiles are less susceptible to the toad's toxin than are freshwater crocodiles, even in large doses (Smith and Phillips 2006).

While our results show that significant declines in the density of freshwater crocodiles coincide with cane toad invasion, other factors may also have contributed to the decline. For example, we cannot reject the possibility that interspecific competition (predation and exclusion) with Saltwater crocodiles, that are more tolerant to cane toad toxin, may have exacerbated the observed declines in freshwater crocodiles. However, several lines of evidence suggest that the arrival of cane toads rather than competition with Saltwater crocodiles is a more plausible mechanism for the observed declines in freshwater crocodiles. First, freshwater crocodiles declined in the upstream sections of the Daly River, where Saltwater crocodiles occur at extremely low densities (Fig. 2). Second, our findings accord with the results of laboratory trials, which suggested that Saltwater crocodiles and larger freshwater crocodiles are less susceptible to cane toad toxin (Smith and Phillips 2006). Third, the size classes that declined most dramatically in our analyses also suffered more serious declines following the arrival of cane toads on a different river system in the Northern Territory (Letnic *et al.* 2008). Fourth, freshwater crocodiles (but not Saltwater crocodiles) were observed feeding on cane toads during surveys and dead individuals with no obvious signs of trauma were in these intermediate size classes. It is also worth noting that crocodile habitats in the study area changed little over the study period (Fukuda *et al*. 2007) and are unlikely to explain our results.

The magnitude of the declines reported here contrast with the results of (Somaweera and Shine 2012) and (Doody *et al*. 2009), which found no effects of toad arrival on freshwater crocodile populations. (Somaweera and Shine 2012) studied freshwater crocodiles immediately following toad arrival in Lake Argyle, Western Australia, a large, permanent man-made water body. They used a different survey methodology (all-terrain vehicle) to that used here (boat), which could be implicated in the different findings. (Doody *et al*. 2009) surveyed by boat, but did so in different sections of the Daly River to those we surveyed. That their surveys (2001-2007) found no differences in crocodile abundance before and after toad arrival is in complete contrast to our results. The longer timeframe of our study may help explain some differences, but the most plausible explanation is differences in survey method. (Doody *et al*. 2009) conducted boat surveys for crocodiles during the day, whereas we surveyed with a spotlight at night (see (Messel *et al*. 1981; Fukuda *et al*. 2013)). Daylight surveys rely on seeing whole crocodiles (rather than eyeshines), and are highly biased towards large individuals (Webb *et al*. 1987). The average total length of crocodiles sighted by (Doody *et al*. 2009) was approximately 3 m, whereas the maximum average length we observed was 1.9 m in 2013, suggesting size estimation biases are also implicated in the different results obtained. During their survey period (2001-2007), which spanned the arrival of toads, the average length sighted in ours surveys increased from 1.1 m in 2001 to 1.7 m in 2007: due to the disappearance of small and intermediate sized animals. (Doody *et al*. 2009) reported no consistent change in the mean length of crocodiles sighted, which is to be expected if the surveys focus on only the largest animals. The very different conclusions that we have drawn concerning the impact of cane toads on freshwater crocodiles in the Daly River, highlights the importance of using survey methods that are appropriate for the management objective.

In contrast to the studies mentioned above, (Letnic *et al*. 2008) reported that freshwater crocodile populations in the Victoria River decreased by 45% two years after toad arrival, and that intermediate size classes were most severely impacted. Similarly, (Britton *et al*. 2013) counted 23 freshwater crocodiles smaller than 1.4 m in isolated pools of the Bullo River, Northern Territory in 2008, but recorded only one crocodile in 2009 after toad arrival.

Our results demonstrate significant changes in the relative densities of freshwater crocodiles following the arrival of toads in section A of the Daly River in 2005, but densities of some size classes appear to have started declining before cane toads were consistently observed throughout this section of the river. This may be because many freshwater crocodiles in section A are migrants from the upstream sections where cane toads were already abundant in earlier years. Freshwater crocodiles require soft-sanded banks for nesting, typically on rock shelves near the water's edge (Webb *et al*. 1983; Webb and Manolis 2010), and these breeding habitats are only available in sections B-D. However, we could not tell precisely when crocodiles started declining in sections B-D because of the absence of survey data between 1998 and 2008. It is also possible that the initial cane toad invasion front went undetected until the toad population reached appreciable densities.

These observations illustrate the difficulty of confidently attributing population declines to the arrival of an invasive species using correlative observational data. In most cases, it is unclear at what density an invader is likely to impact a population, and this density level will be species-specific. We used the date when toads became routinely seen by researchers (Doody *et al*. 2009), local residents, and naturalists to examine population-level impacts, as toad sightings before this time were unverified and infrequent. However, (Brown *et al*. 2013) documented rapid declines in a population of monitor lizards (*Varanus panoptes*) at another site in the Northern Territory only months after the arrival of cane toads, when the toads were still at low density. The establishment of cane toads appears to involve two distinct phases. Initially, larger individuals colonize an area (Phillips and Shine 2005), but exist at low density. This initial phase is then followed by increases in abundance due to smaller animals colonizing from already established areas and breeding from the initial arrivals. Although the expanding edge of the toad invasion front can move quite rapidly (Phillips *et al*. 2007; Urban *et al*. 2007), it can sometimes take years for toads to reach appreciable densities (Freeland 1986; Seabrook and Dettmann 1996; Shine 2010; Brown *et al*. 2013). While some species may exhibit a regional population-scale response to the toad front, for other species, declines may not be apparent until the toad population has become firmly established. This temporal lag in response may be particularly pronounced in species with slow life histories such as crocodiles, and means that even accurate records of the early arrival of cane toads may not correlate tightly with a decline in the local fauna.

The current status of the Australian freshwater crocodile on the IUCN Red List at global, national and state levels is "least concern", but was last reviewed in 1996 (Webb and Manolis 2010). However, across the entire Daly River, the density of freshwater crocodiles decreased by 69.5% between 1997 and 2013. As freshwater crocodiles are sexually mature at approximately 12 years of age (Webb and Manolis 1989), this decline occurred in less than one and a half generations. Declines of this magnitude, which have not ceased or are not reversible, meet the IUCN decline criteria for Endangered at a regional level (IUCN 2001, 2012; Maes *et al*. 2012). Cane toads are likely to continue to impact freshwater crocodiles on the Daly River, as toads are difficult to eradicate (Lever 2001). Critically, the declines we report here suggest that other populations of freshwater crocodiles across northern Australia may also be at risk from cane toads, and thus assessments in other river systems are warranted. Such data would enable a global-level assessment of extinction risk for this endemic species, and may guide management decisions, such as annual egg harvest quotas.

In this regard, it is worth noting that cane toads spread through areas of Queensland inhabited by freshwater crocodiles decades ago (Urban *et al*. 2007). Although the impact that toads have had on these populations has not been thoroughly studied, the populations are currently not considered to be declining or endangered. This suggests that toad impacts may vary spatially (eg according to climate: Letnic *et al*. 2008), or that crocodile numbers may eventually recover. Continued monitoring of the Daly River population will be needed to ascertain whether declines continue, and whether the population structure stabilises or remains in a state of flux. The management program in the Northern Territory allows for landowners to engage in limited commercial use of the wild population, although little harvesting has actually taken place for the last 15+ years. The results suggest that more caution needs to be exercised should landowners request harvests, particularly of some life stages.

Finally, this investigation would not have been possible had not long-term, standardized monitoring programs been implemented and sustained for crocodiles within the Northern Territory, as a management safeguard. The ability to use the results to assess the impacts of cane toads on the abundance and population structure of crocodiles was thus serendipitous. Nevertheless, our findings highlight the importance of long-term monitoring programs, at least for key species.

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Appendix

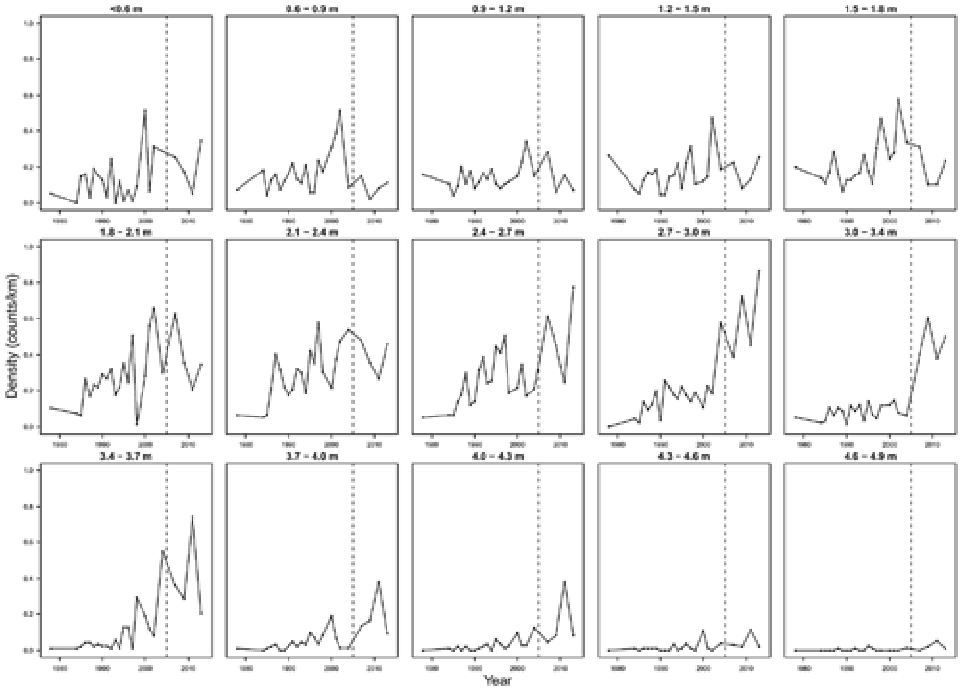

Figure A1. Trends in the relative densities of different size classes of Saltwater crocodiles (*Crocodylus porosus*) in section A of the Daly River, NT, Australia. The dotted line demarcates the point at which invasive cane toads became common in section A.

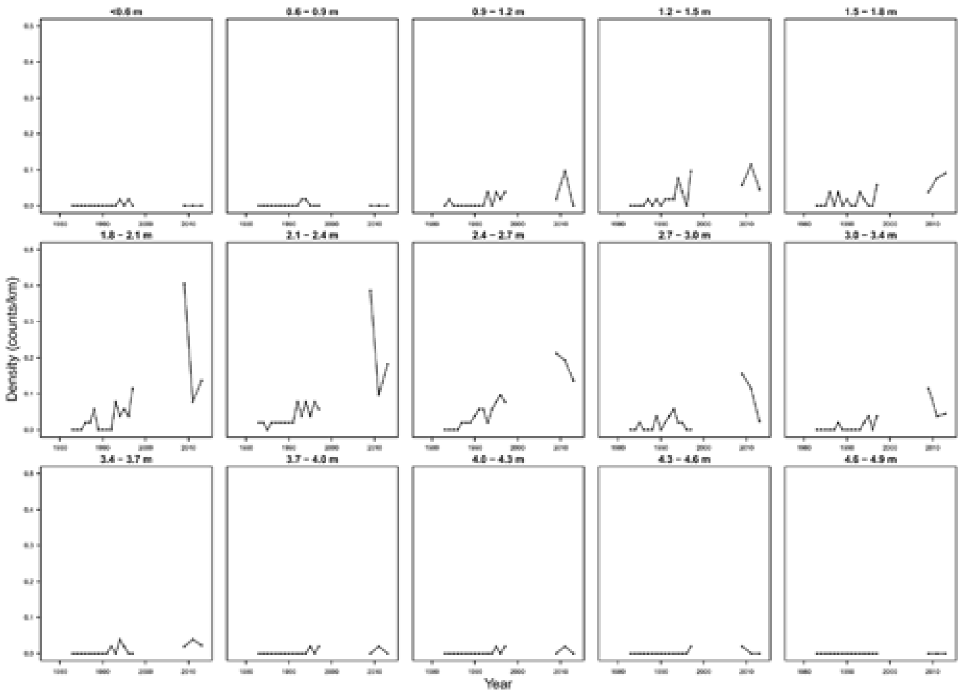

Figure A2. Trends in the relative densities of different size classes of Saltwater crocodiles (*Crocodylus porosus*) in section B of the Daly River, NT, Australia.

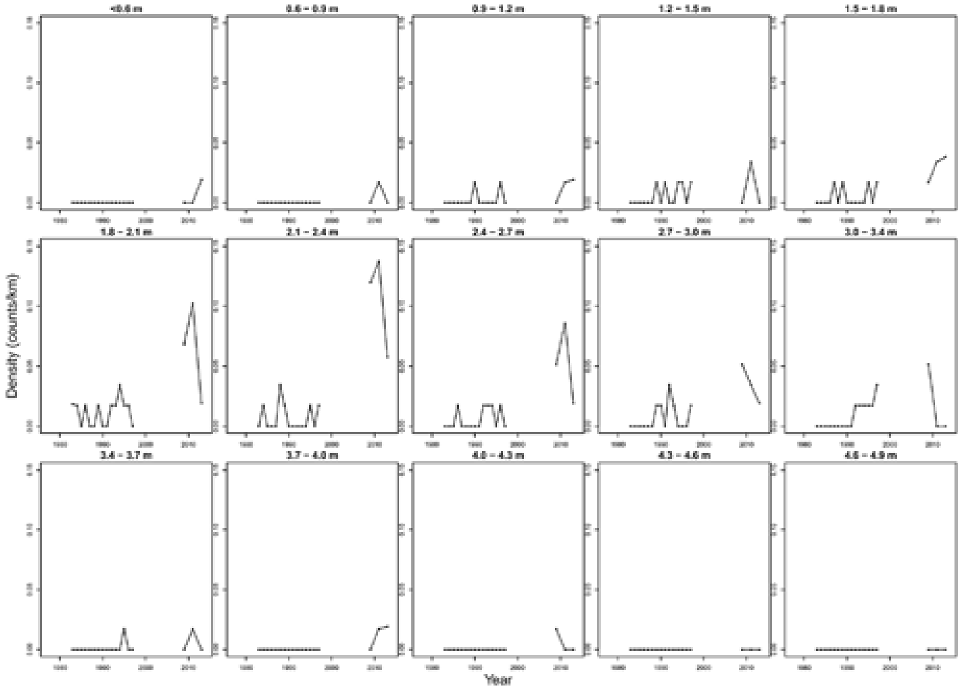

Figure A3. Trends in the relative densities of different size classes of Saltwater crocodiles (*Crocodylus porosus*) in section C of the Daly River, NT, Australia.

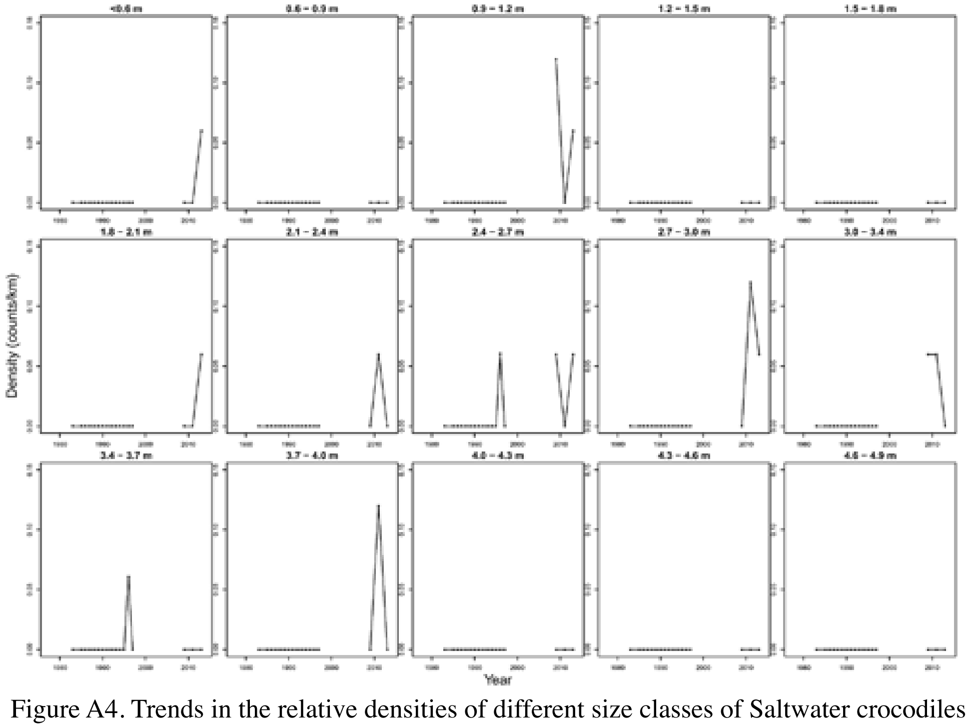

Figure A4. Trends in the relative densities of different size classes of Saltwater crocodiles (*Crocodylus porosus*) in section D of the Daly River, NT, Australia.

Table A1. Frequency of spotlight surveys for freshwater and Saltwater crocodiles in the Daly River of the Northern Territory.

Year	A (87.64 km)	B (51.36 km)	C (57.81 km)	D (16.54 km)
1978	P			
1979				
1980				
1981				
1982				
1983		P	P	P
1984	P	P	P	
1985	P	P	P	P
1986	P	P	P	P
1987	P	P	P	P
1988	P	P	P	P
1989	P	P	P	P
1990	P	P	P	P
1991	P	P	P	P
1992	P	P	P	P
1993	P	P	P	P
1994	P	P	P	P
1995	P	P	P	P
1996	P	P	P	P
1997	P	P	P	P
1998	P			
1999				
2000	P			
2001	P			
2002				
2003				
2004	P			
2005				
2006				
2007	P			
2008				
2009	P	P	P	P
2010				
2011	P	P	P	P
2012				
2013	P	P	P	P

The Gharial (*Gavialis gangeticus*) in Corbett Tiger Reserve

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Abstract

Historically, Corbett Tiger Reserve (CTR) was not considered to be able to maintain a viable population of Gharial and was subject to just a few ad hoc releases of the species. However, surveys in 2008 and since have confirmed that this Protected Area has a substantial Gharial population that lives and breeds largely in the lentic habitats of the Reserve and is perhaps the only known Gharial population that does so. Based on its current population status and increasing trend the Gharial population in CTR may well be considered home to the third largest breeding population of this critically endangered crocodilian. Unlike other Gharial populations which were subject to intensive conservation efforts by the Crocodile Conservation Project in India, the Gharial population in CTR has never been subject to management interventions. Such interventions are now deemed necessary in sustaining and maintaining this Gharial population.

Introduction

The Gharial, *Gavialis gangeticus*, an endemic crocodile of the north Indian sub-continent is considered to prefer free-flowing river systems. Its numerous wild populations have under gone significant reductions throughout much of the species' former range (Ross and Magnusson 1990). The wild populations in India, excluding that in Corbett National Park (CNP) were subject to an intensive conservation programme in the 1970s supported by the United Nations Development Programme (UNDP) - Food and Agriculture Organization (FAO) which included 'head-starting', establishment of protected areas, partial mitigation of anthropogenic pressures and physical enforcement of wildlife laws. In 1975, a conservation project which focussed on the breeding of Gharial and other crocodilians was initiated by the Government of Orissa at the Nandankanan Zoological Park (Achariyo *et al*. 1996).

These wild populations recovered significantly due to these efforts which were hailed a success. However, in 2007 an IUCN Red List Assessment of the Gharial upgraded it from a "Endangered" to "Critically Endangered" (Choudhury *et al*. 2007). Factors identified for this decline in the past 60 years have been over hunting for skin and trophies, egg collection for consumption, killing for indigenous medicine, and retaliatory killing by fishermen. Dams, barrages, irrigation canals, with their associated siltation, changes in river courses, artificial embankments, sand mining, riparian agriculture and land use changes to accommodate domestic and feral livestock have all caused irreversible loss of riverine habitat contributing to limiting the range of the taxon. These threats continue to increase further threatening the very survival of the species (Whitaker *et al*. 2007).

Methods

Review of scientific literature

Scientific literature related to crocodiles or crocodile conservation specifically in CNP, is scant prior to 2010 with just one significant research manuscript relating to Gharial in CNP, published in 1979 and in a PHVA workshop report that was published in 1995. CNP was initially surveyed for crocodiles in 1974 during which only 5 adult Gharial were documented with no evidence of successful breeding either in the form of nests, eggshell remnants or the presence of hatchlings. In addition, the best known Gharial habitat in this protected area was being inundated at that time due to the establishment of the Kalagarh Reservoir caused by the construction of the Kalagarh Dam on the Ramganga River (Whitaker 1979). Eight adult Gharial were estimated on the Ramganga River from 257 captive-reared stock (Rao *et al*. 1995), with these releases being conducted in 8 batches between 1985 and 1994 (D. Basu, pers comm. 2008 in Chowfin and Leslie 2014). As such Corbett Tiger Reserve (CTR) was not a part of the larger crocodile conservation efforts in the country as it was considered primarily unsuitable, marginal habitat for Gharial as evidenced by its small population size.

Field surveys

CTR (which consists of the CNP and the Sonanadi Wildlife Sanctuary) was surveyed in 2008, after the IUCN Red List uplisting of the Gharial to Critically Endangered, and a baseline of 42 adult Gharial was recorded for the the entire reserve of the CNP [N= 35 (9)] and the Sonanadi Wildlife Sanctuary [N= 7 (1)], with nesting recorded in two spatially discrete locations (Chowfin 2010).

Following the 2008 surveys *in-situ* studies were initiated in CTR to investigate population trends and dynamics, nesting ecology and hatchling survivability and mortality of both Gharial and Mugger (*Crocodylus palustris*) with one of the goals being the preparation of a Species Action Plan for Crocodiles in the Reserve. In addition to the crocodilians, freshwater turtles are simultaneously counted.

The resultant project, the Crocodilian and Freshwater Turtle Research and Conservation Project, is characterised by the use of scientifically advanced census and survey techniques. The project attempts to maintain a low carbon footprint by the use of electric boat motors (ie Torqeedo 1003s 3HP Electric outboard motor from 2012-2015 and a Torqeedo Cruise 4.0 TS 8 - 9.9 HP electric outboard motor from 2016 onwards).

Census techniques

Census techniques include the use of trail cameras (Fig. 1), stationary counts and surveys along the shoreline by boat together with photo documentation. Estimates of gharial populations in the Reserve are primarily based on the Maximum + Minimum Method (Messel *et al*. 1981) with estimates being pooled across locations to determine the population size of Gharial in the reserve.

Figure 1. Camera trapping of Gharial using trail cameras in Corbett Tiger Reserve.

The largest Gharial sub-population along the Dhikala shoreline in the Dhikala Range was estimated by using three independent methods which included: i) a first population estimate based on the Maximum + Minimum method; ii) a Two Phase sampling method (also known as Double Sampling) where the mean of counts from intensive surveys form the basis of the second population estimate; and, iii) a population estimate based on the relationship of N= C/p which is estimated from probability (p) derived from the intensive surveys and the count (c) of the rapid surveys of the two phase sampling method. Population size is then estimated as the mean of these three estimates (Chowfin and Leslie 2014). Census techniques and analyses were standardised from 2012 and the population size of Gharials for CNP was estimated annually using these protocols.

The most recent data from these surveys in 2015 indicated a population size of 90 gharial in the CNP areas of the reserve consisting of 21 adult males with 59 adults making up the breeding population [n =80 (21)] and 6 sub-adults with 4 juveniles making up the non-breeding population (Fig. 2). (Chowfin and Leslie, in prep.).

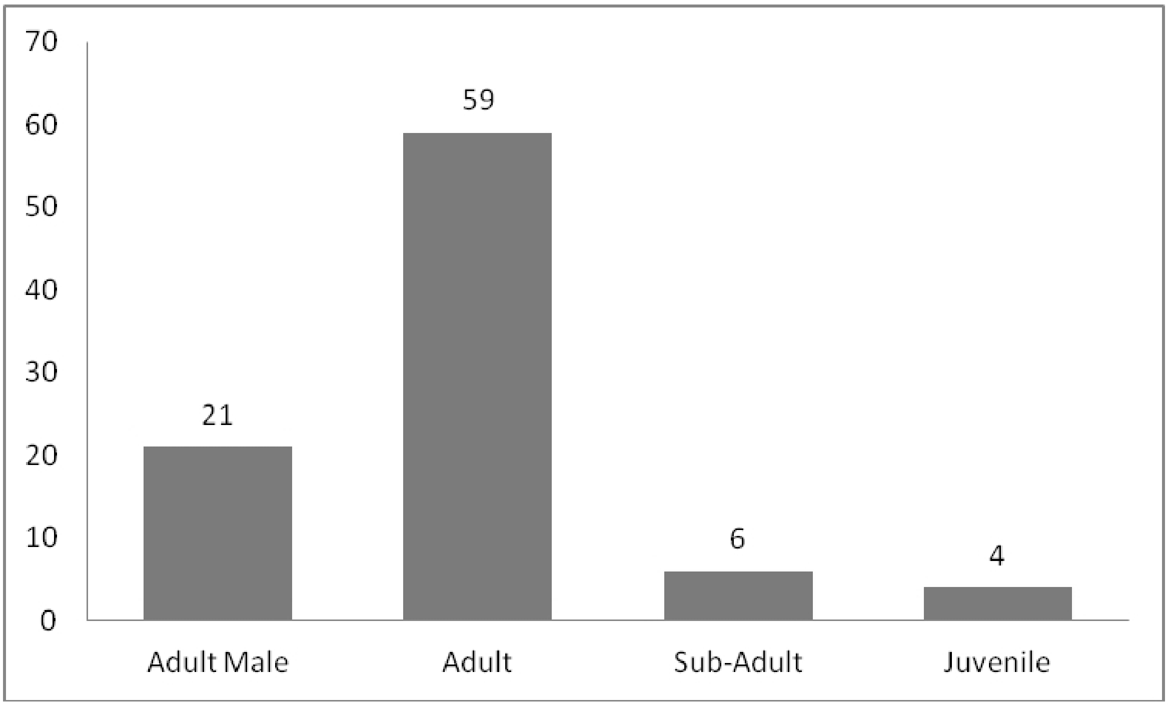

Figure 2. Population structure of Gharial in Corbett National Park (2015).

Comparison with earlier records and present trends

When considering that 257 Gharial were released in CNP between 1985 and 1994 and that 80 adult Gharial were recorded in CNP in 2015 it broadly indicates that in the CNP areas of the Reserve these releases have led to the establishment of an adult Gharial population with a reintroduction success of 31% within one generation of the species since the last releases in 1994 (Chowfin and Leslie, in prep.). Comparing trends in the Gharial population in CNP from its discovery in 2008 to its population status in 2015, the adult population in these areas has more than doubled with a (straight line) growth rate of 128%. However, the non-breeding population (consisting of sub-adults and juveniles) declined by an estimated 50% between 2013 and 2015 due to inadequate recruitment based on high hatchling mortality. It is estimated that Gharial hatchling (Fig. 3) mortality is as high as 99% based on surveys conducted in Boksar in the core area of the CTR (Chowfin and Leslie, in prep.).

Figure 3. Gharial hatchlings in Corbett Tiger Reserve.

Summary

CTR is perhaps the only protected area where a Gharial population, which once originally inhabited a lotic system, has shown adaptability to a predominantly lentic system. It is of significance that prior to the construction of the Kalagarh Dam on the Ramganga River in 1974, the Gharial population was a small non-breeding population predicted to not survive the large-scale changes in habitat parameters that would take place in its aquatic habitat post-dam construction. Since the construction of the dam there have been no further alterations to the aquatic habitat parameters in this protected area. Gharial appear to have adjusted to changes in the habitat which has largely been free of all the aforementioned disturbances for the last 42 years.

It appears that even though CTR habitat may not be considered to be ideal for Gharials, the protection afforded to the species along with unaltered shorelines and river banks over the last four decades have worked to the advantage of the species enabling it to colonise and utilise lentic habitat to its advantage.

Supplementation has also had positive results in CTR with the establishment of an adult population of 80 Gharial from the release of 257 individuals, indicating a reintroduction success of 31% within one generation and based on its present population status - CTR can be considered to be the third largest breeding population of Gharial globally.

Recommendations

While most river-based Gharial populations have faced immense declines due to anthropogenic pressures, these pressures are at present non-existent in CTR, allowing the Gharial population to fare better than its river-based counterparts even though habitat in CTR is not considered to be ideal Gharial habitat.

It is however important to understand that due to high Gharial hatchling mortality a conservation program is necessary in CTR which will include supplementation together with a long-term monitoring program so as to maintain and monitor this viable population of Gharial in Corbett Tiger Reserve.

Acknowledgements

We thank the Columbus Zoo and Aquarium, The CZS CBOT Fund for Endangered Species and the Brookfield Zoo, the Cleveland Zoological Society and the Cleveland Metroparks Zoo for an Asia Seed Grant, the PPG Conservation and Sustainability Fund and the Pittsburgh Zoo, Idea Wild and the University of Stellenbosch for valuable support for this project. We thank Dr. DVS Khati, IFS, PCCF (Wildlife)/Chief Wildlife Warden, Uttarakhand Forest Department, Mr. Samir Sinha, IFS, CCF & Field Director, Corbett Tiger Reserve and Dr. Saket Badola, IFS, Deputy Director, Corbett Tiger Reserve for issue of permissions and logistics in Corbett Tiger Reserve. Range Forest Officers, Field staff and Office Staff of Corbett Tiger Reserve are thanked for their assistance. We would specially like to thank The Gadoli and Manda Khal Wildlife Conservation Trust for its continuing support.

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Restocking, Monitoring, Population Status, New Breeding Record and Conservation Actions for Gharial in the Gandak River, Bihar, India

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Abstract

Once common in the river systems of Pakistan, India, Bangladesh, Myanmar, Bhutan and Nepal, the Indian Gharial (*Gavialis gangeticus*, Gmelin 1789) is now restricted to a few isolated locations in Nepal and India. Three wild breeding populations (National Chambal Wildlife Sanctuary, Katerniaghat Wildlife Sanctuary and Son River) and two non-breeding (Ken and Mahanadi Rivers) populations of Gharial have been recorded in India (Choudhury *et al*. 2007).

Gharial recovery programs in India and Nepal included restocking with captive hatched animals (Choudhury *et al*. 2007). Over 5000 individuals have been released into the river systems of India and Nepal since 1978. Gandak River, which is the Indian stretch of the Narayani River of Nepal, has historically supported Gharial population (Shahi 1976). Choudhary (2010) reported at least 15 Gharial individuals including adult male and female in a stretch of about 300 km, based on the survey conducted in 2010. Nair and Katdare (2014) recorded 50 Gharials including yearlings, juveniles, sub-adults and adults in the same stretch of the river in 2014.

To strengthen the remnant population, the Wildlife Trust of India, under guidance from the first author released 30 captive reared sub-adult Gharials from the Sanjay Gandhi Biological Park, Patna during April, 2014 and February, 2015 in collaboration with Environment and Forest Department, Government of Bihar (India), following the reintroduction guideline of IUCN/SSC (2013).

For implementing a successful monitoring protocol of the released Gharials (4 VHF and 2 satellite tagged, and all individually marked animals), we surveyed 320 km of river length from its entry in India at Valmikinagar (27° 26' 16"N, 83° 54' 27"E) to its confluence with the Ganga River (25° 38' 50"N, 85° 11' 02"E) in March 2015 and recorded 54 individuals (26 adults, 26 sub-adults and 2 juveniles), which is the highest recorded Gharials so far for the Gandak River in India. Of these, 8 Gharials were the released ones under the current restocking program, 35 were wild individuals and one was Nepal released individual. Identity of the remaining individuals could not be ascertained. During survey, secondary information from locals indicated breeding of the species in the river. Based on this information and regular sightings of young ones, we rigorously monitored the probable river stretches suitable for Gharial nesting, and in April 2016 located five nests and observed the presence of 8-10 adults in the nesting area. The located nests are being monitored and protected against egg depredation.

The present findings establish Gandak River stretch in India as one of the important distribution range of Gharial, and the fourth breeding population of the species in the country.

Quality Mapping of Gharial Habitat in National Chambal Sanctuary

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Abstract

Using remote sensing techniques to develop land use classification mapping is useful to improve the management of areas designed for wildlife habitat and biodiversity assessment. Landsat images constitute a major data source for habitat monitoring, capturing broad scale information on changes in habitat extent and spatial patterns of fragmentation that allow disturbances in protected areas. Gharial habitat quality mapping has been prepared from National Chambal Sanctuary (NCS), India. The NCS is the only riverine sanctuary in India which contains wide variety of unique fauna like Gharial (*Gavialis gangeticus*), Mugger (*Crocodylus palustris*), Gangetic River Dolphin (*Platanista gangetic gangeticea*), hard and soft shelled turtles, numerous aquatic birds and fishes. Due to the anthropogenic activities the biodiversity has been disturbed in the NCS. Wildlife in the NCS is very much influenced by various factors like habitat suitability and protection to their habitats. Their distribution is depending on availability of deep water pools, which are present in around 13 zones from Pali to Pachnada in a 400 km linear river sanctuary. Another important factor on which distribution of animals depends is long stretches of sand banks. Sand banks of different categories like steep, flat, mid-river islands are present in different zones. Such areas provide good habitats for nesting of Gharial. Existing data are, however, less able to provide information on changes in habitat quality, species distribution and fine scale disturbances, and hence data from other space borne optical sensors are increasingly being considered. Liss III and Land Sat -8 data are used for generating the thematic map of the study area and also GPS is used for ground survey. Remote sensing softwares like Arc GIS 9.3, ERDAS 9.1, WINRAR are used for generating the habitat maps. The normalized difference vegetation index map of the National Chambal Sanctuary was generated using the IRS- LISS-III satellite imagery & ERDAS Imagine software. NDVI value fluctuates from -0.24 to 0.69. From this value 0.69 indicates high vegetation area and -0.24 indicates water bodies, barren land. The characteristic feature of the Chambal River is that it has perennial, rapidly flowing water during monsoon and slowly flowing water in other seasons, which makes this river fit to accommodate flora and fauna of two different kinds of river flow systems. The Chambal River in the study area has different habitats characterized, particularly the river depth, flow and nature of banks. Animals occupy various suitable habitats to their requirements like basking, nesting and feeding. The river bank with clay/loamy soil substratum, aquatic vegetation, rocky substratum, mid Islands, shallow water pools are the ideal habitats of Gharial for basking, nesting and feeding. The Gharial were sighted at about163 locations respectively in the National Chambal Sanctuary. More than 15 steep sand banks are used by Gharial for nesting. The study on the biomap of Gharial habitat will through light on the conservation of Gharial and success of the habitat restoration programs.

Introduction

Over the last century humans have been changing ecosystems more rapidly than in any comparable period in history, as a result biodiversity or the variety of genes, species and ecosystems has declined rapidly (Balmford *et al*. 2003). This loss is compounded by the loss of knowledge of biodiversity especially among people with close relationship with the natural ecosystem. India is known for its genetic and species richness in a wide variety of ecological zones. Increasing human intervention and excessive exploitation of resources have resulted in great changes and provide alarming signals of accelerated biodiversity loss. The conventional species level approach for biodiversity management has major limitations and a major change in understanding the priorities of biological conservation and management. This has resulted in a policy shift from conservation of single species to their habitats.

For many of the conservation ecologists, question remains unclear to estimate species richness, as there is rapid decline in species diversity. Scientifically sound of biodiversity conservation and management requires frequent and spatially detailed assessments of the species diversity and distribution. Such information can be prohibitively expensive to collect directly. Measuring the distribution and status of biodiversity remotely, with airborne or satellite sensors, seems to be an ideal way to gather these crucial data. This remote sensing based information on vegetation and land cover provides a potential spatial framework and works as one of the vital input layers in assessing and monitoring of biodiversity.

Similarly, Land use and land cover (LULC) is an important component to understand global land status; it shows present as well past status of the earth surface (Yadav *et al*. 2012). On global to local scales, the only feasible way to monitor the Earth's surface is to prioritize and assess the success of conservation efforts through remote sensing. The remote sensing based vegetation type maps and species distribution maps help in prioritizing the areas of bio prospecting and mapping of target economically useful species (Joshi *et al*. 2006). In India due to many factors the wildlife in different states is in danger. No systemic data is available on the species diversity and the habitat management programmes in different protected areas particularly in Madhya Pradesh. The Chambal River is one of the riverine Sanctuary in India. The river is flowing in North Indian states of Rajasthan, Madhya Pradesh and Uttar Pradesh and it is the home for wide variety of unique biota. They are Gharial (*Gavialis gangeticus*), Mugger (*Crocodylus palustris*), Gangetic River Dolphin (*Platanista gangetica gangetica*), hard shell and Soft shell turtles and numerous aquatic birds. Chambal River attracted various research works, surveys have been carried out to evaluate biodiversity, population status of different species, their ecology, habitat and distribution (Bustard 1982; Bustard and Choudhury 1982; Whitaker and Basu 1983; Singh and Sharma 1985; Sharma *et al*. 1989; Hussain 1999; Rao 1999; Rao and Singh 1994; Sharma and Basu 2004; Hussain 2009; Singh and Rao 2010). But no work has been done on biodiversity mapping in National Chambal Sanctuary, Madhya Pradesh which gives the exact location of different biodiversity in the sanctuary. With the help of new technology ie, Remote Sensing & GIS a study has been done to prepare the thematic habitat maps of Gharial in the National Chambal Sanctuary.

Study area

Information for the present study was collected from National Chambal Sanctuary located in North Madhya Pradesh (Fig. 1). A 600 km stretch of the Chambal River, between Jawahar Sagar Dam and Pachhnada, has been protected as the National Chambal Sanctuary. The main study area lies between Pali to Pachhnada within the Sanctuary. Chambal River, a principal tributary of Yamuna River, originates in the Vindhyan hill range near Mhowin Indore District of Madhya Pradesh (M.P). It flows northeast through Rajasthan, Madhya Pradesh and Uttar Pradesh (U.P) where it conjoins the Yamuna River to form the greater Gangetic drainage system. Geographically the Chambal River lies between 24°55’ and 26°50’ N and 75°34’ and 79°18’ E and at an altitude of 120 m. The Chambal River flows for about 320 km in northerly direction before entering a deep gorge in Rajasthan at about 96 km upstream of Kota. The deep gorge extends up to Kota and the river then flows for about 226 km in Rajasthan, and then forms the boundary between M.P and Rajasthan for about 252 km. Thereafter, the river forms the boundary between M.P. and U.P. for about 117 km, enters U.P. near Chaker Nagar village and flows for about 40 km before joining Yamuna River. The main tributaries of Chambal River are Siwana, Retam, Shipra and Choti Kalisindh in M.P. Kalisindh, Parwati, Parwan and Banas in Rajasthan.

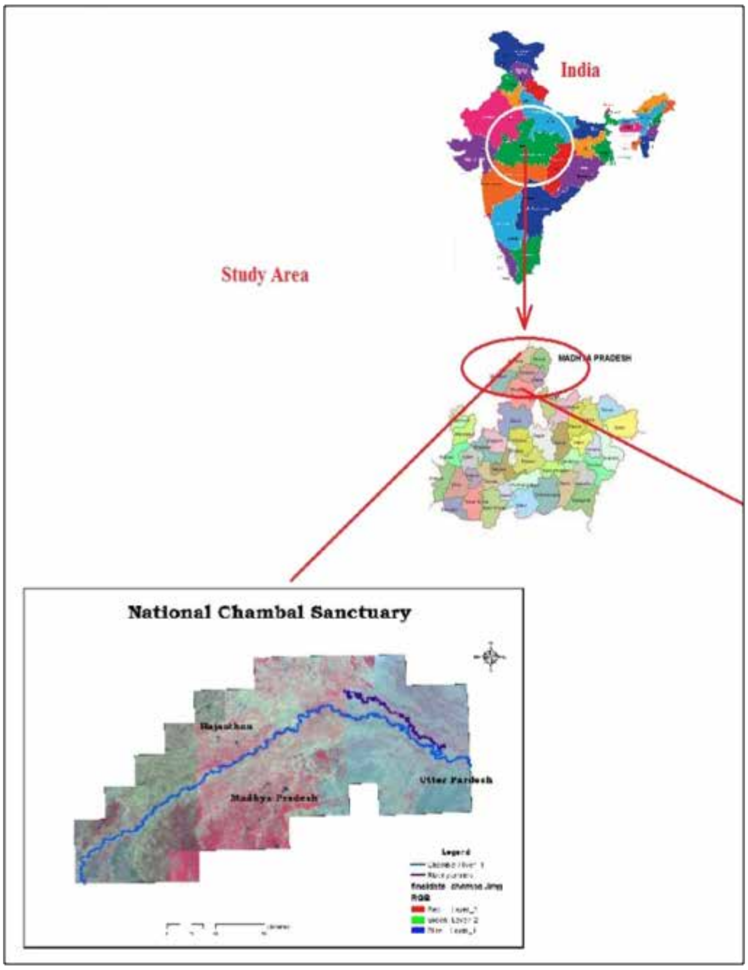

Figure 1. Map of India showing study area National Chambal Sanctuary.

The study area lies within the semi arid zone of Northern Madhya Pradesh and Southern Rajasthan. The study area has got a subtropical climate with moderate to hot temperature, ranges between 7ºC to 45ºC and relative humidity drops to about 20% during March, April and May. July-August is most humid period averaging 70% to 80% humidity. The Kharif-wet season is spread over from July to September. Rabi winter season is between the months of October to February while the hot season or pre monsoon season is occurring from April to May. The monsoon usually starts from second half of the June but heavy rain fall occurs during July-August and early September. The average rainfall of the area is 850 mm and precipitation largely depends on the south-western monsoon. The south-west monsoon lasts from the third week of June till late September. The effect of north-east monsoon in the study area is low, lasting 5-10 days during December, with 50-60 mm precipitation.

Land use pattern

The area along the Chambal River is primarily a grazing pasture, now predominately used for irrigation cultivation of cash crops and vegetables. Land put to non-agriculture uses such as quarrying (for sandstones, limestone, sand and murram) and industrial development is marginal. Construction of multipurpose dams in the upper reaches of the river and their association canals downstream have intensified agricultural practices in the entire area. A special development plan launches with World Bank assistance is enhancing agricultural practices, ravines reclamation and the soil moisture conservation in the command area (Sale, 1982). Whilst the population of the area is predominantly rural, within 25 km of the study area, there are three large and three to four small towns. The forest area comes under the Revenue Forest of Rajasthan, M.P and U.P Forest Departments.

Materials

Data sets

To carry out the study, the following data sets were used: *IRS-1D LISS - III (for National Chambal Sanctuary); GPS (Global Positioning System); and, Secondary Data.

IRS-1D LISS - III

The IRS (Indian Remote Sensing) satellites form a large family of Earth observation satellites operated by the Indian space agency. IRS P6/Resourcesat-1 and Resourcesat-2 ensure continuity of medium and high resolution data supply provided by the twin satellites IRS-1C and IRS-1D. These two, launched in 1995 and 1997 respectively, have completed their missions after more than 10 years of service. Like their predecessors, Resource sat satellites carry a LISS-III sensor as well as a wide field A WiFS sensor, but the high resolution (5.8 m) LISS-4 sensor replaces the panchromatic sensor.

Table 1. Spectral bands of LISS III*

Band	Spectral band	Resolution
2	0.52 - 0.59 μm	23 x 23 m
3	0.62 - 0.68 μm	23 x 23 m
4	0.77 - 0.86 μm	23 x 23 m
5	1.55 - 1.70 μm	23 x 23 m

The high-resolution data are useful for applications such as urban planning and mapping, while the average resolution is used for vegetation discrimination, land mapping, and natural resources management. The LISS-III (Linear Imaging Self Scanning Sensor) is an optical sensor working in four spectral bands (green, red, near infrared and short wave infrared). It covers a 141 km-wide swath with a resolution of 23 m in all spectral bands (Tables 1 and 2).

Table 2. Specifications of description of LISS III.

Type	Imaging multi-spectral radiometers (vis/IR)
Spectrum	VIS (~0.40 μm to ~0.75 μm); NIR (~0.75 μm to ~1.3 μm); SWIR (~1.3 μm to ~3.0 μm)
Resolution class	Medium (20 m - 200 m)
MS resolution	23.5m
Swath	141 km
Revisit Time	24 days
Other characteristics	LISS - III camera provides multispectral data in 4 bands. The spatial resolution for visible (two bands) and near infrared (one band) is 23.5 m with a ground swath of 141 km. The fourth band (short wave infrared band) has a spatial resolution of 70.5 m with a ground swath of 148 km. The repetitively of LISS - III is 24 days.
On-board of	IRS 1C; IRS 1D; IRS P6 (ResourceSat-1); IRS P7 (ResourceSat-2)

Landsat Satellite-8

Landsat-8 images consist of 7 spectral bands with a spatial resolution of 30 m for Bands 1 to 5 and 7. Spatial resolution for Band 6 (thermal infrared) is 120 m, but is re sampled to 30-m pixels. Approximate scene size is 170 km north-south by 183 km east-west (106 miles by 114 miles) (Table 3). Software included Arc Gis 9.3, Erdas Imagine 9.1 and Win Rar.

Methods

The aim of the study was to prepare the habitat maps of Gharial in th National Chambal Sanctuary in North Madhya Pradesh. The data were collected from the visual based surveys (GPS Survey) in the National Chambal Sanctuary. The data for the study were downloaded from the National Remote Sensing Centre (www.bhuvan.nrsc.gov.in). Satellite images of LISS III and LandSat-8 were rectified in ERDAS IMAGINE by Geo-referencing the satellite data with the help of already rectified Survey of India Topo maps. After Geo-referencing the satellite data, the images were mosaiced and the area of interest was subsetted using ERDAS Imagine tools. For data collection Garmin 78s GPS was used for recording the location of Gharial present in the National Chambal Sanctuary. About 366 GPS Points were taken along the whole National Chambal Sanctuary to identify habitat features. After taking the GPS points they were overlapped on that satellite images to prepare habitat maps.

Table 3. Specifications of the description of Land Sat satellite-8 (National Remote Sensing Centre; bhuvan.nrsc.gov.in).

	Landsat 4-5	Wavelength (μm)	Resolution (m)
Thematic Mapper (TM)	Band 1	0.45-0.52	30
	Band 2	0.52-0.60	30
	Band 3	0.63-0.69	30
	Band 4	0.76-0.90	30
	Band 5	1.55-1.75	30
	Band 6	10.40-12.50	120* (30)
	Band 7	2.08-2.35	30

Results

The National Chambal Sanctuary was segmented into 13 different zones (Pali to Pachnada), which varied between 22 and 45 km in length (Fig. 2): 1. Pali, 2. Rameshwar, 3. Rijheta, 4. Gaughat, 5. Atar Ghat, 6. Sarsaini, 7. Raj Ghat, 8. Bamboo Singh Gher, 9. Used Ghat, 10. Ater, 11. Barhi, 12. Chaker Nagar, 13. Pachnada.

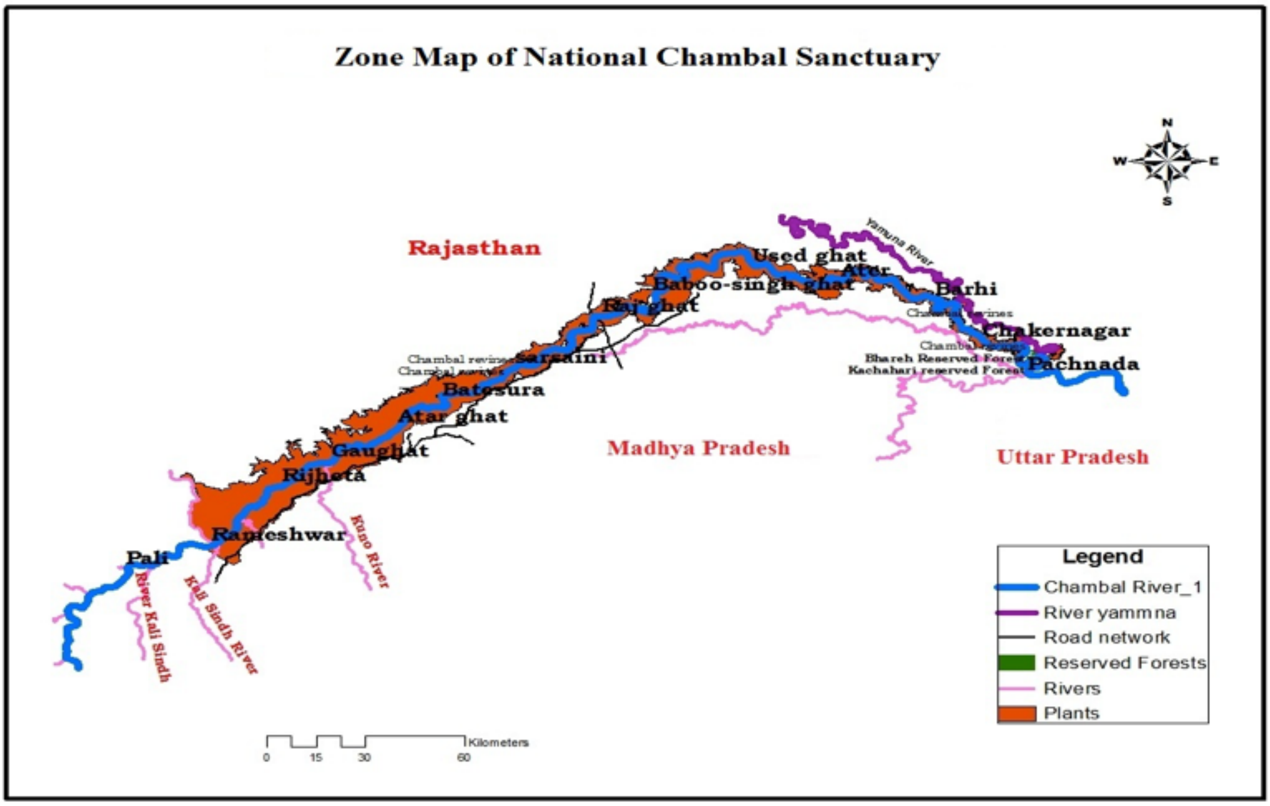

Figure 2. Zone map of National Chambal Sanctuary.

Biodiversity of National Chambal Sanctuary

The Chambal River is the only riverine Sanctuary in India. The river is home for wide variety of unique biota. They are Gharial (*G. gangeticus*), Mugger (*C. palustris*), Gangetic River Dolphin (*Platanista gangetica gangetica*), hard shell and soft shell turtles.

Habitats

The wildlife habitat in NCS is mostly aquatic with terrestrial habitat within 1 km from the mid-river bank. The micro-habitats are: deep water pools, shallow riffle areas, sand peninsulas, muddy banks, sand banks (steep and flat sand banks), rocky banks, nallas, steep riverside cliffs, xerophytic vegetation on the banks, etc.

The microhabitats are: deep water pools, shallow riffle areas, sand peninsulas, muddy banks, sand banks (steep and flat sand banks), rocky banks, nallas, steep riverside cliffs, xerophytes vegetation on the banks, etc. Habitat studies provide crucial information about the ecological requirements of a species or community. While it is important to assess the habitat usage, it is equally important to conduct studies addressing the pattern of usage. It is assumed that high quality resources will be selected more than low quality ones and use may change with availability when the latter is not uniform. Wildlife in the NCS is very much influenced by various factors like habitat suitability and protection of their habitats. Their distribution is depending on availability of deep water pools, which are present in many 13 zones from Pali to Pachnada as per the survey, and also it has been estimated that maximum biodiversity is found in northern part of the Sanctuary. Another important factor on which distribution of animals depends is long stretches of sand banks. Sandbanks of different categories like steep, flat, mid-river islands are present in different zones. Such areas provide good habitats for nesting of crocodiles, turtles and birds.

Gharial habitats

The characteristic feature of the Chambal River is that it has perennial, rapidly flowing water during monsoon and slowly flowing water in other seasons, which makes this river fit to accommodate fauna of two different kinds of river flow systems. The Chambal River in the study area has different habitats characterized, particularly the river depth, flow and nature of banks (Plate 1). Although Gharial occupies in various habitats to their requirements sand banks are the most important habitat requirements (Table 4). The crucial microhabitats of the Chambal River are defined as:

a. River bank with sandy substratum: Crucial habitat for nesting and basking of aquatic reptiles. Sandy banks are also crucial habitat for otters for rest, play and rolling.
b. River bank with clay/loamy soil substratum: Crucial habitat for Mugger for nesting and habitat for several birds for feeding.
c. River bank with aquatic vegetation: Crucial habitat for fishes to breed. Nursery ground for several species of chelonians, Gharial, Mugger and birds, as well as shelter and feeding ground.
d. River bank with rocky substratum: Crucial habitat for Mugger and chelonians for basking and halting site for otters. Habitat for several species of rocky fauna, which are major food for several birds and fishes.
e. Mid-river Islands: Several small islands occur in the river, which acts as excellent basking and nesting ground for Gharial, Mugger and chelonians.
f. Littoral zone: Littoral zone or the banks of the Chambal River is very crucial not only for several breeding fishes and chelonians as feeding ground, but also protect the habitat from further erosions and produce necessary primary producers such as plankton, etc.
g. River bed with rocky substratum: Important habitat of otter for feeding and mating.
h. River bed with deep water: Habitat for river dolphins, Gharial, Mugger, birds for foraging, breeding and playing.
i. River bed with deep running water: Crucial habitat for endangered dolphin and otter for feeding and diving.
j. River bed with shallow water pools: Crucial habitat for several fishes to breed. Feeding ground for otter, Mugger, Gharial and birds.

Table 4. Status of sandbanks in National Chambal Sanctuary.

No.	River Bank	State	Latitude	Longitude	Area (km^2)
1.	Pali (Island)	Raj/MP.	N 25° 50' 41.0"	E 076° 33' 47.8"	1.1
2.	Rameshwar	Raj	N 25° 54' 20.3"	E 076° 44' 15.9"	2.3
3.	Bagadia Sand	M.P.	N 25° 56' 46.4"	E 076° 45' 66.8"	1.46
4.	Maharajpur/Nimach	Raj.	N 26° 04' 72.8"	E 076° 53' 89.1"	0.233
5.	Baroli	M.P.	N 26° 05' 58.4"	E 076° 55' 06.4"	2.3
6.	Nadigaon	M.P.	N 26° 08' 29.6"	E 077° 00' 05.9"	0.206
7.	Barotha	M.P.	N 26° 13' 56.3"	E 077° 09' 11.4"	6.01
8.	Atar	Raj	N 26° 16' 33.2"	E 077° 16' 47.6"	10.07
9.	Banwara (Island)	Raj.	N 26° 20' 67.2"	E 077° 20' 23.8"	1.3
10.	Batesura/Shankarpur	Raj.	N 26° 21' 64.2"	E 077° 26' 4.4"	2.42
11.	Bharra	M.P.	N 26° 24' 13.6"	E 077° 26' 51.8"	2.5
12.	Sarsaini/Gargoli	Raj.	N 26° 30' 07.0"	E 077° 40' 34.2"	5.39
13.	Tursipura/Chalipura	Raj.	N 26° 32' 43.6"	E 077° 48' 57.5"	5.66
14.	Barwasin	M.P.	N 26° 35' 34.2"	E 077° 49' 40.9"	1.37
15.	Bindwa/Dev ka pura	Raj.	N 26° 37' 22.7"	E 077° 53' 54.4"	6.12
16.	Rajghat	M.P.	N 26° 39' 55.1"	E 077° 54' 14.3"	4.23
17.	Tighari Rithora	M.P.	N 26° 41' 05.5"	E 078° 02' 35.4"	2.98
18.	Mahuwa kheda	M.P.	N 26° 41' 05.5"	E 078° 02' 35.4"	4.79
19.	Kuthiana	Raj.	N 26° 41' 53.8"	E 078° 05' 44.3"	13.48
20.	Babu S. Gher	M.P.	N 26° 44' 23.2"	E 078° 06' 37.9"	5.02
21.	Puraini	Raj.	N 26° 44' 29.9"	E 078° 06' 14.4"	4.03
22.	Daljit ka pura	M.P.	N 26° 47' 24.5"	E 078° 08' 37.3"	18.88
23.	Barsala	Raj.	N 26° 47' 43.6"	E 078° 11' 09.4"	5.98
24.	Sukhdyan ka pura	M.P.	N 26° 48' 36.4"	E 078° 10' 19.8"	4.21
25.	Chursalai	Raj.	N 26° 49' 46.5"	E 078° 11' 53.9"	3.03
26.	Holapura	M.P.	N 26° 49' 27.9"	E 078° 12' 28.2"	4.87
27.	Kachiara	U.P.	N 26° 49' 03.6"	E 078° 16' 37.4"	5.45
28.	Baranda/Rudawali	M.P.	N 26° 50' 41.1"	E 078° 16' 25.7"	12.01
29.	Useth	U.P.	N 26° 51' 44.5"	E 078° 20' 01.2"	3.84
30.	Bijila/Bindwa	M.P.	N 26° 50' 14.2"	E 078° 23' 06.3"	4.97
31.	Jalalpura (Island)	U.P.	N 26° 47' 23.3"	E 078° 27' 30.1"	6.23
32.	Sahas pura	M.P.	N 26° 46' 41.1"	E 078° 31' 36.0"	3.92
33.	Godha	U.P	N 26° 44' 99.2"	E 078° 34' 56.0"	19.6
34.	Ranipura	U.P.	N 26° 45' 19.5"	E 078° 36' 55.8"	2.86
35.	Ater	M.P.	N 26°45' 23.4"	E 078° 36' 58.9"	7.86
36.	Maghera ka pura	M.P.	N 26° 47' 10.0"	E 078° 40' 57.6"	4.89
37.	Kosad	U.P.	N 26° 46' 82.4"	E 078° 43' 61.4"	2.83
38.	Nakhloni	M.P.	N 26° 47' 11.8"	E 078° 45' 34.8"	4.56
39.	Gadayata/Bijora	U.P.	N 26° 45' 51.6"	E 078° 48' 32.3"	8.29
40.	Jori	M.P.	N 26° 43' 69.9"	E 078° 50' 62.7"	3.81
41.	Baroli	U.P.	N 26° 42' 77.4"	E 078° 50' 99.2"	5.97
42.	Barai	M.P.	N 26°42' 23.3"	E 078° 54' 34.5"	6.23
43.	Khera ajab singh	U.P.	N 26° 40' 20.5"	E 078° 56' 47.8"	7.6
44.	Gyanpura	M.P.	N 26° 40' 36.8"	E 078° 59' 36.3"	7.98
45.	Sankari	M.P.	N 26° 38' 27.3"	E 078° 59' 38.2"	3.82
46.	Kanakpura	M.P.	N 26° 36' 55.4"	E 079° 00' 42.2"	6.78
	Total area				247.439

Landuse/Landcover of National Chambal Sanctuary

Land is the most important natural resource, which embodies soil, water and associated flora and fauna involving the total ecosystem (Rao *et al.* 1991). The levels of detail that can be obtained in a land cover or land use classification depends on number of factors including first the quality of the satellite images, defined by the spatial, spectral and radiometric resolution and second the nature of the land use classes being observed. Digitally land use and land cover map have been prepared using supervised & knowledge classification method in ERDAS Imagine 9.1, and satellite remote sensing data ie IRS-P6 LISS-III data. Land use of the study area comprise mostly of Agricultural fields, Barren Land, Dense Forests, Flowing Water, Forest, Grass Fields, Sand banks, Settlement, Water Bodies (Figs. 3 and 4; Table. 5).

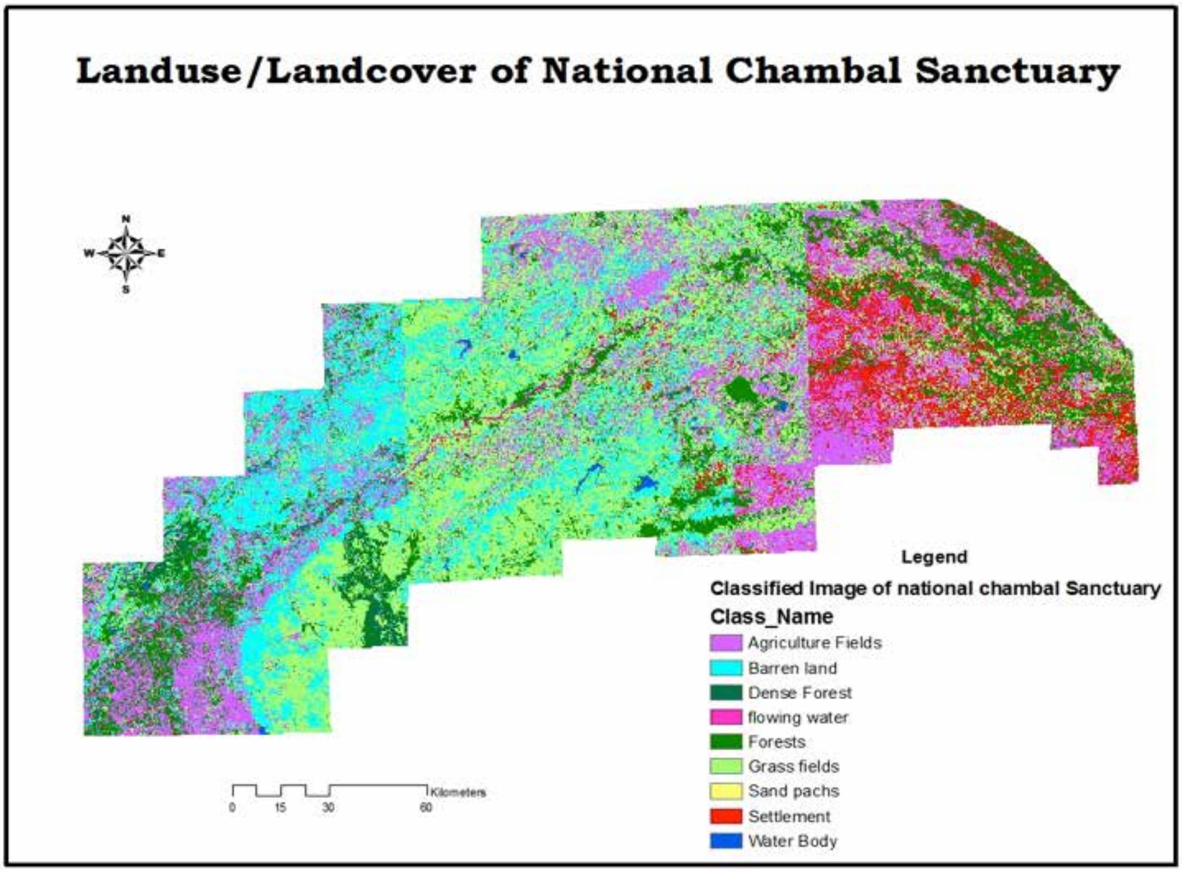

Figure 3. Landuse/Landcover of National Chambal Sanctuary.

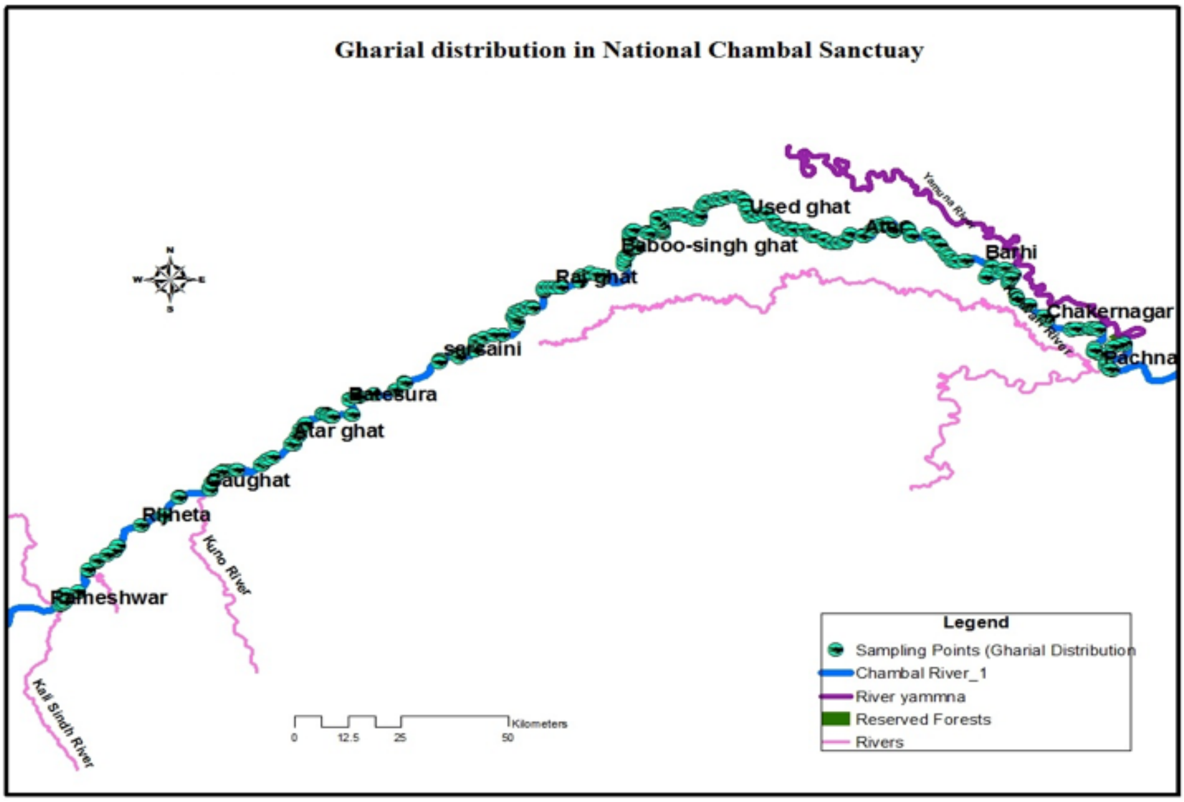

Figure 4. Gharial distribution in National Chambal Sanctuary.

Table 5. Landuse/Landcover data of NCS.

No.	Landuse/Landcover	Area (ha)
1	Agricultural Fields	681,345
2	Barren land	500,244
3	Dense Forests	37,152.3
4	Flowing Water	7075.64
5	Forests	512,509
6	Grassland	979,869
7	Sand Patches	13,319.7
8	Settlement	200,156
9	Water bodies	17,352

Discussion

Biodiversity in the National Chambal Sanctuary is mostly aquatic with terrestrial habitat within 1 km from the mid-river bank. The microhabitats are: deep water pools, shallow riffle areas, sand peninsulas, muddy banks, sand banks (steep and flat sand banks), rocky banks, nallas, steep riverside cliffs, xerophytes vegetation on the banks, etc. Habitat studies provide crucial information about the ecological requirements of a species or community. While it is important to assess the habitat usage, it is equally important to conduct studies addressing the pattern of usage. It is assumed that high quality resources will be selected more than low quality ones and use may change with availability when the latter is not uniform. Wildlife in the NCS is very much influenced by various factors like habitat suitability and protection of their habitats.

Their distribution is depending on availability of deep water pools, which are present in many 13 zones from Pali to Pachnada as per the survey, and also it has been estimated that maximum biodiversity is found in northern part of the Sanctuary. Another important factor on which distribution of animals depends is long stretches of sandbanks. Sandbanks of different categories like steep, flat, mid-river islands are present in different zones. Such areas provide good habitats for nesting of crocodiles, turtles and birds.

Indian Gharial in the NCS is very much influenced by various factors like habitat suitability and protection of its habitats. Their distribution is depending on availability of deep water pools, which are present in many zones, maximum in northern part of the Sanctuary. Another important factor on which distribution of ghrial depends is long stretches of sandbanks. Sandbanks of different categories like steep, flat, mid-river islands are present in different zones. Such areas provide good habitats for basking and nesting of Gharials.

In this study, Remote sensing (RS) and GIS technique is applied to identify biodiversity in the NCS. The results obtained from this study indicate that the integration of Remote Sensing and GIS and application of multi criteria evaluation could provide a superior database and guide map for decision makers considering biodiversity substitution in order to conserve and manage the biodiversity in North Madhya Pradesh and also Geospatial techniques provides a powerful tool for assessing geospatial information for monitoring land use and land cover changes, changes in landscape, mapping potential, species distributions and monitoring and biodiversity losses. Understanding the biodiversity fragmentations, species distributions and levels of species richness and how they operate in different geospatial contexts is a fundamental challenge of modern conservation biology. This challenge is considered important with the ongoing simplification of native ecosystems, declining populations and rising loss of biodiversity. It may be concluded that not 'either or' but a hybrid approach (both ground sampling and satellite tool) play a major role in assessing biodiversity at landscape level.

Habitat maps appear to be capable of providing information on the distribution of Gharial in India. The protection received by Gharial is a major beneficial parameter for the Gharial distribution in the NCS. The habitats are well protected in the NCS. Various Government departments like forest, police and irrigation have major role in protecting the Gharial.

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Plates - Gharial habitats along the National Chambal Sanctuary.

a. Chambal River showing habitat features.

b. Locations of Gharial nesting sites.

c. Gharial in different age groups basking.

d. Chambal River showing sandbanks.

e. Female Gharial basking on sandbank.

f. Male Gharial basking on sandbank.

Chambal Gharial Ecology Project - 2016 Update

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Abstract

Ecological studies (2008-2016) of resident, wild Gharial have been conducted in the National Chambal Sanctuary (NCS), north India, utilizing telemetry to record their movements, activities, and behaviours relevant to the die-off of 2007-08, and critical to the long-term conservation of the species. Major project activities from 2013 onward have been: 1) tracking Gharial tagged in 2013 (20), in 2014 (7), and in 2015 (10), plus several immature Muggers, tagged in 2013, 2) making detailed observations on crèches of Gharial hatchlings, attended and guarded by adults, and 3) monitoring nesting as well as hatching success of Gharial on the lower Chambal in the NCS.

Long distance seasonal movements of 200+ km are now well documented in the NCS population, indicating the importance of an open, dynamic river ecosystem. Adult females frequently move long distances upstream from monsoonal feeding sites, to breeding and nesting areas. Adult males have small, dry season residences and move less. In contrast, juveniles and sub-adults live throughout the year in restricted sections (<10 km) of river. GPS loggers incorporated with VHF tracking radios, capable of remotely downloading location data acquired over months, are providing fine scale movement and activity patterns for resident Gharial, ranging in size from 2-5+ m total length.

The exceptional hatchling care behaviours, exhibited by adult females and males guarding large crèches (100-1000+ hatchlings) have been documented in detail, with game cameras for weeks prior to the onset of monsoon. At nest colonies, one of the larger females is the dominant guardian; she attends groups of young at frequent intervals day and night. Also in attendance, and prominent especially when the young are threatened is a large ghara male who resides near the crèche. At times, both female and male guardians together attend and defend hatchlings. A male guardian is a regular feature of all large (>10 nests) hatchling crèches, but sometimes a male guardian is associated with smaller hatchling groups (2-4 nests/site). In several cases, "alloparental care" by guardian adults, other than one of the presumed parents, occurs. Overall, Gharial social interactions and behaviours appear to be decidedly complex and markedly seasonal, and not restricted to particular ages, sizes, and/or sexes.

Nesting sites and hatching success on the lower Chambal (29-163 km from Yamuna confluence) has been tallied from 2010 onwards. Over 6 nesting seasons, 21 nest sites were utilized on this 134 km stretch, but nesting occurred in every year at only 2 sites. Nest location varies with local river topography, changes every monsoon depending on its intensity, occurs on high sand banks, adjacent to deep water, and is communal. In 2015, 153 nests were counted on the lower Chambal, of which 124 hatched, producing 4500+ hatchlings. At 2 upstream sites, "industrial scale" sand mining occurred 24/7 from Sept into July, during nesting, incubation, and hatching of 27 nests, of 31 laid. Other immediate threats include water extraction and unregulated fishing with nets.

Introduction: Brief Synopsis of Gharial Conservation

Historically, the Gharial (*Gavialis gangeticus*) inhabited the deep rivers of India, Nepal, Pakistan, Bangladesh, Bhutan, and Myanmar, but today the species is limited primarily to isolated pockets in northern India and Nepal (Whitaker and Basu 1983; Whitaker *et al*. 2007). A recent rapid decline led to a "critically endangered" status in 2007 (Sharma and Basu 2004; Choudhury *et al*. 2007), followed shortly thereafter by an unexplained mass die-off (Whitaker *et al*. 2008; Huchzermeyer *et al*. 2008). In response, the Gharial Species Recovery Plan was drafted (GCA 2009), resulting in a CSG Action Plan (Stevenson and Whitaker 2010) and a national plan for Gharial conservation within India (NTRIS-CASMACC 2011; GSRP, 2011) in which the Chambal River population is highlighted.

These conservation initiatives have their origins in the Indian Crocodile Conservation Project (Bustard 1999; Choudhury 1999; Singh 1999), centered on range-state rehabilitation programs (Singh 1979; Basu 1980; Rao and Sharma 1986). Captive breeding and head starting entailed egg incubation and rearing of all Indian crocodilians (Whitaker and Whitaker 1984; Lang *et al*. 1989; Lang and Andrews 1994; Andrews and Whitaker 2004). For decades, the Chambal River system has contained the largest, self-sustaining Gharial population (Rao *et al*. 1995), and this region has been the focus of ecological studies (Singh 1978, 1985; Whitaker and Basu 1983; Rao 1988; Hussain 1999, 2009), and conservation landscape analyses (Hussain and Badola 2001). Annual surveys have been conducted (Sharma 1999; Sharma and Basu 2004), augmented by recent surveys (Katdare *et al*. 2011; Nair *et al*. 2012a,b). Interactions between Mugger and Gharial have been commented on at Katerniaghat (Choudhary, 2011) and in the Chambal system (Sharma and Singh 2015).The Gharial Ecology Project, initiated in 2008 following the mass die-off of the winter of 2007-08, is now in its ninth field season (Lang and Kumar 2013; Lang 2016; this report). Recent commentaries on Gharial conservation include Stevenson (2015) and Lang (2016).

Background

The Gharial Ecology Project, also known as the Gharial Telemetry Project, and summarized in Lang and Kumar (2013), was designed to investigate the mass die-off of Gharials in the 2-4 m size class, totalling 110+ individuals in the lower Chambal River, National Chambal Sanctuary (NCS).

Previously, 20 radio-tagged Gharials have been tracked successfully through the annual monsoon and dry periods, for an average of 2+ years/animal, since June 2008 through August 2013. Individual Gharials show different patterns of seasonal movement and residency, dependent on size/age. Adult f males move as far as 80-120 km each to join dry season basking-breeding aggregations (>60 adults), and to locate suitable nesting areas. In contrast, sub-adult Gharials moved little, only 10-30 km seasonally, and occupy residencies only 5-15 km. Most sub-adults are sedentary, virtually not moving, either upstream or downstream any time.

In July 2013, project continuation was approved to provide a comprehensive picture of the spatial and reproductive ecology of Gharial and Mugger living in the NCS. Permission was granted for 25 Gharial and 5 Mugger to be tagged and monitored. Support from the international zoo community funded previous tagging and monitoring of radioed animals in 2013 and in 2014.

The overall study demonstrates that individual Gharial utilize the entire river for specific habitats as well as corridors, and aims to provide baseline information on the status of Gharial populations within the NCS. In 2015, the Gharial Ecology Project continued in two phases: a) monitoring radioed animals throughout the year, and b) capture and tag in November 2015. The tagged individuals serve as indicators of what other members in the population may be doing at different times of year. These animals provide a "window" on the specific daily behaviours and activities of the population as a whole. Detailed behavioural observations are routinely made on all Gharial, as well as locating and tracking those with radio tags.

Long Distance, Seasonal Movements

The most exceptional result to date are the extraordinary distances some of the tagged Gharial are moving each season, up and down the river, in seasonal patterns related to nesting. One of the tagged Gharial moved 210 km downriver to the confluence where she remained during the monsoon. Previously, she moved upriver 100+ km to the site where she nested and protected hatchlings that emerged from her nest in early June (female 24; Table 3). After the monsoon, in late 2015, she moved back 210 km upriver to the same site where she remains, as of March 2016. A sub-adult female made similar movements to the same nesting site upriver, but returned during the monsoon, a distance of 180 km to the site where she was tagged in 2013 (female 66; Table 1).

Tracking Radio-Tagged Gharial

The international zoo community supported the final monitoring throughout the year for the remaining Gharial and Mugger tagged in November 2013. Their movements and activities are presented in Tables 1 and 2, and described in the following narrative. As late December 2015, it is noted that nearly all of these Gharial and Mugger no longer had functioning, trackable radios. Two juvenile Gharial (90 and 94) were last recorded in January 2016. In particular, all of these animals were captured and tagged at a wide range of river locations, extending from 35 km downstream to 105 km upstream, or over approximately 70 km of river. Of special interest were 3 adults which included two adult females (50 and 54) and a young adult male (44/46). The movement patterns of these three adults contrasted markedly with the few adult females monitored in earlier groups (described and discussed in previous reports). This result, in turn, suggests that adults generally may show variable movement patterns, presumably related to differences in their sizes/ sexes/ ages and reproductive status.

The movement parameters for the 20 Gharial and 2 Mugger are shown in Tables 1 and 2. With the exception of the three adults referred to above (ID 44,50, 54), the remaining 17 Gharial consisted of 6 juveniles (4 females, 2 males; Table 1), and 11 sub-adults (3 females, 8 males; Table 3), ranging in size from 1.4 to 2.8 m total length. The two Mugger crocodiles (*Crocodylus palustris*) were juveniles, a male and a female, both less than 2 m total length. These animals were captured, tagged, and released at sites ranging from Gohera (103 km upriver from Yamuna- Chambal confluence) to Jagtouli (34 Km upriver from Y-C confluence), in areas frequented by Gharial tagged earlier.

In total, for these 20 Gharial, 1869 animal-specific locations have been recorded, and 224 instances for the two Mugger, or 2093 locations overall during the past 2+yrs. In general, juvenile Gharial were located less frequently than were sub-adults; animal-specific locations averaged 80 locations/animal for 6 juveniles and 100 for 11 sub-adults, ranging from 11 to 143 for juveniles and from 57 to 140 for sub-adults. In fact, some individuals proved difficult to locate regularly, eg ID

92, 96, 40, and 58, while others were reliably located at frequent intervals, eg ID 60, 62, 90, 94. The two juvenile Mugger were regularly tracked at frequent intervals, with 117 and 107 locations for 82 (upstream at Gohera; = 103 km) and 56 (downstream at Barecha; =38 km), respectively. Monitoring was spaced evenly throughout the monitoring period, with 4-8 locations per animal per month (Tables 1 and 2).

In brief, nearly all of the tagged Gharial exhibited restricted, seasonal movements, similar to the sedentary sub-adults that were monitored earlier in the 2010 radio-tagged group. The two adult females were the only notable exceptions. One of these, ID 50, though apparently not nesting in 2014, moved extensively up and down 50~64 km of river, from her capture site at Ater upstream to Rhea, as least several times back and forth during the study months. The other female, ID 54, moved mostly downstream to two potential nesting areas, Nachnouli (~18 km downstream) and Dinnpura (~12 km downstream) prior to the monsoon high water, and then during the monsoon shifted upriver +14 km to Gohera, just above her capture site, during the monsoon. Both females nested in 2015. Female 54 nested at Dinnpura (see Figure 47), and female 50 disappeared during the nesting/incubation/ hatching period, bu t reappeared post monsoon back at Ater near where she was tagged. Unlike these two adult females living in the same general area, the young adult male (ID 44) showed no dry season movements, and only shifted +7 km upstream during the monsoonal months of July--
-September in both years monitored (Table 1).

Table 1. Tracking summary for 20 resident wild Gharial (*Gavialis gangeticus*) in National Chambal Sanctuary, 2013-2016. Movements are summarized for 20 Gharial tagged in November-December 2013. Most were tracked biweekly, weekly or more frequently; as of end January 2016, all radios were not functioning. Animal-specific locations from December 2013 through end January 2016 totaled 1869; transmitter temperatures were recorded in 2060 instances (external transmitters mounted on tail or neck). Records are across all months, monsoon= Jun, Jul, Aug, Sept; rest= dry. Chambal River loc: GD= Godha; GH= Gohera; MG= Maghera ka Pura; DN= Dinnpura; KH= Khera Ajab Singh; KS= Kasaua; CT= Chikni Tower; TK= Tumrakola; PN= Pituwan ka Nagla; BR= Barchouli; SG= Sashon Ghat; Mahua Sunda= MS; PA= Patharra-Bihar; CF =Y-C confluence; SA= Sahson; JG= Jagtouli; RH= Rhea (upstrm= 163) YM= Yamuna; below confl: PD= Pachnada, BW= Bawain; cap/release sites=PN, KH, KS, BR. BA= Baroli, RN= Ranepura, GY= Gyanpura, BD= Badpura, BC= Barecha. Legends: tl-m= total length, metres; cap= capture location; mn= months tracked; locs= animal-specific locations recorded ; km= maximum movement distance, in km; loc to CFL= location on Chambal, relative to confluence (CF; in km)= 0; D= seasonal movement, in km (minus= downstrm; plus= upstrm). Neither 50 nor 54 nested in 2014, but both moved to and remained near nesting areas, upstream for 50, and downstream for 54. In 2015, both nested, 50 near home area, and 54 nested at the same downstream site, which she frequented in 2014 (DN, -14 km from AT).

ID	sex	tl	cp	mn	locs	km	river loc	village locations	trans temp (N)	monsoon	D	shift	home
98	jF	1.8	GH	24	83	8	97 to 105	Ater to Ranipura	14-40C (93)	no move		none	AT
52	saM	2.5	GH	25	100	6	97 to 103	Ater to Gohera	15-36C (110)	no move		none	AT
50	aF	3.3	AT	22	44	66	97 to 163	Ater to Rhea	14-40C (57)	no move	+50	up, +50	AT
54	aF	3.3	AT	23	116	26	79 to 105	NK to Ranipura	15-46C (130)	upstrm	+14	dwn, +14	DN
96	jF	1.7	MG	9	11	12	93 to 105	Naan to Ranipura	27-32C (13)	no move		none	GH
42	jM	1.8	MG	23	95	3	88 to 91	Hathkant to NaanTpl	14-38C (109)	no move		none	MG
48	saM	2.6	MG	23	97	2	89 to 91	Magerpura to NTpl	14-38C (111)	no move		none	MG
44	aM	4.3	MG	25	125	7	90 to 97	Magerpura to Ater	15-46C (183)	upstrm	+5	up, +5	NT
94	jF	1.9	KM	26	130	2	73 to 75	Chilonga to Murong	15-36C (125)	no move		none	MU
92	jF	1.3	MU	11	20	4	64 to 68	Udi Bdge to Baroli	15-35C (21)	no move		none	BA
90	jM	1.4	CH	26	143	4	72 to 76	Lakan to Murong	15-36C (167)	no move		none	CH
40	saM	2.1	CH	22	57	25	93 to 118	Naan to Harlalpura	19-39C (47)	variable		up, +24	RN
58	saF	2.6	BR	23	72	41	65 to 105	Barhi to Ranipura	16-35C (75)	variable		up, dwn, 30	BA
60	saM	2.5	GY	25	129	5	49 to 54	Gyanpura to Khera C	14-37C (132)	no move		none	GY
62	saM	2.8	GY	23	140	5	49 to 54	Gyanpura to Khera C	14-38C (145)	no move		none	GY
66	saF	2.3	GY	22	100	195	15 to 210	Mahua S. to Tighra	16-40C (106)	dwnstrm	+180	up, +180	PN
64	saM	2.7	PN	25	118	12	30 to 42	Sashon to Tumrakola	15-40C (122)	variable	-8	dwn, -8	BC
68	saM	2.6	PG	22	92	11	33 to 44	Barchauli to Kasaua	15-41C (96)	no move		none	PN
76	saF	2.0	PG	18	89	16	36 to 15	PN to Mahua Suda	15-36C (104)	dwnstrm	-4	none	JG
80	saF	2.5	JG	25	108	56	0 to 56	Bhareh to Khera	15-37C (114)	dwnstrm	-23	dwn, -15+	BC

Table 2. Tracking summary for resident wild Mugger crocodiles (*Crocodylus palustris*) in National Chambal Sanctuary, 2013-2015. Movements are summarized for 2 Mugger tagged in November-December 2013. These were tracked biweekly, weekly and/or 2-3 times per week; Radios ceased to function by December 2015. Animal-specific locations from December 2013 through November-December 2015 to end sampling period= 224; transmitter temperatures= 247 instances (external transmitters mounted on tail). Records for monsoon= Jun, Jul, Aug, Sept; rest= dry. Chambal River loc: GD= Godha; GH= Gohera; MG= Maghera ka Pura; DN= Dinnpura; KH= Khera Ajab Singh; KS= Kasaua; CT= Chikni Tower; TK= Tumrakola; PN= Pituwan ka Nagla (Philmunnagara); BR= Barchouli; SG= Sashon Ghat; Mahua Sunda= MS; PA= Patharra-Bihar; CF= Yamuma-Chambal confluence; YM= Yamuna; below confl: PD= Pachnada, BW= Bawain; cap/release sites= PN, KH, KS, BR. JG= Jagtouli. Legends: tl-m= total length, in metres; cap= capture location; mn= months tracked; locs= locations recorded; km= maximum movement distance, in km, maximum river distance upstream to downstream site; loc to CFL= location on Chambal, relative to confluence (= 0); Dkm= seasonal movement, in km (plus= upstrm; minus= dwnstrm).

ID	sex	tl	cp	mn	locs	km	river loc	village locations	trans temp (N)	monsoon	D	home	shift
56	jF	1.9	MK	24	107	8	97 to 105	Ater to Ranipura	14-39 C (119)	no move		Gohera	none
82	saM	1.7	BC	23	117	49	0 to 49	Bhareh to Gyanpura	14-37 C (128)	variable		KS, JG	none

The majority (11of 17) of the recently-tagged Gharial (4 females= 92, 94, 96, 98; 7 male= 42, 90, 48, 52, 60, 62, 68) as well as the two juvenile Mugger exhibited little or no movements during the study period. Of the remainder (6), two shifted dry season residencies soon after release (40, 58), moving upstream and then showed monsoonal movements, with a seasonal return to previous residences post monsoon a year post tagging. One (80) showed short, downstream monsoon movements both years. Two (64, 76) showed downstream movement the first year, but were stationary the second monsoon season. Finally, a sub-adult female (66) moved downstream the first monsoon (2014), then upstream 195 km to the large nesting colony at Tighra (= 210 km above the Yamuna-Chambal confluence) where another long-distance mover, female 24, nested in 2015. Then, she moved back 190 km downstream during the monsoon.

The two juvenile Mugger exhibited variable movements that contrasted with each other. The upstream individual (ID 56; female) resided within +5 km of Gohera, where it was located most often. It did not shift residences nor migrate during the monsoon high water (Table 2). The other Mugger (ID 82; male) was a dry season resident in the lower section of river. It made a monsoonal movement of ~25 km downstream, then returned to the same dry season residence it occupied prior to the monsoon (Table 2). In general, the movement patterns of the immature Muggers were similar to those of the immature Gharial with respect to the parameters listed in Table 1.

Large Gharial With GPS Loggers

Last year, in 2014, for the first time, radio transmitters equipped with GPS loggers which could be remotely downloaded periodically, while still attached to free-ranging, wild resident Gharial were utilized. Thus, units were capable of being remotely downloaded at intervals, but also emitted a VHF location signal that could be tracked manually. These tags have been successful in producing very fine scale data on daily locations, resulting in 2-10 records per day, on an almost daily basis, for each tagged Gharial. A total of 7 GPS tagged Gharial were monitored during 2015, and a summary table of results to date is presented here as Table 3. Representative movement maps for GPS tagged Gharial are shown in Figures 2-4.

To date, as of March 2016, three Gharial (28, 30 and 32) have been successfully tracked and the GPS data downloaded for the previous 15 months since tags were affixed (Figs. 1-4). For these three, 3599 animal locations were logged, ranging from 991 for sub-adult male 28 to 1438 for large ghara male 30 (Table 3). These three Gharial have been monitored for 462-466 days, more than a year (= November 2014 to March 2016), and daily records were obtained on 859 of 1390 total days, or 62% of the total days with the radios attached. In general, the least successful months for obtaining daily locations are the monsoon months (July through September) and the most successful months are the winter months (November through February) when Gharial are basking on land on sunny, warmer days.

Partial location records were obtained for two other Gharial equipped with GPS loggers, 931 records for a sub-adult female (=22) monitored for 301 days (with 213 daily locations or 71% of the total days monitored), and 472 records for an adult female (=26) monitored for 175 days (with 85 daily locations or 49% of the total days monitored). For two additional Gharial (20, 24), the GPS loggers failed to allow remote download, and these are noted as "fail" in Table 3, and were only tracked successfully using the regular manual tracking procedure followed for all VHF radioed Gharial during the course of study. Five of these large Gharial were equipped with both VHF and GPS logging radios (20, 22, 24, 26, 30), and two Gharial in this group (28 and 32) were only equipped with GPS units. The GPS units have a built-in VHF signal that allows limited manual tracking, but the signal has a short range, relative to the regular VHF radios utilized, which do not have GPS downloading capability.

In Table 3, the correspondence between the maximum river distance occupied by each animal is shown for the values determined using the manual tracking method, and the maximum river distance determined for each animal, based on the GPS locations obtained. In the cases of two Gharial (22, 30), the distances obtained using either method is very close, 10 vs. 13 km for 22, and 65 vs 67 km for 30. GPS determined values were only slightly higher in both instances, +3 km for 22, and +2 km for 30. This difference for Female 26 was almost double, =2X for the GPS determined locations (=19 km) vs. the manual tracking locations (=10 km). For Female 32, one long trip upstream was not detected manually, but is evident from the GPS records, more than doubling the maximum range of river locations, from 30 km (=manual tracking) to 69 km (=GPS tracking).

In one instance, sub-adult male 28 was not detected at all for a full season, from November 2014 until September 2015 using the regular manual tracking procedure. However, during the monsoon months, this individual was observed visually at a downriver location for the first time in over 9 months (Figs. 5-8). Shortly thereafter, the GPS records were downloaded, and revealed that this individual had been living in and using a familiar stretch of river being routinely monitored on a weekly basis during this entire 9-month period. During this time, 547 GPS locations were logged, over 290 days, with 163 daily locations determined. Using the conventional manual tracking procedures, it was thought that this individual was no longer in the area or the radio had malfunctioned. In retrospect, neither of these scenarios were correct. This example demonstrates the value of the GPS logging system to successfully record animal locations and activities undetected using other conventional techniques. For instance, male 28 had a maximum range of upstream vs. downstream river locations that was very limited, from kilometre marker 34 to 30, or just 4 km, based solely on 10 locations using manual tracking methodology (Table 3). In fact, this individual ranged over 116 km of river, and made an undetected upstream visit over several months, with multiple daily stopovers along the way upstream and downstream. The extensive use of a much larger section of river was only revealed when the GPS locations were downloaded and plotted.

Table 3. Tracking summary for 7 resident wild adult Gharial (*Gavialis gangeticus*) in National Chambal Sanctuary, 2014-2016. Movements are summarized for 7 Gharial tagged in mid Nov 2014. These larger Gharial were tracked manually, utilizing the VHF radios attached to base of tail and/or neck. Tracking frequency was biweekly, weekly, or 2-3 per week, depending on season and daily conditions. In addition, each animal was outfitted with a custom designed GpS logger (Wildlink W510; Advanced Telemetry Systems, Isanti, MN USA) that was scheduled to take location "fixes" and log these, according to a pre-determined daily program, adjustedseasonally to maximize attempted readings when animals were likely to be on land, either basking during the cooler months, or resting on land in evenings and at night during the warmer months. Legends: tl-m= total length, metres; cap= capture location; mn= months tracked; locs= animal-specific locations recorded ; km= maximum movement distance, in km; loc to CFL= location on Chambal, relative to confluence (CF; in km)= 0; D= seasonal movement, in km (minus= downstrm; plus= upstrm). Animal-specific locations from Nov 2014 onward are summarized below, for locations determined manually and also by GPS locations. Periodic downloading of the GPS loggers was attempted during the cool months when animals were on land for hours on sunny days. The Wildlink system downloads via UHF coupled with on-board VHF signal preset for fixed daily times, and depends on proximity of 200-500 m and a strong, clear VHF signal when the radio GPS logger is not submerged and out of water. Consequently, downloading is limited to times when tagged Gharial can be approached closely, and the radioed individual is out of the water. Records are across all months, monsoon= Jun, Jul, Aug, Sept; rest= dry, and are comparable to the records of radio-tagged Gharial and Mugger shown in Tables 1 and 2.

ID	sex	tl	cp	mn	locs	km	river loc	village locations	trans temp (N)	monsoon	home	GPS rec	day rec
20	aM	4.1	CH	12	70	61	38 to -10 0 to -13	BA, Gopiakar Gohani (Yamuna)	15-38C (81)	BA,PA	BA,PA	fail	none
22	saF	2.9	AT	12	57	10 (13)*	93 to 103 (92 to 105)*	Naan to Gohera Naan-dwn to RN*	14-40C (71)	no moves	GH	931	213/301
24	aF	3.1	GH	12	16	210	0 to 210	Bhareh to Tighra	23-32C (23)	CF=0	TG	fail	
26	aF	3.2	GH	12	29	11 (19)*	114 to 103 (116 to 97)*	Khndra to Gohera Halapura to Ater*	14-38C (36)	no move	RN	472	85/175
28	saM	3.1	JK	15	10	4 (116)*	34 to 30 (135 to 19)*	Jagtauli to Sahson Pinhut to Kheda*	-------------	SA SA	JG KS	991**	282/462
30	aM	5.3	KS	15	89	65 (67)*	-10 to 55 (-9 to 56)*	Gopiakar to KH GP up to KH frnt*	15-40C (99)	CF=0, GP	KH, KS	1438**	298/466
32	aF	3.2	JK	15	25	30 (69)*	55 to 25 (91 to 22)*	Khera to Kundaul NaanT to Palighar	-------------	SA	GY	1170**	279/462

Figure 1. Male 30, a 5.3 m (totl) "large ghara" male radio-tagged with VHF radio on his back, and another radio with GPS logger affixed to his tail. Tagged in 2014, he was tracked manually at frequent intervals during the intervening 15+ months (as of March 2016). 89 locations indicated a limited range of 65 km, primarily downriver, monsoon movements to the Yamuna River confluence.

Figure 2. GPS plot, with location points shown as green dots, for male 30. Over 466 days, a total of 1438 locations were logged, and are plotted on this Google Earth image of lower Chambal. This animal had two distinct residences, an upstream, dry season site at Khera, Chikni, and Kasaua, and a summer, monsoon residence near the Yamuna River. He ranged 10 km below confluence during this period, but primarily stayed above the confluences in lower Chambal section (Table 3).

Figure 3. GPS plot of dry season locations for male 30, a large ghara, dominant male that maintained a breeding "territory" at Khera Channel, where he was often located.

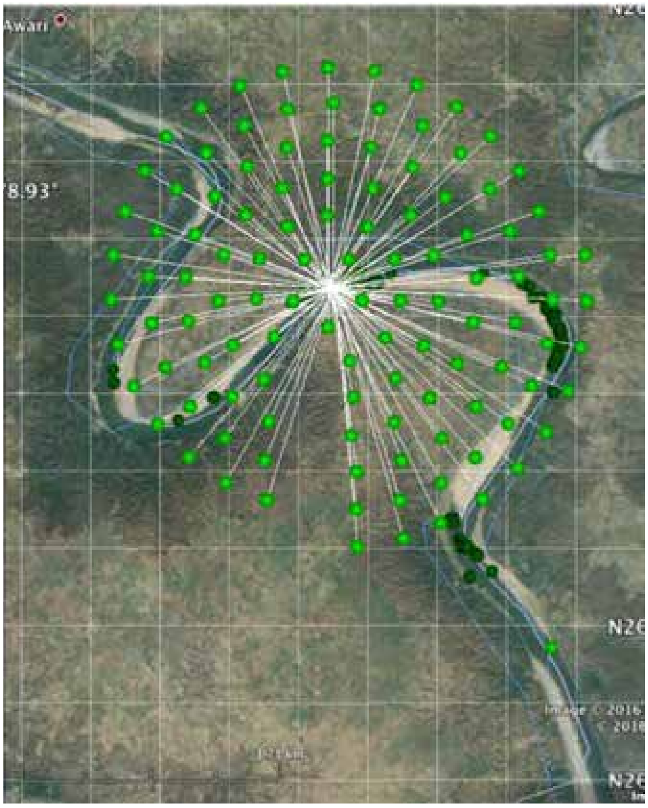

Figure 4. GPS plot, on Google Earth, of multiple occupancies by male 30 of the same location at Khera Channel, during dry season, pre-monsoon period in 2015. This male remained at this site where one female nested, and he acted as guardian for hatchlings when they appeared in early June.

Figure 5. Male 44, a "junior" male, not yet fully grown, and not yet dominant, swims near a large creche of hatchlings at a communal nest site, Naangoan Temple site, where 11 nests hatched in 2015. At this site, 13 sites hatched in 2014. This male was tracked by VHF radio for 25+ months, November 2013-December 2015. He was the dominant guardian male at this communal nest site in two successive years, 2014 and 2015. He was definitely not the breeding male in the area, where a larger sized, large ghara male is the dominant breeder (Table 1).

Figure 6. Hatchlings remain tightly grouped in a dense creche at nest site where there was communal nesting. Hatchlings spend the early morning and late evening on land, midday at the shoreline or in the water, and then disperse in shallow water in the nest vicinity at night (see Figs. 9-12 - game camera night images at creches). After 2-4 weeks post-hatching, young actively feeding and widely dispersed at night near the nest site.

Figure 7. A female "guardian" at a nest site typically remainsin close proximity to the creche, but she is not necessarily always present. At each nest site, a singular female, one of the larger sized animals, assumes a dominant, guardian role, and appears to actively exclude other females from the area she occupies while attending the creche. Here, a hatchling perches on the female guardian's head while other hatchlings float in the water nearby. Females remain with creches until high water floods the shoreline near the nest site, and then leave the young when adults disperse downriver, or upriver, and away from the nest site.

Figure 8. Batteries of game cameras, modified to record near-continuous, short video clips, were installed at each of two communal nest sites, one upriver and one downriver. These were operated for 3-6 days continuously, 24 hrs early (within a week to 10 days) and late (3 wks) post-hatching. Using this et-up, it has been possible to monitor guardians at the creche, and determine who is guarding when among the adults in the nest vicinity. This site, Khera front side, had 17 nests, and all of these hatched, producing 500+ hatchlings in 2015.

Figure 9. Game camera infrared image at creche site: a large ghara male “guardian” comes ashore amidst a shoreline lined with hatchlings (= small eyeshines in distance at creche shore).

Figure 10. Game camera infrared image at creche site: a female attending the creche, approaches hatchlings at shore. Other cameras are visible in the distance. Note open jaw position of the young in the water, an effective “fish catching” posture.

Figure 11. Game camera infrared image at creche site: a “guardian” female rests at shoreline while a hatchling perches on her back.

Figure 12. Game camera infrared image at creche site: Female “guardian” rests at shoreline, with jaws agape. Hatchlings visible in foreground, and in distance on shore and at shoreline (= small eyeshines in distance).

Figure 13. Close-up telephoto of tractors and trolleys, removing sand from Rithora (MP)-Tighra (Raj) sandbank on Madhya Pradesh side of river. Tractors shown here were only some of the 35-40 tractors counted at site in late afternoon, early June 2015. Wide angle image of same scene in Figure 14.

Figure 14. Panoramic view of sandbar on Chambal River, 5 km below Rajghat, at Rithora-Tighra villages. Here, 35-40 tractors operated almost 24 hours in the several pre-monsoon months of May-June to remove trolley loads of sand, on an industrial scale, to be used for building and construction in major metropolitan areas. This activity is strictly illegal, but widely engaged in by a sand mining "mafia".

Figure 15. At Rithora-Tighra sandbar, there were two large, communal nest sites used by gharial, an upper site with 16 nests and lower site with 13 sites. A total of 25 of 29 nests hatched in 2015; two nests were predated at each site. Shown here is a tractor-trolley in the distance, and two gharial on the shoreline in foreground. Nesting area (upper site) was located just to the right of photo on shoreline.

Figure 16. Concentration of nests at upper communal site (16 nests; 14 hatched) at Rithora-Tighra. Three females are visible on land near excavated nests (evident as sand disturbances on surface), and one female remains at shoreline. Jackals closely approached unattended nests and scavenged damaged eggs and consumed injured hatchlings. Nest excavation and/or predation by jackals was usually interrupted by guarding females. Sand removal was underway on an industrial scale, almost 24/7 at this season, immediately behind these nests.

Creche Attendance and Hatchling Guardians

During the nesting season in 2015, a concerted effort was made to monitor several large crèches in the lower Chambal with infrared game cameras for continuous 24-hour periods for days at a time in order to determine which adult individuals were acting as protectors and guardians at each crèche, and to characterize the patterns of attendance, care, protection and guarding exhibited by adults remaining in the vicinity of large groups of hatchlings assembled at colonial nesting sites. The emphases during this year's hatching and post hatching period were to make detailed observations, supported with still, video, and remote game camera video clips, without interfering with the natural progression of daily activities. In other words, disturbance by human presence was minimized as much as possible. Creches were observed at safe distances that resulted in minimal, if any disturbance, in most cases. Whenever possible, observations were confined to opposite river banks, at distances of 100+ m, so direct disturbance was avoided.

Creches at two sites were sampled with game camera video continuously during hatching, within a week to 10 days post hatching, and later at 2-3 weeks post hatching (Figs. 5-8). Sets of game cameras, modified to take sequential 55 second video clips for 24+ hrs were installed, and changed daily to record complete records of activities at the crèche during the daytime and at night (Fig. 8). Representative stills from this extensive data set are shown in Figures 9-12.

Importantly, these crèche observations indicate two special features associated with the patterns of attendance and guarding of hatchlings. First, one of the nesting females, apparently among the largest at each site, appears to be dominant in protection of hatchlings. She visits the group(s) of young often, but does not remain constantly with them. Only one female seems to do the majority of the guarding, and she actively excludes other females from close approach to the hatchlings, under most circumstances. Typically, the dominant guarding female is assisted in hatchling protection by a large ghara male who associates himself with the crèche of young. When disturbances pose major threats occur to the hatchlings, the male replaces the dominant female at the crèche, and displays his presence with acoustic jawclaps, and loud exhalations that resonatethrough the large, cartilaginous ghara at the tip of his snout. The male assumes "guard" duty, providing protection for hatchlings against potential predators.

Detailed behavioural observations during the breeding and nesting and hatching periods in 2014 and in 2015 at one site upstream, Naangoan Temple nesting colony, strongly suggest that the guardian male at this site is a junior, not yet dominant male, with a ghara that has enlarged in size over the past 3 years. Male 44/46 was tagged in November 2014 and so is relatively easy to locate; his river travel is very limited to a small area, eg within 7 km (Table 1). He has not been actively engaged in breeding, but has been the persistent guardian at a relatively large crèche consisting of 13-17 hatched nests in both 2014 and again at the same site in 2015 (Fig. 5).

Nesting Success On The Lower Chambal

Nesting effort has been monitored for the past six years on the Uttar Pradesh section of the lower Chambal, in the National Chambal Sanctuary; the river distance above the confluence with the Yamuna is 163 km. In 2015, several of the radio-tagged females ventured even farther upstream, to a river distance of 210 km above the Yamuna confluence, at Tighra. Consequently, observations were frequently made at Tighra on two large nesting groups (Figs. 13-16). In 2015, a longer river stretch (210 km) was surveyed from Tighra down to the confluence for evidence of nesting.

Within this stretch, 153 nests were located, based on either predation signs prior to hatching and/ or evidence of hatching. Of these, 126 showed evidence of successful hatching, and many groups of young were observed in the vicinity of the numerous nest sites examined within several weeks of hatching. The survey was conducted during daylight hours, from pre-dawn through evening dusk, during 17-20 June, and followed up several weeks later to ensure that all hatched nests were tallied. A summary of nesting (2010-16) is shown in Table 4; in addition, 49 km above the upstream Rhea site were examined and resulted in 66 nests from Barsala to Tighra, with 59 of these producing hatchlings. Male guarding was prevelant at larger communal sites; nest lost from predation occurred at nest sites with relatively few nests, though some of these small groups of nests were guarded by males.

As noted above, during the 2015 nesting season, observations were targeted at larger nest sites, where creches were guarded by both male and female guardians. An effort was made to not disturb these groupings when making observations, by remaining on opposite banks, or at distances from the creche that left the hatchlings and adults undisturbed to determine the natural pattern of care.

Table 4. Spatial distribution of Gharial nest sites on lower Chambal River, referenced on distance from Yamuna confluence (= 0 km), left is upstream, right is downstream. Twenty-one nest sites were used during 6 nesting seasons, producing 38, 76, 80, 62, 96, and 85 nests respectively from 2010-15. The number of nests that produced hatchlings in each year are shown for each location in the bottom panel. For example, the Rhea site (RH) had 11, 20, and 30 total nests, and 11, 19, and 28 nests hatched in 2010, 2011, and 2012, respectively. Bolded,underlined entry indicates that a male was observed guarding hatchlings at nest site, and resided nearby during hatch-post hatch.

km Loc.	161 RH	133 KP	126 GA	118 HL	107 GD	103 GH	97 AT	91 NT	90 MG	89 HK	87 DM	85 DU	79 NK	74 CH	56 KF	54 KC	49 CT	46 KS	39 BA	38 PN	29 SG	Total Nests	Nest Sites
2010	11	2	-	-	4	3	-	-	-	-	-	-	3	3	4	4	-	3	-	1	-	38	10
2011	20	4	-	-	3	3	-	4	-	-	-	-	6	6	7	7	3	7	-	6	-	76	12
2012	30	-	-	-	-	-	-	-	-	-	12	8	-	-	20	-	3	-	-	7	-	80	6
2013	26	2	-	4	8	-	2	-	2	-	-	8	2	-	4	-	-	2	2	-	-	62	11
2014	20	4	3	21	-	-	3	15	-	1	7	-	-	2	13	-	-	3	-	1	1	96	14
2015	16	5	-	15	-	-	3	11	-	-	5	-	-	4	17	1	-	7	-	1	-	85	11

	RH	KP	GA	HL	GD	GH	AT	NT	MG	HK	DM	DU	NK	CH	KF	KC	CT	KS	BA	PN	SG	Nests hatch.	Hatch sites
2010	**11**	pd	-	-	4	3	-		-	-	-	-	2	**3**	**4**	**3**	-	pd	-	1	-	31	8
2011	**19**	pd	-	-	pd	pd	-	pd	-	-	-	-	**6**	**6**	**3**	pd	**3**	**5**	-	**6**	-	48	7
2012	**28**	-	-	-	-	-	-	-	-	-	**11**	**8**	-	-	**17**	-	pd	-	-	**5**	-	69	5
2013	**24**	2	-	4	**8**	-	2	-	2	-	-	**8**	pd	-	**2**	-	-	pd	1	-	-	53	9
2014	**18**	4	**2**	21	-	-	**3**	**13**	-	1	**7**	-	-	**2**	**13**	-	-	**3**	-	pd		88	12
2015	**16**	2	-	6	-	-	**3**	**11**	-	-	**2**	-	-	**2**	**17**	**1**	1	**7**	-	pd		67	10

Conservation Benefits Of Gharial Ecology Project

The Gharial Telemetry Project has been recognized by the National Tri-State Committee for Coordination of the Conservation in the National Chambal Sanctuary, and the recommendations of the project solicited for incorporation into the National Plan for Gharial Conservation, under consideration for adoption and implementation. The Principal Investigator presented a plenary paper in January 2015 at the Society for Integrative and Comparative Biology (SICB) meeting in Florida, and delivered talks at the University of Florida in Gainesville, St. Augustine Alligator Farm, and in late January 2015, at the Wildlife Conservation Society head office in New York City, based at Bronx Zoo. In April 2015, I visited the Herp Tag Meeting in Atlanta, Georgia and presented a talk on Gharial ecology to the Crocodilian AZA group associated with Herp Tag. In late May and early June, I visited the Madras Crocodile Bank, and did a presentation for the Board of Trustees, and discussed the project and conferred with Romulus Whitaker, founding Trustee, and Zahida Whitaker, Director and Founding Trustee at MCBT. Publication in peer-reviewed journals is in preparation, as are popular articles. A summary of recent activities related to Gharial conservation is scheduled to appear in the January-March 2016 issue of the Crocodile Specialist Group Newsletter.

There is now wide recognition of the importance and significance of the biological findings of the Gharial Ecology Project, and in particular, how these inform and are directly relevant to ongoing conservation and management efforts. This is especially important for the remaining Gharial populations in the National Chambal Sanctuary, living in a protected, free-ranging, wild river habitat. The success of efforts to date to continue the study attest to the interest and commitment by the state and central governments, particularly the MoEF and the Forest Departments of Madhya Pradesh, Rajasthan, and Uttar Pradesh for the shared objectives outlined for this project.

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Notes on the Current Status of the Saltwater Crocodile, *Crocodylus porosus*, within East Nusa Tenggara Province, Indonesia

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Abstract

East Nusa Tenggara is a province in Indonesian consisting of much of the eastern Lesser Sunda Islands (including Flores, Sumba and the western half of the island of Timor). Historically the Saltwater crocodile, *Crocodylus porosus*, was present within suitable habitat throughout virtually all portions of East Nusa Tenggara, but hide-hunting during the mid-20th Century combined with widespread habitat destruction resulted in the disappearance of the species from many areas. In recent years a sharp increase in reports of crocodile attacks on humans suggests that the species may be on the rebound within the province. Most of the reported attacks came from West Timor, but a small number of attacks were also reported from Flores, Lembata, Rote, and Sumba. Recent human-crocodile conflict surveys of the Kupang and Malaka regencies of West Timor and the West Manggarai regency of Flores have included short visits to crocodile habitat which revealed resident populations of crocodiles existing in these areas. In addition, information and photos from wildlife officials in Sumba have shown the species to present within eastern portions of the island, even far upriver in some areas.

Introduction

The Indonesian province of East Nusa Tenggara is a part of the Lesser Sunda Islands archipelago and comprises an area of 47,349.9 km^2 and consists of more than 96 islands, the largest of which are Timor (the eastern half of which comprises the sovereign nation of Timor-Leste), Flores and Sumba. The human population of East Nusa Tenggara is 4,683,827 (as of the 2010 census) with nearly equal ratios of male and female citizens and 80.66% of which were living in rural areas (UN OCHA). The Saltwater crocodile (*Crocodylus porosus*) is considered the world's largest crocodilian species, with some individuals on rare occasions reaching over 6 m in length (Britton *et al*. 2012). Saltwater crocodile populations have been drastically reduced throughout much of their range, with extirpation from most of mainland Southeast Asia. Within the Indonesian archipelago the Saltwater crocodile persists in many areas, but the status of these populations is largely unknown (www.crocodilian.com). A combination of factors, including bounties for the capturing or killing of crocodiles, massive human population growth, and the demand for crocodile skins, caused severe declines of Saltwater crocodile populations in Indonesia during the last half of the 20th Century (Boomgaard 2007) and today the species is absent from most of Java and West Nusa Tenggara (www.crocodilian.com). In East Nusa Tenggara the Saltwater crocodile populations persist in a number of areas (BBKSDA-NTT, pers. comm.).

The Saltwater crocodile has a reputation as being one of the crocodilian species most likely to prey upon humans (Webb *et al*. 2010). Attacks on humans by Saltwater crocodiles are well-documented, particularly within Indonesia (with the exception of Papua and West Papua Provinces), with East Nusa Tenggara having the highest number of reported attacks, along with East Kalimantan Province. It is possible that significant numbers of attacks go unreported throughout the country (CrocBITE). In many portions of East Nusa Tenggara the Saltwater crocodile is viewed as a "totem" animal in the same way that it is viewed in Timor-Leste (BBKSDA-NTT, pers. comm.). This cultural importance has led to people in these areas being very tolerant of crocodiles, even when they attack and kill people.

We collected details on crocodile attacks throughout East Nusa Tenggara Province using a variety of means. In addition, in late July and early August of 2015 we visited three locations in East Nusa Tenggara (two locations in West Timor and one location in Flores) to assess the extent of human-crocodile conflict (HCC) in the area (which included collecting the details of unreported attacks), conduct interviews with villager in an effort to gain a better understanding of local attitudes towards crocodiles and to conduct short classes on crocodile safety. We also made short visits to crocodile habitat near each of these locations which resulted in opportunistic crocodile sightings and a better understanding of habitat quality.

Materials and Methods

Crocodile attack records were collected from a number of sources - media reports, the local BBKSDA-NTT offices and through interviews with villagers. Visits to villages which had recently experienced incidents of HCC included the collection of unreported crocodile attacks from previous years. The local BBKSDA-NTT office in Betun near Maubesi Mangrove provided us with a large number of previously unreported attacks in the region from the period 2000-2015.

Our HCC surveys consisted of a questionnaire in-order to determine the general attitude towards crocodiles in the villages and a conference with villagers in order to determine what crocodile attack incidents had gone unreported. Questions asked of villagers included what they believed should be done with problem crocodiles, if they believed crocodiles had an importance (and what that importance was), if crocodile attacks/numbers had increased during the previous 10 years, etc. Most of the villagers given the questionnaire were fishermen, which are the most frequent victims of crocodile attack in West Timor. We also included a brief power point presentation on crocodile awareness and safety.

Results

Crocodile attacks

For the period 1 January 2006 through 31 October 2016 there are records of 86 attacks resulting in 55 deaths in East Nusa Tenggara, for a fatality rate of 63.9%. Of these, 77.9% (67) of attacks and 80% (44) of deaths were reported from West Timor. The remainder of the attacks were reported from the islands of Lembata (8 attacks, 5 fatal), Sumba (6 attacks, 2 fatal), Flores (3 attacks, 2 fatal), Rote (1 attack, fatal), and Semau (1 attack, 1 fatal). The highest numbers of attacks were recorded from the Kupang (33 attacks, 19 fatal) and Malaka (22 attacks, 15 fatal) regencies (both in West Timor). When combined these two regencies account for 63.9% (55) of attacks and 61.8% (34) of deaths.

Surveys

Menipo Island

On 31 July 2015 we arrived at the local BBKSDA office in Enoraen village, which lies approximately 800 m from the Menipo Island estuary. The area consists of a large, mangrove-lined estuary separating Menipo Island from the mainland. The park is also known as an important Saltwater crocodile habitat and the site of recent HCC. After a short lecture on crocodile biology and safety we asked villagers if they had any knowledge of crocodile attacks. This inquiry resulted in the collection of three previously unrecorded crocodile attacks (two of them fatal) from the Menipo Island region. Upon completion of attack data collection we provided a questionnaire to 12 villagers in order to gain information on attitudes towards crocodiles in the village. The results of the questionnaire revealed unanimous support for crocodile protection, despite all respondents agreeing that crocodile populations and crocodile attacks (on humans and livestock) had increased compared to 10 years previous. All 12 of the residents stated that they believed crocodiles should be protected, but also that they were well aware that crocodiles were dangerous. The results of this questionnaire are not surprising, given that there are deep cultural connections to the Saltwater crocodile in West Timor, similar or identical to the cultural significance the species has in neighboring Timor-Leste (BBKSDA-NTT, pers. comm.).

On the afternoon of 31 July and the morning of 1 August 2015 we conducted short surveys of crocodile habitat in the Menipo Island estuary. We encountered 4 different crocodiles during these surveys, estimated as 3.5-4 m, 3-3.5 m, 2-2.5 m and 1-1.5 m in length. During these surveys we also encountered other wildlife, including potential prey species. These included a troop of long-tailed macaques foraging on exposed mangrove mud-flats and a large colony of flying foxes. The information collected in Enoraen and the crocodiles sighted during our survey suggest that the Menipo Island region may hold a significant population of the species. In addition, immediately to the east of Menipo Island are the Noelmina River estuary and Lake Kubai, which are two locations also known to hold Saltwater crocodile populations (BBKSDA-NTT, pers. comm.).

Maubesi Mangrove

The Maubesi mangrove is a nature preserve located along the southeastern coast of West Timor, near the border with Timor-Leste, and is known to hold a population of Saltwater crocodiles. On 4 August 2015 we visited Kletek village, which is located around 1 km from the Maubesi mangroves along the southeastern coast of West Timor near the border with Timor-Leste. We provided the village with the same brief lecture on crocodile biology and safety that we provided to Enoraen village, as well as the same attack survey and the same questionnaire. The attack survey resulted in 29 new records (20 of which resulted in the death of the victim) spanning from 2001 to 2015. All of the attack records were from the Maubesi mangrove area (Central Malaka, Kobalima and Wemataus sub-districts). The results of the questionnaire were very similar to those obtained from Enoraen village - the villagers in Kletek also wanted crocodiles to be protected and felt they should be left alone. In fact, the only notable difference in responses between the two villages was that in Kletek some of the villagers (3 respondents) claimed to swim in crocodile habitat. During this visit we also interviewed a 72 year old "Pawang" (a traditional "crocodile charmer") who claimed that the Maubesi mangrove region currently held 1000 crocodiles. He also claimed that some of the residents in the Benain River estuary (which is located a short distance to the west of the Maubesi mangrove region) had been unsuccessfully attempting to kill crocodiles by poisoning the water, suggesting that the positive view of crocodiles we found to be prevalent in Enoraen and Kletek is not universally held throughout West Timor.

On the morning of 5 August 2015 we conducted a short survey of the Maubesi mangroves using a large and very loud diesel powered boat. During the survey we encountered two crocodiles - an individual of approximately 3 m length which quickly entered the water when we came into view and a small (<1 m) individual which also quickly scattered into the water. It is possible that the noise produced by the vessel resulted in fewer sightings. During the survey we also encountered many fishermen, all of them using small dug-out canoes that could easily leave the victim vulnerable to crocodile attack. In addition, we encountered two children in a small dug-out canoe dangling their arms into the estuary.

Nanga Nae River

On 6 August 2015 I arrived in Labuan Bajo which is located within the West Manggarai regency of Flores and is a popular tourist destination with the main attraction being tours to the nearby islands of Rinca and Komodo to view the Komodo dragon (*Varanus komodoensis*). In recent years there have been frequent reports of crocodiles from the Nanga Nae River, which lies south of the town, and sporadic sightings are even reported from Rinca Island. In April 2015 a man was reported to have been killed by a crocodile while bathing in the Nanga Nae River approximately 6.2 km upstream from the river mouth. On the evening of 6 August, shortly before dusk, we conducted a short survey of the Nanga Nae River from the attack site to the river mouth and back. Our boat driver displayed a photo he had taken with his phone of a large (3.5-4 m) crocodile basking on the shore of the river near where the man was killed and he informed us that while the local residents feared the crocodiles and wanted them moved, they would not kill them. He responded positively to the idea of crocodile exclusion enclosures (CEEs). Our short survey yielded no crocodile sightings, but we did observe ample human activity along the river, including sand-mining. Upon our return we witnessed two teenage boys bathing not far from the location where the man was killed in April. Unlike our experiences in West Timor, here residents used the river for bathing and washing clothes.

On 10 August 2015 I met with a man who had survived a crocodile attack along the Nanga Nae River in 2013. The man was apparently attacked by a crocodile he estimated to be 2-3 m in length while collecting water from the river. He sustained an injury to his left leg just below the knee during the attack. He stated that goats had been killed by crocodiles and that on one occasion multiple crocodiles arrived to feed on a single goat. He also stated that he had previously seen baby crocodiles in a freshwater spring nearby and that multiple crocodiles lived in the river, the largest of which he estimated was between 4-5 m long. Like our boat driver, he also responded positively to the idea of CEEs being placed at important locations along the river.

Other regions

The Saltwater crocodile is reportedly present throughout many other portions of East Nusa Tenggara that we were unable to visit during our time there. While we visited the best areas of crocodile habitat in West Timor, crocodiles are likely present within some of the limited habitat along the northern coast of West Timor as well (evidence to this is 3 attacks, including 1 death, reported from the Biboki Anleu sub-district of North Central Timor regency in 2009, as well as fatal attacks being reported from Belu regency in 2016). Crocodiles are apparently also found on Rote Ndao, an island which lies to the southwest of Timor. In late December 2014 a man was killed by a crocodile at Pantai Baru in eastern Rote Ndao. Outside of the Nanga Nae River region, other known locations in Flores include Reo sub-district, where there have been reports of predation on livestock. Crocodiles are also reportedly present in Lake Tiwu Rhewu, a small freshwater lake in the Maukaro sub-district of Ende regency (BBKSDA-NTT, pers. comm.) and within Lake Waibelen (also known as Lake Asmara), a crater lake in the Tanjung Bunga sub-district of East Flores regency (Colin Trainor, pers. comm.), and within the mangroves at Lewolaga in Titehena sub-district of East Flores regency (BBKSDA-NTT, pers. comm.). Other known West Manggarai regency locations include habitat within the Lembor (where a child was reportedly killed by a crocodile at Siru village in October of 2012) and Boleng sub-districts, as well as near Bari in Macang Pacar sub-district (BBKSDA-NTT, pers. comm.).

On the island of Sumba crocodiles are still known from various portions of the East Sumba regency. Crocodiles have been photographed 12 km up the Kambaniru River at Tanau (Simon Onggo, pers. comm.) and in 2015 and 2016 crocodiles injured bathers around 15 km up the same river at Malumbi. Crocodiles are also known from the eastern and southeastern coasts of the regency, including Kaliuda (the site of a fatal attack in 2016), Watuparunu (where a man lost his left arm to a crocodile in 2015), Pindu Hurani (Laiwangi Wanggameti National Park) and Walakiri (the site of a fatal attack in 2014) (BBKSDA-NTT, pers. comm.).

Attacks on humans have been relatively frequent on Lembata Island (at least 8 attacks resulting in 5 deaths since 2011). These attacks occurred in shallow seas along the coast and in some small estuaries, particularly within the Omesuri, Nubatukan and Nagawutung sub-districts. The status of crocodiles throughout the other islands within the province is unknown, although a crocodile was recently captured in Kalabahi Bay of Alor Island (BBKSDA-NTT, pers. comm.).

Discussion

The results of our surveys and inquiries suggest that significant saltwater crocodile populations may exist in some parts of East Nusa Tenggara Province (particularly within West Timor) and, as is the case in neighboring Timor-Leste (Sideleau *et al*. 2016), HCC is becoming a serious issue in West Timor. While the most important population locations have been noted for West Timor, the status of the species elsewhere within East Nusa Tenggara Province is largely unknown. In addition to providing more detailed information on the species within West Timor, future surveys should also focus on determining the status of the species in eastern Sumba and northern Flores (including within some land-locked lakes) since crocodile populations clearly persist in some of these areas. The BBKSDA-NTT has stated it is possible that a significant number of crocodile attacks go unreported in Flores, Lembata and Sumba. The frequency of attacks in the coastal portions of Lembata Island is of interest, particularly given the habitat limitations (Lembata contains virtually no freshwater habitat and limited mangrove) (Colin Trainor, pers. comm.). In West Timor crocodiles are totem animals, just as they are in Timor-Leste (BBKSDA-NTT, pers. comm.), and the same is true on Sumba Island (Simon Onggo, pers. comm.). Based on articles reporting of crocodile attacks, the species is a totem on Lembata Island as well. This cultural importance appears to have allowed the species to persist in areas of human presence, even following increased incidence of HCC. CEEs may be of use along the freshwater sections of rivers in Sumba and Flores where bathers have been in conflict with crocodiles, but would be of little use in West Timor and Lembata where the majority of attacks occur on fishermen in estuaries and shallow ocean. Crocodile awareness and safety education may prove more useful in West Timor and Lembata, given the circumstances of attacks in these areas. In West Timor suspected man-eaters and other nuisance crocodiles are often captured and placed into captivity in Kupang, which has led to a new issue - where to put all of these captured crocodiles? We hope to help address this and many other issues in future visits to East Nusa Tenggara.

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Banking on Crocodiles in India

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Abstract

In 1975 Rom and Zai Whitaker bought land on the southeast coast of India and with a measly dozen crocodiles and lots of advice from croc people worldwide, set up India's first crocodile farm. We called it a 'bank' because crocs were facing extinction in India and needed a gene bank. Ten years later the Croc Bank had over 500 crocodiles of 10 species and was termed an 'international crocodile bank'. Forty years later we've got over 2000 crocs of 18 species, half a million annual visitors, and three field stations.

We began the Croc Bank with the goal of setting up a sustainable use program with the beneficiaries being the local Irula tribe, based on models we experienced in Papua New Guinea, Australia, Zimbabwe and USA. Animal welfare lobbies in India soon put an end to that dream (even though we argued that F3 generations of crocs are no longer wildlife but domestic animals) and the Croc Bank supplied 1500 of its surplus animals for wild restocking programmes around India. Ironically, now we destroy thousands of eggs each year and help States deal with the growing problem of human-crocodile conflict.

In line with the mitigation of human-crocodile conflict (HCC), a Croc Bank project funded by UNDP trains regional Forest Departments, imparts community education, and gets information direct from the field on conflicts, via several CBOs that function in these areas. Current focus is on HCC in the Andaman Islands, beginning with base-line croc surveys in areas inhabited by people. The plight of the Gharial was addressed in the CSG meeting in 2004 at Darwin, resulting in the creation of the Gharial Conservation Alliance. The future Croc Bank will be equipped with live web-cams, interactive educational tools, stone sculptures to illustrate mythology, and a state-of-the-art veterinary lab.

Rom outlines the visions and achievements of the Croc Bank for the first 25 years until he quit as Director in 2001. Nik Whitaker summarizes the period between 2001-2015, along the lines of conservation, education and research activities of the Madras Crocodile Bank.

Advances in Knowledge, Conservation and Sustainable Use of *Crocodylus moreletii* in Mexico

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Abstract

In recent years, Mexican authorities, researchers and crocodile farmers have worked together to improve the information on *Crocodylus moreletii* to strengthen the decision-making processes on its conservation, management and sustainable use. In 2010, the populations of Mexico and Belize were transferred from Appendix I to Appendix II of CITES with a zero quota for wild specimens for commercial purposes, as a precautionary measure. Results of the National Monitoring Program (2011-2015), financed and coordinated by CONABIO in collaboration with experts, suggest an average Encounter Rate of 3.34 ind/km and a Mexican population of around 76,000 individuals, estimated by considering a very conservative potential distribution area of 22,833 km (permanent water bodies in undisturbed areas). In this sense, the available information on *C. moreletii* reflects that the wild populations in Mexico are in a good state and that there is the potential for the development of sustainable productive projects to benefit local communities and the conservation of the species. Within this context, Mexico will present an amendment proposal to remove the zero quota from the *C. moreletii* Mexican population listing in Appendix II.

The species has a great potential for international high value markets and up to 86 captive breeding Management Units for Conservation of Wildlife are registered to manage *C. moreletii* in Mexico at the General Office of Wildlife of the Natural Resources Ministry (DGVS-SEMARNAT). However, among them, 34 had authorized harvest (captive bred) between 2005 and 2015, only 10 of these harvested with commercial purposes, and from these, only 5 have exported. The captive population in Mexico is approximately 28,000 specimens, and annual harvest (captive bred) is close to 4,500 individuals. Potential production in captive breed facilities is around 15,700 individuals and 6,800 skins per year (5,600 for international trade). Based on favorable results of the Monitoring Program and successful conservation activities, the CITES Mexican Authorities decided to explore sustainable use options for the Morelet's crocodile, with a strong *in-situ* conservation component. The objective is to set up an integrated production system of *C. moreletii*, based on conservation of the species and its habitat, as well as the sustainable, legal and traceable use with fair and equitable sharing of the derived benefits among the actors in the value chain. The initial implementation of such activities will be achieved through the "Pilot project on the sustainability, production systems and traceability of skins of Morelet's crocodile (*Crocodylus moreletii*) in Mexico", in collaboration with the Responsible Ecosystems Sourcing Platform (RESP), in a few pilot sites. In 2016, an intensive monitoring of nests (which will continue in the following years) will be carried out in the pilot sites in order to obtain detailed information to estimate sustainable harvest rates according to a ranching protocol being developed by CONABIO in conjunction with national and international experts.

Form February 29th to March 2nd the workshop on "Traceability of Morelet´s crocodile skins in Mexico" was held in UMA Cocodrilos Maya (exporting farm) in Ciudad del Carmen, Campeche. Participants in the workshop included local communities (Santa Isabel and UMA Cocodrilos de Palizada), producers (Manuel Muñiz), tanneries (Grupo Cuadra), Authorities (CONABIO, PROFEPA) and representatives of RESP and Anteleon. The objective was to test the biometric identification technology developed by RESP within the usual process of skin production in a farm. Results will be presented at the 17th meeting of the Conference of the Parties of CITES (Johannesburg, September-October 2016). Also, on March 4th, 2016 a meeting of the National Coordination Committee of the Pilot Project was held in Mexico City, where progress to date was reviewed and next steps were determined. In 2016, the local communities will be officially registered to the Mexican Ministry of Environment (SEMARNAT) as Management Units, they will be provided with training on monitoring populations, nests and habitat, and complimentary monitoring data on habitat, populations and nests will be obtained at the pilot sites, among other activities. A formal ranching quota for 2017 will be authorized, based on monitoring data, Non Detriment Findings and the ranching protocol, only to the pilot sites of the project, which will then be revised in the following years as part of an adaptive management scheme and in line with the ranching protocol provisions.

It is hoped that the results of these activities can be replicated in other local communities and farms in order to broaden the effects of the project at the national level and in the long term. It is also hoped that the results will be useful for similar projects in other countries.

Balamkú: Morelet's Crocodile Farm in Mexico

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Abstract

Balamkú, Morelet's Crocodile Farm for its conservation and sustainable use, established in 2013 in the southeast of Mexico. With advice from experts such as Manuel Muñiz and Maria de la Paz López, a high performance intensive breeding system was created, it develops infrastructure with with controlled atmosphere facilities and individual enclosures for finalization. With an ongoing programme of research in nutrition and rapid growth has been a result of growth of average 8 cm per month for hatchlings up to one year of age and average 6 cm per month in juveniles, thus reaching a production cycle of two years for each generation of copies. It is projected to reach for 2021 a production for at least 4,000 skins per year and a population in the farm of more than 10,000 crocodiles, and growing up to 2026 to a production of 15,000 skins per year and a population of at least 50,000 crocodiles. We are working on a ranching program linked to the social sector, with communities living in areas with natural distribution of morelet's crocodile. Also on a project for the establishment of microclimates to ensure the preservation of the habitat of the species and the health of watersheds and micro-basin water, in addition to fighting climate change with the reduction of ambient temperature and water retention in soils, addressing erosion areas.

Abstract: Balamkú, granja para la conservación y aprovechamiento sustentable del cocodrilo moreletii, establecida en 2013 en el sureste de México. Con la asesoría de expertos como Manuel Muñiz y María de la Paz López, se desarrolla un sistema de cría intensiva de alto rendimiento, con infraestructura con recintos con ambiente controlado y encierros individuales para finalización. Con un programa permanente de investigación en nutrición y crecimiento acelerado se ha logrado un resultado de crecimiento de 8 cm promedio por mes para crías hasta de un año de edad y 6 cm promedio por mes en juveniles, llegando así a un ciclo de producción de dos años para cada generación de ejemplares. Se proyecta alcanzar una producción para 2021 de al menos 4 mil pieles al año y una población en granja de más de 10 mil ejemplares, y creciendo para 2026 a una producción de 15 mil pieles anuales y una población de al menos 50 mil ejemplares. Se está trabajando en un programa de rancheo ligado al sector social, con comunidades que habitan zonas con distribución natural del cocodrilo moreletii. También en un proyecto para la creación de microclimas para garantizar la preservación del hábitat de la especie y la salud de las cuencas y microcuencas hídricas, además de combatir el cambio climático con la reducción de temperatura ambiental y retención de agua en suelos, subsanando erosión de áreas.

Population Status of *Caiman latirostris* in the "Managed Nature Reserve El Fisco"

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Abstract

Inside "El Fisco" reserve (S 30° 15', W 60° 55') located in San Cristobal department, northwest of Santa Fe Province, Argentina, nests have been harvested for the sustainable use program "Proyecto Yacaré", since 1992. For many years, the effectiveness of reintroduction of young crocodilians to wild population from ranching programs has been questioned, arguing that individuals bred in captivity and released in the wild could not survive, possibly as a result of having to learn to hunt for food and having to adapt to a new environment. However, not only survival but also the reproduction of individuals who were released by Proyecto Yacaré had been confirmed. This work is a preview of an ongoing research, entitled "Dynamic Population of Released and Wild *Caiman latirostris* in El Fisco". We analyzed data recorded using night counts during 2015, in months before nest eclosion (October to February). From these data, preliminary results are presented, obtained by two methods. On one hand, the population size was analyzed by the method of Messel *et al*. (1981), which showed an estimate between 357 and 406 animals. Furthermore, the method of Maximum Value (King and Messel in Cerrato 1991) was used, and it was obtained a result of 497 animals. Historical records for 1992 recorded an estimate of less than 1 ind / km population density, then increased to 10 ind/km in 1996 and according to our current study the density would be 41 ind/km. After over 20 years of the management program in the reserve, can be observe an increase in the population of *C. latirostris* occurred, demonstrating its sustainability.

Principles of Animal Welfare at Slaughter: A Current Perspective

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Abstract

Since the dawn of time, mankind has hunted animals using traps, clubs and spears, while the more recent emergence of farming saw humans forge a closer relationship with animals. In modern livestock production and processing industries, our aims are still the same; to rear and ultimately slaughter livestock. However, the methods available and our understanding of the underpinning animal welfare science has changed considerably.

The requirement for a humane slaughter method has arisen from the need for a method that is fast and safe. Nowadays, our choice of method is determined by a number of factors, including:

- Available resources, including economic impact and technical support
- External influences, for example, retailer standards and consumer opinion
- Local laws, including religious and cultural requirements
- Product safety and quality
- Animal welfare science and the scientific basis of stunning and slaughter
- Livestock species and use

Our ethical obligation to prevent animals suffering is arguably nowhere more important than when we choose to end an animal's life. Understanding and concern for animal welfare during slaughter has increased steadily over the years, probably in parallel with increasing affluence in societies. The differentiation between individual consumers is large, each having their own unique set of values and perceptions, and their demands, as interpreted by retailers, regulators and other organizations are presented in the media on an almost daily basis. This public pressure has led to the development and implementation of a range of standards, guidelines and assurance schemes across all aspects of livestock production including slaughter processes.

Animal welfare science has identified a number of core criteria deemed necessary to ensure a positive animal welfare outcome during the slaughter process, namely:

- Appropriate and effective restraint
- Accurately and reproducibly applied method
- Production of immediate unconsciousness that lasts until the animal is dead
- Minimising distress related to the emotions of fear, anxiety, rage and anger

The requirement for immediate unconsciousness, to prevent animals from feeling any pain associated with the procedure, has led to the development of pre-slaughter stunning methods. Early 'stunning methods' have been used since Roman times, when a process to incapacitate the animal was often used to facilitate slaughter. In the UK, commercial stunning methods were first introduced in the early 1900s, though largely ignored by the industry. By 1925, after demonstrating the positive improvements to welfare, safety and quality brought the use of a 'humane killer', the equipment was introduced throughout the UK and many European countries. The adoption of humane slaughter principles is now evident in the majority of livestock industries, with a set of global guidelines designed to improve animal welfare during slaughter, introduced by the World Organisation for Animal Health (OIE) in 2005.

This paper presents an overview of the principles of animal welfare at slaughter, the development and mode of action of currently available methods, and provides an up-to-date review of the scientific assessment of existing methodologies.

Implementation of the New Model for the Sustainable Use Extraction *In Situ* in the Intensive Breeding Farm (UMA) Caimanes y Cocodrilos de Chiapas (CAICROCHIS), Chiapas, Mexico

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Abstract

So far the use of Mexican crocodile (*Crocodylus moreletii*) was performed exclusively under a scheme of captive breeding closed intensive cycle model within the Management Units for the Conservation of Wildlife (UMA), this model contributes little to the conservation of wild populations of Crocodylus moreletii and their habitat. In recent years we have been working with CONABIO, RESP, researchers, academics, crocodile producers and especially with the communities living with crocodiles for the implementation of the Pilot Ranching Program. With the legal framework and current classification of the species in Mexico, the major "Retailers and / or House of Fashion" are interested in acquiring the best crocodile skins with very high quality (no osteodermos, small scales and wide skin). To enter this international trade of high-top fashion and well dress, we have to obtain and deliver products and subproducts of very high quality, which should include an industrialized process, safety, packaging and an elite presentation that meets the standards of the prestige brands or retailers of fashion and well dress. To Caicrochis this means and involves a big transformation of the environmental educational system, the social model and the current production system, we're transforming the incubation systems, the growth enclosures of specimens, and the implementation of individual enclosures, also including the transformation of the slaughterhouse system and procedures for exportations. This program involves the local community in the conservation of the species and its habitat through the Ranching Program, backed up by rates of sustainable development and Opinions Non-Detriment Findings (NDF) in compliance with national legislation and CITES. Above, under a system of fair sharing of benefits among actors in the production chain (PIC and MAT). The community will receive training, environmental education and in time, will extract the eggs from the wild, they will incubate and in the future sell the hatchlings to CAICROCHIS, which will grow the crocodiles and in proper time the juveniles and sub-adults will be sale to another company which will get high quality products for exportation to the major "Retailers".

Implementación del Nuevo Modelo para Aprovechamiento Sustentable Extractivo *In Situ* en el Criadero Intensivo (UMA) Caimanes y Cocodrilos de Chiapas (CAICROCHIS), Chiapas, México

Hasta ahora el aprovechamiento del cocodrilo mexicano (*Crocodylus moreletii*) se ha realizado exclusivamente bajo un esquema de cría en cautiverio de ciclo cerrado dentro de Unidades de Manejo para la Conservación de la Vida Silvestre (UMA) de modalidad intensiva, mismas que contribuyen de forma limitada a la conservación de las poblaciones silvestres de C. moreletii y su hábitat. En los últimos años se viene trabajando con la CONABIO, RESP, Investigadores, Académicos, Productores y en especial con las comunidades que viven con los cocodrilos para la implementación del programa piloto de rancheo. Con este marco legal y clasificación actual de la especie en México, comerciantes y principales "Retailers y/o Casas de la Moda" se han interesado en adquirir la mejor piel de cocodrilo de muy alta calidad (sin osteodermos, escama pequeña y ancha). Para ingresar a este comercio internacional elite de la moda y del buen vestir, hay que obtener y entregar productos y subproductos de muy alta calidad, los cuales deben incluir un proceso industrializado, de inocuidad, envasado y con una presentación elite que cumpla con los estándares de prestigio de las marcas o retailers de la moda y del buen vestir. Para Caicrochis esto ha implicado una transformación del modelo educativo, social y productivo actual transformando los sistemas de la incubación, desarrollo y crecimiento, hasta el año de finalización de los ejemplares, así como el sistema de aprovechamiento (rastro) y los procedimientos para la exportación. Lo anterior, bajo un esquema de reparto justo de beneficios entre los actores de la cadena productiva (PIC y MAT). Se está involucrando a una comunidad local en la conservación de la especie y su hábitat a través del Programa de Rancheo (UMA en vida libre), esto respaldado por tasas de aprovechamiento sustentables y Dictámenes de Extracción no Perjudicial (NDF) en cumplimiento de la legislación nacional y CITES. La comunidad recibirá capacitación, educación ambiental y extraerá los huevos, los incubará y venderá las crías a la UMA CAICROCHIS la cual crecerá y desarrollará en encierros individuales los ejemplares y está a su vez se los comercializará a otra empresa la cual aprovechará los ejemplares y obtendrá los productos y subproductos de alta calidad para su exportación con los principales "Retailers".

POSTERS

Philippine Crocodile Head-Start Program in Northeast Luzon

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Abstract

The endemic Philippine crocodile *Crocodylus mindorensis* is critically endangered with a very limited distribution and population size in the wild. In 1999 an *in-situ* conservation project started for one of the last wild populations of this species in the municipality of San Mariano, Isabela Province in northeast Luzon. A community-based conservation strategy by the Mabuwaya Foundation has led to the establishment of eight local crocodile sanctuaries. Sanctuary guards protect these community-managed areas and are also involved in crocodile monitoring. Although increasing, the crocodile population is still very small in San Mariano with less than 100 individuals. Wild crocodilian hatchling mortality is generally very high and to help the Philippine crocodile recover, a head-start program is ongoing since 2005. Nests are searched for by sanctuary guards and protected in the wild. Hatchlings are collected and reared in captivity. Before release back to the wild, juveniles spend at least 6 months in groups in semi-natural soft-release ponds to learn how to swim, hunt and interact. Head-started crocodiles are released back into the wild after two years when they are large enough to withstand most predators and strong river currents. More than 250 hatchlings, from 44 nests, have been collected since 2005. The survival rate in the rearing station is higher than 70% and 134 head-started juveniles have been released back into the wild to date. Of 32 crocodiles closely monitored after release in a study in 2008, at least 50% survived after one year in the wild. Released crocodiles have been recaptured several kilometers from release sites, and in different wetland areas, suggesting substantial post-release dispersal. In 2015, 9 head-started juvenile crocodiles were fitted with radio transmitters to track post-release movements. Smaller crocodiles stayed near their release site while larger crocodiles moved distances of up to 500 m. within 30 days after release. Released crocodiles had a preference for dense vegetation and greater distance to human trails although crocodiles also moved into rice fields nearby release sites. Two tagged crocodiles were killed by people. Head-starting seems to be an effective way to increase survival rates of hatchling Philippine crocodiles and released crocodiles seem to adapt well to wild conditions. The killing of crocodiles and the continued conversion of natural wetlands, however, continue to pose a threat to wild Philippine crocodiles in San Mariano.

Occurrence of Nest of Crocodile *Crocodylus palustris* in Kadavi River at Sarud, Tal-Shahuwadi, Dist-Kolhapur, MS, India

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Abstract

River Kadavi is tributary of River Warana. The nest of crocodile *Crocodylus palustris* had been found in the month of May for two successive years 2014 and 2015 on left bank of River Kadavi at Sarud, Tal, Shahuwadi, Dist. Kolhapur, MS, India. The clutch size was 26 and 22 for year 2014 and 2015 respectively. The average nest depth was 1.5 ft. The average egg size was 8.5 cm. This site was chosen for nesting because of soil type, suitable climate and adequate food. Hatchlings were about 11 inches (from snout to tail). There are four records or evidence of human and livestock-crocodile conflict known from Sarud and nearby region. Conservative and management steps should be taken to protect crocodiles from River Warana.

Introduction

Mugger crocodiles are found basically in freshwater, including marshes, ponds, lakes, irrigation canals, and reservoirs, as well as saltwater lagoons. As the name "*Crocodylus palustris*", "crocodile of the marshes", suggests, they are found mainly in marshes. During the dry season Mugger crocodiles dig burrows in the mud to protect themselves from the heat, and they may travel long distances over land to find water if their pool of water dries up. The crocodile will excavate a hole nest during the dry season (December to February). Most females choose sloping banks, but some opt for other locations. Once the female finds a suitable site to dig her nesting hole, it is usually utilized for most or all of her breeding years. Approximately one month after mating occurs; the eggs are deposited by the female into the nesting hole she has formed. This takes place in February-April and consists of an average 28 (10-48 range) eggs per clutch. The Mugger has been known to lay two clutches in one year while being kept in captivity, but little is known about the individuals in the wild. The incubation period is relatively short, 55-75 days. When the eggs finally hatch, they are transported by the mother and sometimes even the father to nearby water. Young crocodiles remain in loosely organized groups with the adults for up to one year before dispersing. Present paper deals with the study of nest of *Crocodylus palustris* observed at the left bank of River Kadavi at Sarud, Tal, Shahuwadi, Dist. Kolhapur, MS, India. This is the first attempt taken towards the exploration and conservation of crocodiles in Warana tributaries.

Review of Literature

The Mugger is a hole-nesting species, with egg-laying taking place during the annual dry season. Females become sexually mature at approximately 1.8-2 m, and lay 25-30 eggs (Whitaker and Whitaker 1989). Nests are located in a wide variety of habitats, and females have even been known to nest at the opening of, or inside, their burrow. In captivity, some Muggers are known to lay two clutches in a single year (Whitaker and Whitaker 1984), but this has not been observed in the wild. Incubation is relatively short, typically lasting 55-75 days (Whitaker 1987). Whitaker and Whitaker (1989) provide a good review of the behaviour and ecology of this species. Like a number of other crocodilians, *C. palustris* is known to dig burrows. Whitaker and Whitaker (1984) referred to Mugger burrows in Sri Lanka and India (Gujarat and South India) and noted that yearling, sub-adult and adult Mugger all dig burrows. In Iran they are sometimes known to dig two burrows close to each other, which may be used by one or more crocodiles (Mobaraki 2002). These burrows are presumably utilized as an effective refuge from hot daytime ambient temperatures. These burrows play a critical role in the survival of crocodiles living in harsh environments (Whitaker *et al*. 2007), allowing them to avoid exposure to excessively low and high temperatures (<5ºC and >38ºC respectively) for long periods of time, which may be lethal (Lang 1987). The sex of the offspring is determined by the temperature in the nest, with a temperature of 32.5°C (90.5°F) leading to an all-male batch. Below and above this more females will be produced, and a temperature of 28-31°C (82-88°F) leads to females only. The emerging juveniles are roughly 30 cm long and their mother will aid them by opening up the nest and carrying them to the water.

Methods

When the authors came to know about the frequent visits of crocodile at the site in the month of April 2014 they visited the study area and noted the occurance of a nest of crocodile. Further frequent visits were made and observations at the nesting sites were noted. The local evidences like slide, footprints and eggshells were noted and photographed. The persons inhabiting near the nesting site were interviewed and their observations and opinions were noted. The photograph of hatchlings with empty eggshells had been taken from the site. The morphometric measurements of nest, clutch, egg and hatchlings were noted. In present investigation, nesting observations were carried out during the year 2014 and 2015.

Study Area

River Kadavi is a major tributary of river Warana of Deccan Plateau of India. It begins its course in the Sahyadri ranges (17° 1' 40" N, 73° 50' 10" E) at the border of Kolhapur and Sangli district, on the west side of village Kokrud (16° 59' 51" N, 73° 58' 53" E), Tal-Shirala, Dist.-Sangli. A small dam is constructed at Parle Ninai (17° 0' 15" N, 73° 52' 14" E) on this river. In the beginning River Kadavi runs north to South through Sahyadri ranges towards Malkapur. Further it takes eastward turn near Malkapur (16° 55' 12" N, 73° 55' 55" E) of Kolhapur district and merges in river Warana near village Thergaon (16° 54' 24" N, 74° 4' 47" E). Total length of river Kadavi is about 46 km. The village Sarud (160 54' 38" N, 74° 2' 41" E) is on the left bank of river Kadavi. Present nest of crocodile observed at Sarud is at 16° 54' 9" N, 74° 2' 7" E (Figs. 1 and 2). It is on the left bank of River Kadavi. The red coloured soil of clay type with mixture of sand, clay and gravel is observed at the location.

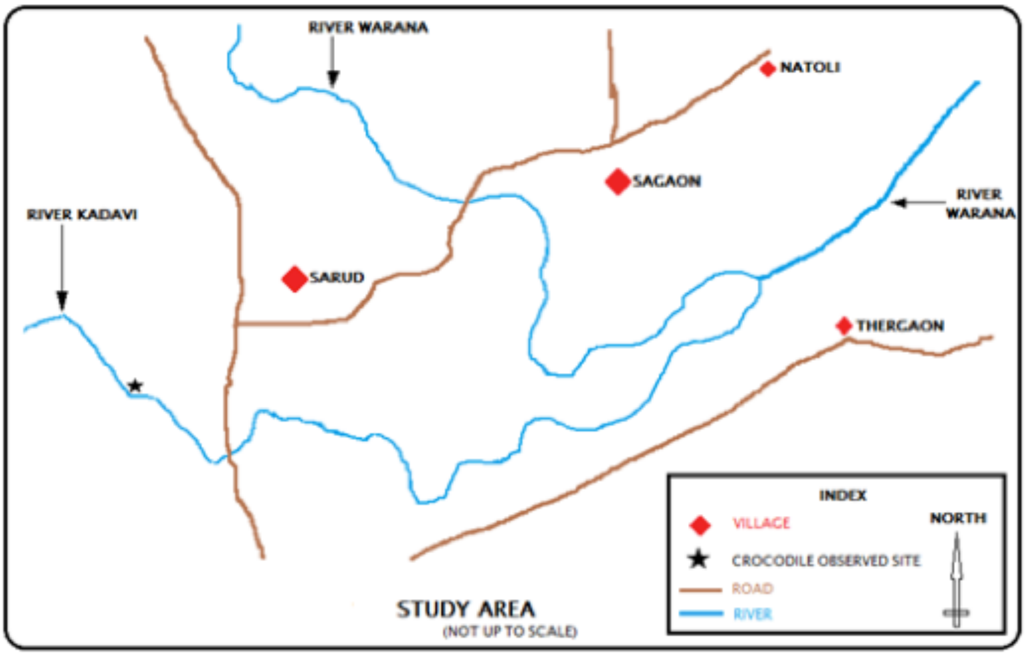

Figure 1. Map of study area.

Figure 2. Imagery of nest site from Google Earth.

Results

Currently in the year 2014 and 2015, nesting of crocodile is reported from river Kadavi-tributary of river Warana at Sarud (Fig. 3). The village Sarud is located on left bank of river Kadavi in Shahuwadi Tahasil of Kolhapur district. *Crocodylus palustris* was also observed in basking state by many farmers at the nest site in the month of December, January. According to the local peoples the size of crocodile observed at Sarud was about 7 to 7.5 feet (2.1-2.3 m) in length. The nest was on the left bank of river and about 10 feet (3 m) away from the water line. It was 1.5 feet (0.4 m) deep. The nest of 2015 was only about three feet (1 m) away from nest of 2014.

Figure 3. (left) Hatchlings at the nest; (right) Empty eggshells and tail mark of crocodile observed at nest next day after sparse rain.

A clutch of 26 and 22 eggs was observed in May 2014 and in May 2015 respectively. Average egg size was about 8.5 cm in length. The incubation temperature recorded was in the range of 31^{o}C to 35^{o}C. In 2014 hatching was observed on 29 May and in 2015 hatching was observed on 28 May. The hatchlings observed were about 28 cm in length from snout to tail. Number of empty eggshells observed in 2014 was 26 (ie no loss of eggs has been recorded in 2014 but in 2015 only 12 eggshells were observed at the nest). Thus, about 45% loss of eggs was recorded. This loss is due to human interference. Mainly the eggs are collected and eaten as a food by some tribal people. In the first week of June 2015, when subsequent survey was conducted, about 5 to 8 hatchlings were seen around the nesting site in a small eroded, muddy hole near waterline (Fig. 4). The presence of crocodiles with two young ones was also witnessed at nesting site by Mr. Arun Balaso Patil in June 2015. Human-crocodile conflict was not recorded from the study area during the study period.

Figure 4. Hatchling observed in a muddy hole near waterline.

Discussion

Present finding of crocodile nest for successive two years indicate that crocodile nesting site is relatively undisturbed even though farmers visit the river banks regularly for their routine work. The crocodile might have selected this sight for nesting because of the type of soil. The river Kadavi and its both banks have clay type of soil with mixture of sand, clay and gravel, which is useful in making hole in it. Also the temperature of this area in summer ranges between 31 to 40°C which is suitable for incubation of crocodile eggs. Present location of nesting is a good source of food in the form of fingerlings to the young ones of crocodiles. Thus adequate food and suitable climatic conditions might be also the reason behind selection of this sight by crocodiles. The egg laying and incubation was recorded in the summer months. This is in contrast to the observations made by Joanen (1969) in *A. mississippiensis*. It indicates that this species is still recovering. The incubation period of about 68 to 90 days is similarly recorded by Webb (1983) for *C. johnstoni*.

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Size of Caimans Killed by Humans at a Hydroelectric Dam in the Madeira River, Brazilian Amazon

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Abstract

Hydroelectric dams change river shorelines and often force caimans into closer contact with people. In this study, I recorded crocodilians killed by humans in the newly flooded Santo Antônio dam, Madeira River, Amazon. From September 2010 to May 2013, I recorded floating dead caimans on the banks of the Madeira River and its tributaries. The reservoir area of UHE Santo Antônio (8° 47'S, 63° 57'W), Madeira River, extends 100 km, and was formed in November 2011. Each dead caiman was inspected to determine cause of death based on physical evidence, such as bullet wounds or the presence of a hook. The length of the head (HL cm) of dead individuals was measured using a measuring tape, and this measure used to estimate snout-vent length (SVL cm). Over the period of 2 years, I encountered 42 dead *Melanosuchus niger*, with estimated SVL (cm) between 58.6 and 247.0 cm, 6 *Caiman yacare* between 68.4 and 89.6 cm SVL, 3 *Paleosuchus palpebrosus* between 74.9 and 82.4 cm SVL, and 3 *P. trigonatus* between 36.0 and 78.1 cm SVL. The caimans had been killed by gunshots (36), hooks (9), water turbulence near the dam walls (4) and unidentified causes (5). In this latter study, 52% of the killed *M. niger* individuals had an estimated total length of more than 4.0 m (ie SVL>2.0 m). In conclusion, with regard to the region of the Madeira River, the conflict between humans and crocodilians is focused on large individuals of *M. niger*.

Human-Crocodile Conflict in Madagascar

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Abstract

Human-crocodile conflict (HCC) involving *Crocodylus niloticus* is a significant management issue in Madagascar. We assessed HCC for the periods 1892-1938 and 1987-2015, to better understand the distribution and cause of attacks, and to assist in the development of measures to mitigate HCC. The results of a recent assessment of HCC by Maheritafika *et al. (*2016) can be summarized as:

<u>Number of Attacks</u>: Data on attacks were compiled from various sources (eg government records, media reports, CrocBite), and incorporated in the Direction Générale des Forêts (DGF) database. In all, 388 records of attacks were identified: 121 from 1892-1938 (the colonial period); and, 267 from 1987-2015. The number of attacks between 1892 and 1938 is considered to be under-reported, as is the lack of records between 1939 and 1986.

<u>Fatality Rate</u>: The fatality rate for 1892-1938 was 80.2%, higher than 56.2% for 1987-2015.

<u>Sex and Age of Victims</u>: Most victims (71.7%) were males. In 61 cases where the age of victims was known, a high proportion (36.1%) of victims were children (1-15 y), and the average age of victims was 26.4 y (range 2 to 70 y).

<u>Month of Attack</u>: Most attacks occurred in warmer months (October-May), which coincides with the rainy season (most rainfall is in December-March). There was an average of 14.5 attacks/mth in Oct-May relative to 6.3/mth in Jul-Sep.

<u>Trends</u>: Within 1987-2015 there were two distinct periods of high attack frequency: 1990-1995 (average= 24.8 attacks/year); and, 2008-2015 (average= 12.0 attacks/year). Reasons for the decline in attack reports between 1990-1995 and 2008-2015 are not known. The 1990-1995 period coincides with Madagascar's attempts at CITES to maintain *C. niloticus* on Appendix II pursuant to ranching, and extensive fieldwork allowed information on attacks to be collected by direct interview. After the successful Appendix-II listing was achieved in 1997, efforts to collate information were largely restricted to media and Provincial authorities, although some data were collected during egg collections. Reporting of attacks is also affected by local beliefs about crocodiles.

<u>Activity at Time of Attack</u>: There are few data available on the activity of victims at the time of the attack, but fishing and crossing rivers appear to be the most common activities of victims.

<u>Size of Crocodile</u>: Information on size is only available for 7 crocodiles involved in attacks, of which 5 were estimated to be 3-5 m long, and 2 were relatively small (0.7 and 1.5 m TL).

<u>Distribution of Attacks</u>: The post-1986 distribution of attacks across the country is considered to represent the current areas with high levels of HCC - the north, west and southeast of the country. The western distribution has the highest densities of *C. niloticus,* and population size (human and crocodiles) may be implicated in the distribution of attacks.

<u>Conclusions</u>: A more detailed analysis of HCC in Madagascar is constrained by the lack of details for many attacks, and the fact that many attacks almost certainly go unreported. The absence of records over a 48-year period (1939-1986) is evidence of a lack of reporting, rather than a lack of attacks. Although information from that period would be of historical interest, it would not necesarily be important for guiding management activities now. Despite the lack of detail for many records, the available data nonetheless do provide insights that can improve crocodile management. The activities that put people at risk of attack are associated with water, such as bathing, washing, fishing, etc. Rural livelihoods depend on waterways to large degree, and Crocodile Exclusion Enclosures (CEEs), such as those used in India and Sri Lanka, could assist in the mitigation of HCC in Madagascar. The distribution of attacks identifies areas where public awareness and education programs can focus in the first instance.

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Leptospiras in *Caiman latirostris* in Argentina

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Abstract

Leptospira is a genus of spirochetes to which belong a number of pathogenic species that may produce leptospirosis, a disease transmitted by wild and domestic animals, and important for human health. Know the variables related to the eventual acquisition of infection in animals have great importance for the design of sanitary policies. Our objective was to assess the prevalence of antibodies anti-leptospira and the presence of *Leptospiras* spp. in *Caiman latirostris* in captivity and in the wild, in Santa Fe Province. We extracted blood samples of 32 individuals (7 wild, 25 captive). Prior to the extraction we cleaned the neck of caimans, in order to prevent samples contamination; then we put each sample in two vacutainers: 1- with heparin and we used for agar media, 2- with serum separator and we used to perform MAT (microscopic agglutination test) and PCR (polymerase chain reaction) tests. For MAT test, we excluded 9 of 32 samples because we detected presence of lipemic serum. Of the MAT analyses, 15 of 19 samples in captivity and 3 of 4 samples in the wild were positive, and they showed the presence of antibody anti-leptospira. The 78.3% of samples (18 of 23) showed the presence of antibody anti-leptospira from the serogroup Pyrogenes. Besides, we detected in 31.8% of samples (7 of 23) the presence of Pyrogenes and Icterohaemorrhagiae, and only 1 sample of 23 hosted three serogroups (Pyrogenes, Icterohaemorrhagiae and Grippothyphosa). Moreover one sample from captivity analyzed with PCR was positive but unfortunately the culture was contaminated. On the other hand, 40% (13 of 32) of the blood samples cultivated in agar media were contaminated and could not be studied; but the other 60% (19 of 32 samples), none presented growth of the bacteria. In the present work it was possible to determinate the presence of *Leptospira* spp. in caiman without evidence of clinical symptoms. A high prevalence of antibodies anti-leptospira, in captivity and in the wild, was detected. In this way, we understand those animals were in contact with the bacteria at some point in their lives. In addition one animal presented an active infection without any symptoms. Studies of leptospira in crocodilians are important in order to understand the role exerted by the bacteria, and if caimans are natural reservoir or accidental host.

Nesting Behaviour and Nest Excavation of the West African Slender-snouted Crocodile (*Mecistops cataphractus*) at the St. Augustine Alligator Farm Zoological Park

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Abstract

At the St Augustine Alligator Farm in St Augustine, Florida, USA, on 9 September 2015 it was observed and documented that the female *Mecistops cataphractus* excavated her nest and gathered one hatchling at a time, and carried them in her mouth to the shallow end of the enclosure's pool. In 2010, the West African Slender-snouted crocodiles were bred for the first time at the St Augustine Alligator Farm resulting in 23 eggs. Two of the offspring hatched from the nest inside the enclosure in August 2010, and it was observed that eggshells were floating in the main pool. It was speculated that the adult crocodiles carried the hatchlings to the pool from the nest. In May 2014, the juveniles were removed from the enclosure, and the adult crocodiles mated and built a nest in 2015 with 25 eggs. This time, we wanted to observe and document whether or not these parents would respond to the calls of the hatchlings.

The Neurophysiologic Basis of Acupuncture and the Therapeutic Benefits for Crocodilians and other Reptiles

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Abstract

Acupuncture is a safe, non-invasive treatment used primarily as an analgesic. However, it has been shown to be efficacious treating a wide range of veterinary conditions ranging from musculoskeletal, neurological, and gastrointestinal issues, to reproductive and respiratory disorders. Although practiced in the East as a component of Chinese medicine for over 2000 years, only in the past half-century has this ancient therapeutic approach been incorporated into Western medicine. This employment of acupuncture is correlated with advances in understanding of neurotransmitters, neuroanatomy, and the physiology of pain inhibition. By developing a more scientific approach to this therapeutic modality, acupuncture is now an accepted component of integrated veterinary medicinal care. The mechanisms of acupuncture includes activation at various levels of the central and peripheral nervous systems stimulating the release of endogenous opioids (enkephalins, β-endorphins and dynorphins), and other pain-inhibiting neurotransmitters such as serotonin and norepinephrine. Acupuncture also works through the "Gate Theory of Pain" where needle insertion stimulates sensory nerve fibers that inhibit pain transmission in the dorsal horn of the spinal cord. Lastly, acupuncture can have profound effects on the autonomic nervous system. Some acupuncture points are considered sympathetic sites, which have been shown to stimulate respiration and increase blood pressure for animals recovering from anesthesia or suffering from shock. Conversely, other points are deemed "calming sites" due to their parasympathetic response. These are known to have a strong influence over the parasympathetic areas of the brain, and the heart by causing vasodilation, and normalization of bodily systems. Magnetic resonance imaging (MRI) studies further support acupuncture by showing how needle insertion at known acupuncture points, activates specific corresponding regions of the brain with distinct response patterns.

In reptiles, acupuncture has been used for calming patients under restraint, analgesia, resuscitation, muscle spasms, and wound healing. Additionally, acupuncture can impact nerves associated with some internal ailments like egg stasis and ileus. We will explore the neurophysiological model of acupuncture and the potential therapeutic benefits concerning analgesia and stress reduction in a Galapagos tortoise (*Geochelone nigra vicina*), Fiji iguana (*Brachylophus bulabula*), Komodo dragon (*Varanus komodoensis*), and three crocodilian species (*Alligator sinensis*, *Caiman yacare*, and *Crocodylus johnstoni*).

Whether receiving treatments for disease, under restraint for medical examination, or facilitating postoperative recovery, managing stress and pain is vital in crocodilian husbandry, and we believe incorporating acupuncture in a multimodal treatment plan can assist in alleviating these conditions.

Magnetic Resonance Imaging (MRI) to Investigate Crocodylian Phallic Morphology

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Abstract

Phalli often combine tissues with different material properties to impart stiffness to the structure, to allow localized tissue expansion, and to assist in gamete transport. Traditionally, understanding these complex tissues morphologies has relied on histological sectioning followed by tissue staining. While these methods yield fine cellular-level details and valuable information about the tissues' material composition, they also destroy many of their higher-level three-dimensional relationships and make it more difficult to model their mechanical behaviour during copulation and sperm transfer. In contrast, magnetic resonance imaging (MRI) provides a nondestructive 3D imaging modality that makes it possible to study the spatial relationships of soft tissues. When combined with paired histological information, MRI can contribute to 3D models of functional architecture and provide greater insights into tissue structure/function relationships. Here, we compare histological sections of male adult American alligator (*Alligator mississippiensis*) phalli with images obtained through MRI.

Adult male alligator phalli were collected at Lake Woodruff, Florida in 2010 and preserved in Bouin's fluid. Tissues were either paraffin-sectioned and stained or MRI scanned intact after equilibrating in PBS. Seven-micron tissue sections of the medial phallic shaft and distal glans were stained using periodic acid methenamine silver method with gold toning for reticular (argyrophillic) fibers - putatively collagen Type I and III- or with Weigert's resorcin fuchsin for elastin fibers (blue) and counterstained with nuclear fast red. Additionally, an intact phallus was scanned in a Bruker Biospec MRI scanner using a 72 mm diameter Bruker volume RF coil and a 3D FLASH sequence (T/TR/Flip/NA = 6.5ms/25ms/14°/2) at 200 μm isotropic resolution and a total scan time ~2 hr. Images of the phallic shaft and glans were selected from positions in the resulting transverse image stack that approximated the positions of the histological sections.

The MRI scans captured phallic anatomy with sufficient resolution to identify functional regions within the intact tissue (Fig. 1): dense collagen fiber bundles in the phallic shaft and glans that contribute structural strength and rigidity (labeled A), spongiform inflatable regions of the glans which fill with blood and engorges the glans into a copulatory conformation (labeled B), and smooth muscle fiber bundles flanking the sperm-conducting ventral sulcus groove along the shaft and glans regions (labeled C). MRI imaging additionally revealed: 1) how elastin fiber bundle-rich tissue regions (elastin staining, blue noted by an asterisk) presents differing MRI contrasts (MRI asterisk) than those of the adjacent collagen bundles and 2) collagen fiber bundle orientations (compare A vs. B in collagen and in MRI images).

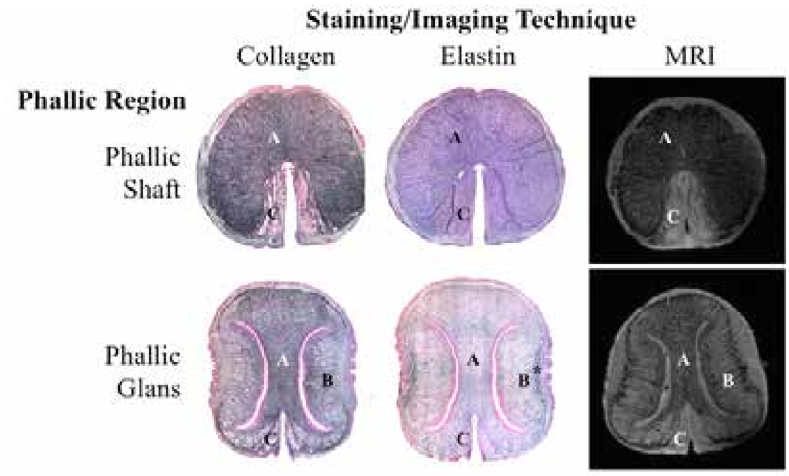

Figure 1. Paraffin histology tissue staining for collagen and elastin fibers and magnetic resonance imaging (MRI) of American alligator phallic cross-sections. Highlighted regions: A) dense collagen fiber bundles, B) spongiform tissues associated with expanding vascularized regions, and C) smooth muscle fiber bundles adjacent to the ventral sperm-conducting sulcus. The asterisk denotes elastin fiber rich regions that are blue with elastin staining and of a differing contrast than collagen in MRI staining.

Therefore, these results facilitate further crocodylian biology investigations of tissue-specific material properties associated with phallic function. This type of noninvasive imaging can streamline the investigation of tissue-specific material properties associated with phallic function by capturing internal morphology prior to materials testing. We are proceeding with 3D reconstructions of MRI image stacks to better understand crocodylian phallic biomechanics. This imaging technology is applicable across crocodylians and can identify both conserved morphologies across species and species-specific phallic novelties. We hope to use this technique to understand phallic variation among crocodylians. Understanding copulatory dynamics will better inform breeding operations producing commercial animals and also conservation efforts.

Relationship between Blood Corticosterone Concentration and Immune Function in Juvenile Broad-Snouted Caimans (*Caiman latirostris*) under Stress

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Abstract

Caiman latirostris (Broad-snouted caiman) is one of the two species of Crocodilians living in Argentina, with ecological and economic importance. In the wild, they have to face numerous stressors including climatic factors, toxicant exposure, infection, etc. Stressors can affect the components of the immune system that might be involved in an organism's survival as they actively participate in resistance and rapid response to infection. The goals of this study were to experimentally investigate the effect of three potential environmental stressors on the corticosterone levels (CORT) and immune parameters: total and differential count of leukocytes (WBC), natural antibodies (NAbs), complement system activity (CS) and growth (snout-vent length and weight). We exposed 96 Broad-snouted caiman hatchlings, randomly distributed to four groups exposed to different stressors during one month. They consisted of limited water availability, food restriction, periods of overheating, and a control. Results showed that CORT levels were lower in heat treatment than control. The titers of Nabs in food restriction and heat treatment were higher than in controls; and changes in the percentage of leukocytes were detected in all groups. There was not difference in CS activity, WBC and clutch effects. Corticosterone was not associated with immune parameters but there were negatively associated with growth (snout-vent length and weight). These results suggest that these immunological indices were not associated with the type of stressors tested; and in the terms of this study, the immune parameters evaluated in *C. latirostris* hatchlings seem to be independent of the hypothalamic-pituitary-adrenal cortical axis activation.

Land-use Intensification, Amplified by Flooding Events, Increases Threat to the Mutale River Crocodiles

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Abstract

Reaching a balance between conservation and development, especially in regard to large predators, will continue to be at the forefront of conservation and management efforts in the developing world. We present a portion of that overlap from a rural community in the Mutale Municipality (formerly a part of Venda), in the north of South Africa, bordering Kruger National Park. The Mutale River has a small, but stable Nile crocodile population despite the source and majority of the river being outside of a protected area. Over a two-year period, including a full-scale river review in 2013, we were able to ascertain potential human influence sites along the Mutale River. We also documented the altered landscape along the riverbanks over the past 40 years. These changes are more intense following flooding events. Indirect effects derive from the agricultural society, where water is pumped from the river into farms and/or small villages, or cattle and cleared land cause bank instability. A 40% change to farmland occurred in a 14-year period. Dams of both small and large scale were found, which can disrupt flow and health restoration following major flooding events. Direct effects include net fishing and revenge killing for loss of cattle, but these instances are limited. Despite increased land use change and population, direct human-animal conflict is still minimal and the crocodile population is growing (0.18 crocodiles/km in 1981 to 0.34/km in 2013). However, this increase is not seen across all size classes and adults over 2 m have reduced from 10 to 4 sighted individuals. The combined effects of climate change, land use change, and flow alteration present juxtaposition in the conservation of the crocodile population and development along the Mutale River.

Expression of Melanocortin Receptor 2 Involved in the Stress Response Pathway in Different Tissues of Broad-snouted Caiman (*Caiman latirostris*)

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Abstract

The interaction between melanocortin and reproduction is associated with various processes related to metabolism, stress, immunity, and neuroendocrine signaling pathways. Melanocortin receptors (MCRs) are involved in a diverse number of physiological functions, including pigmentation, steroidogenesis, energy homeostasis, exocrine secretion, sexual function, analgesia and inflammation. There are five MCR subtypes (termed MC1R to MC5R) that each has a different melanocortin peptide and different patterns of tissue expression. While other receptors are promiscuous for different peptides, the melanocortin MC2 receptor (MC2R) is a specific receptor that mediates different tissue responses to ACTH. Their identification, sequencing and expression were previously analyzed in mammal (human, bovine, rabbits and rodents), avian (*Gallus gallus*), reptilian (*Anolis carolinensis*), and amphibian (*Xenopus tropicalis*) tissues; however, there are no reports regarding their detection in crocodilians. In order to identify the mRNA coding *C. latirostris* MC2R, we designed a set of primers from sequence alignments of phylogenetically related species obtained from NCBI. We detected MC2R mRNA expression in *C. latirostris* adrenal, gonad, liver, and spleen tissues by reverse transcription PCR (RT-PCR) and real time PCR. This study is the initial step to performing an evaluation of the reproductive impact of the ACTH pathway in steroid secretion and function in *C. latirostris* gonads. Since the neuroendocrine system is an essential component of reproduction, any response of this system to stress might influence reproductive success.

Eggshell of *Caiman latirostris* as Indicators of Environmental Disturbance by Human Activities

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Abstract

Natural environments are affected by the growth of human populations and their activities (ie expansion of agriculture). Today, this change persists and affects many species, including reptiles. In this study, we used Landsat-8 satellite images to assess anthropomorphized changes in the landscape during 2013 of 5 Proyecto Yacaré survey sites, and determined if these changes affect eggs characteristics and hatching success of *Caiman latirostris* nests. We collected 5 nests per site and then the eggs were incubated artificially. After hatching, we evaluated eggshell hardness, pore density, and measured the levels of heavy metals. Less anthropic sites (Fisco and Caminos Natural Reserves) produced bigger eggs. Although, we did not find relationship between the degree of human impact and hatching success of *C. latirostris* nests. The levels of heavy metals found in the eggshells from all sites was low (Al, Cu, Cr, Mn, Mo, Se and Zn), and there were very few differences between sites. However, on the most anthropomorphized site (Lucero) we observed higher levels of Cr and Al. Future studies should expand the studied areas and continue to evaluate the relationship between the human-induced changes in habitats, the status of *C. latirostris* females, and the levels of contaminants in the eggshell, albumin and yolk, and the effects on the embryos.

Gharial Field Techniques: GPS Radios and Game Cameras

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Abstract

Innovative advances in GPS telemetry and game camera surveillance have been utilized in the Gharial Ecology Project, underway since 2008 in the lower Chambal River, north India. These field techniques have been employed recently to provide detailed information about movements and behaviours of Gharial, but are applicable to other study populations, as well as other crocodilian species.

GPS logging radios, incorporating a VHF tracking signal and UHF remote download capability, are providing fine scale daily movement and activity records for individually tagged, resident wild Gharial. To date, units have been operating for 16+ months, have provided hundreds to thousands of daily locations per animal, and provide a way to verify the accuracy and limitations of conventional manual tracking. Details of radio attachment procedures as well as tracking methodologies are illustrated, and the routines for programming location/fix schedules and remote downloading at periodic intervals are explained.

For detailed observations of hatching and post-hatching behaviours of adults and young at communal nest sites, game cameras have been customized with long-lasting battery packs to allow continuous video/still image coverage for 24-36 hours per deployment. In addition, conventional game cameras have been modified to provide viewing ports to permit accurate framing and viewing of the field of view for image capture. Details of the necessary modifications to off-the-shelf models are provided, and step-by-step instructions are included in this poster presentation, accompanied by video examples.

Cost-effective Options for Crocodile Monitoring in Developing Countries - a Pilot Study from Timor-Leste

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Abstract

Comprehensive monitoring is the basis for every crocodile management regime. However, monitoring often requires advanced and costly techniques, as crocodile habitat and attack hotspots are often situated in remote areas. Here, many developing and post-conflict countries lack the financial and technical capabilities for successful implementation. Our study area Timor-Leste is one of the least developed countries in the world gaining independence in 2002 after centuries of armed conflict. Crocodiles are worshiped as ancestors particularly in remote communities based on the country's creation myth "Lafaek Diak - The Good Crocodile". Crocodile attacks continue to be serious problem (130 incidents since 1996), with most being fatal. The number of non-fatal attacks is believed to be higher than reported, due to lacking monitoring capabilities and social implications for the victims. Crocodile attacks on humans are often seen in the light of traditional beliefs and culture (eg as a punishment for acts against nature).

We argue that the implementation of an effective monitoring system and public awareness campaign is urgent in Timor-Leste to prevent attacks and enable human-crocodile coexistence. A successful monitoring regime should: 1. be cost-effective; 2. integrate local authorities and traditional knowledge holder; and, 3) maintain a long-term data infrastructure. Here, we test a participative system collating crocodile observations nation-wide using the website "common-environment.org" and the application Crocodile Observation Mapper (COM), and through map-based Community-based Monitoring Systems (CBMS) on the local level. The website will be launched in May 2016, but a CBMS was successfully tested during a pilot study in the villages of Vessuro, Irabin de Baixo (both Viqueque District), Mehara and Malahara (both Lautem District) in 2014 and 2015. Here, a total of 20 crocodile attacks could be located including the exact position of the incident. The compiled information can help to draft a management plan addressing the human-crocodile interactions in Timor-Leste.

A Preliminary Analysis of Recent Crocodile Attacks in Borneo

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Abstract

Borneo is the largest island within the Greater Sunda Islands archipelago, which it shares with the of Sumatra, Java, and Sulawesi. Borneo itself is split amongst three nations - Indonesia (the provinces of West, Central, South, East and North Kalimantan), Malaysia (the states of Sabah and Sarawak), and the sovereign state of Brunei. Three species of crocodilian are known to inhabit Borneo - the Saltwater crocodile (*Crocodylus porosus*), the Siamese crocodile (*C. siamensis*) and the Tomistoma (*Tomistoma schlegelii*). Borneo is fairly infamous for its man-eating Saltwater crocodiles (the legendary man-eater known as "Bujang Senang" is claimed to have killed 13 people in Sarawak before he was eventually caught and killed in 1992), but prior to data collection by CrocBITE (the worldwide crocodilian attack database) detailed information on crocodile attacks in Borneo (with the exception of Sarawak) was mostly limited to the local news media. For the period of 2007-2016 we recorded 221 crocodile attacks in Borneo, resulting in 129 deaths, with Sarawak (Malaysia) and East Kalimantan (Indonesia) having the highest numbers of reported attacks. While the Saltwater crocodile was responsible for the vast majority of attacks, a small number of attacks and deaths were attributed to the Tomistoma, and at least one non-fatal attack was attributed to the Siamese crocodile.

Characterization of *Caiman latirostris* Meat Enriched with Flax Seeds and Chía Seeds

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Abstract

Caiman meat is a non-traditional dietary protein source, but based on its qualities could be presented as a healthy food product. In this work, we evaluated the physicochemical characteristics of meat from captive-bred individuals fed a regular diet, and individuals whose diet was enriched with flax and chia seeds. We found that with the regular diet, caiman meat is reduced in fat, high in protein content, is tender and exhibits aesthetic appeal, with a high representation of oleic, linoleic and palmitic fatty acids. The addition of seeds to regular caiman diets altered the color (b* coordinate) making meat more yellowish. The addition of flax seed altered the lipid profile, increasing the proportion of oleic acid, and with the addition of chia seed we observed increased polyunsaturated fatty acids, which are beneficial to human health.

Evaluation of Natural Antibodies in *Caiman latirostris* Fed Diets Enriched with Fatty Acids of the Omega-3 Family

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Abstract

Previous studies evaluated how the addition of fatty acids (omega-3 family) to the diet contributes to health improvements of not only humans but of farmed animals. Enrichment the diet of captive caimans with flaxseeds could enhance the essential fatty acid content of its meat, thus increasing meat value, promoting growth, and improving their overall health status. In the present study, we fed caimans with five diets: 1. Diet Control (crushed chicken heads and dry ration, 70/30%), 2. Chia diet1 (control diet + 10% crushed chia seeds offered once a week), 3. Flax diet1 (control diet + 10% crushed flax seeds offered once a week), 4. Chia diet3 (control diet + 10% crushed chia seeds offered three times a week) and 5. Flax diet3 (control diet + 10% crushed flax seeds offered three times a week) during 30, 60, and 90-day treatments. During the study all caimans grew, but we did not find significant differences in weight or length among diet treatments or treatment period. Further, we found no significant differences in the immune system (antibodies) of these caimans, but we observed great variability in the immunological status of individuals. To obtain more definitive results, in future studies, we plan to evaluate the active response of the caiman immune system faced with pathogens under different diet treatments.

Development of the Central Nervous System in *Caiman latirostris*

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Abstract

Our study is aimed at describing the histological organization of anterior portion of the neural tubule (brain) during embryonic development. *Caiman latirostris* embryos were incubated at 33°C (male producing temperature) and collected for histological observation at the following stages: 15, 18, 20, 22, 24, 26 and 28 (hatching time). Brains were serially sectioned longitudinally and stained with hematoxylin and eosin. In early stages (15-22), the neural tubule consisted of a germinal neuroepithelium, which is a pseudostratified epithelium with a basic three-zone pattern involving proliferative (on ventricular lumen), mantle (on intermediate), and marginal (on outer border) zones. However, at stage 20 these three-zones were modified in the midbrain, and the mesencephalic tectum was stratified into four layers which lay on the periphery of mantle (mid plexiform, mid nuclear, outer plexiform and outer nuclear). In the dorsal alar plate of the primordium cerebellum, some neural precursors migrated to the marginal zones, forming a thin outer cortex. The most drastic changes in the laminar organization of grey matter occurred between stages 22 to 24. By stage 24, the proliferative zone had turned into ependymal layer lining the ventricular surface. The mesencephalic tectum had expanded and two new layers had developed (inner plexiform and inner nuclear) resulting in a six-layered tectum. The cerebellum cortex had differentiated into a peripheral molecular layer encircling a central granule cell layer. Three vague layers had formed the telencephalic cortex where a main nuclear layer was sandwiched between the inner and outer plexiform layers. Two giant neurons had been found, the Purkinje and the mesencephalic gigantocellular ones. From stage 24 to hatching no substantial changes occurred in the laminar structures, only more gigantocellullar neurons appeared in the tectum. The most significant development of brain vesicles takes place in the midbrain, suggesting that the tectum is far more than a simple center receiving input from retina. Finally, brain development of *C. latirostris* is more similar to reptiles than to birds, though the latter is more phylogenetically related to caimans.

Hematologic and Plasma Biochemical Reference Interval of Captive False Gharial (*Tomistoma schlegelii*) in Non-breeding Season in Taiwan

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Abstract

Tomistoma schlegelii is classified as 'Vulnerable' in the IUCN Red List and also under CITES Appendix They are threatened by continuing loss and fragmentation of habitat and their conservation work has got more and more attention internationally. However, there is no substantial research on reference interval of hematology and biochemistry in Tomistoma schlegelii to assist their conservation work or evaluate individual health, the aim of this study is to establish reference interval of hematology and plasma biochemistry in Tomistoma schlegelii under captivity in Taiwan. The blood samples were collected from ventral tail vein of 29 adult female, 10 adult male and 16 yearlings. Complete blood count and leukocyte differential count were obtained using manual method. Plasma biochemical analysis were evaluated. The reference interval was determined with 90% confidence interval, and there were significant differences between male and female, adult and yearling. The result of this study could be applied to other false Gharial for health evaluation in non-breeding season.

Evaluating the Activity Patterns of *Caiman latirostris* and its Use of the Enclosure in Cordoba Zoo (Argentina): a Preliminary Study

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Abstract

Assessing animal welfare in zoos is a basic requirement to promote management measures that improve life conditions. Welfare may be initially assessed by describing behaviour of captive animals and their interaction with the environment. To date, no studies have described the repertoire of behaviours of *Caiman latirostris* and their use of the environment in zoos. We conducted a preliminary study of a *C. latirostris* population (4 adults and 3 juveniles) during the breeding season, which consisted of developing an ethogram based on behavioural observations and supporting information taken from the literature of wild crocodilians. The frequency of each behaviour was obtained through observations and according to the size of individual (discriminating adults from juveniles). Using a chi-square test contingency table for non-independent samples (McNemar test), we evaluated the association between individual size and behaviour frequency. Behaviours were recorded between 0900 and 1800 h during 26 days (December 2015-March 2016). Simultaneously, the location of each individual was recorded using a scan sampling to analyze use of space by the group as a whole and according to size via a modified Spread of Participation Index (SPI), often used in studies of zoo animals. The enclosure, which consists of a 130 m^2 artificial lake and a central 90 m^2 island, was divided into 10 zones, depending on the presence/absence of food, proximity to the visitors, and/or following the structural limits of the environment. Thirty different behaviours performed in 5533 events (juveniles 2064; adults 3469) were recorded. The most common behaviours were: (a) semi-submerged (20.8%), (b) walking (17.8%), (c) in the shade (12.1%), (d) swimming without immersion (13.3%), (e) basking (10.2%) and (f) swimming with immersion (5.8%), whereas the remaining behaviours, such as aggression, courtship and copulation, had frequencies below 5%. Adults showed a higher frequency of behaviours (a), (d) and (f), related to the lake sector, whereas juvenile showed a higher frequency of behaviours (b), (c) and (e), related to the island sector. Adults' preference for water may be related to thermoregulatory behaviour; therefore, this behaviour could change at different times of the day. Consequently, it would be interesting to assess possible differences in patterns of behaviour associated with different sizes and at different times of day. SPI for the whole population revealed limited use of space (SPI= 0.373), with adults (SPI= 0.340) showing greater use than juveniles (SPI= 0.524). Although preliminary, these results are consistent with a hierarchical behaviour associated with animal size, which could be exacerbated during the reproductive period in mixed groups, such as those housed in Cordoba Zoo. These results are the baseline for the development of a framework project aiming at identifying and assessing key behaviours; this information will be useful for adapting the enclosure of these animals and therefore improve their welfare.

Population Structure and Human Pressure of Two Caiman Species in Southern Brazilian Amazon

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Abstract

Studies in protected areas provide interesting data for population status of crocodilians. However, crocodilians populations in these areas also undergo negative effects of human presence in its abundance, structure, and animal behaviour. In this sense, studies relating anthropic pressure and their effects on population of crocodilians are need for a better management practice and conservation measures. Therefore, we determined and compared the population parameters of encounter rates, size structure, and sex ratio for *Melanosuchus niger* and *Caiman crocodilus* populations among three study sites (Coco River, Rico Lake, and Dentro Lake) in the Cantão State Park, Southern Brazilian Amazon, Tocantins State. Additionally, we tested two hypotheses of relationships between humans and crocodilians abundance/size structure: (1) caiman will be less abundant closer to human community; and (2) human disturbance may have a positive effect on abundance in smaller caiman and a negative effect in larger individuals. Spotlight surveys were carried out during dry season (August-November) between 2013 and 2014 to collect data about: abundance (by encounter rate: individuals/km), species, sex ratio, injury frequencies, and animal size. The human disturbance analysis was conducted just in the Coco River where allowed a comparison between transects (about 8 km each) of different degrees (high, medium and low) of human disturbance (transit, presence of ports, boats, and houses on the banks). We counted 4787 caiman (2506 *C. crocodilus*, 201 *M. niger* and 2080 unidentified) and we captured 60 *C. crocodilus* and 24 *M. niger* for sex ratio and injury studies. We found that *M. niger* and *C. crocodilus* populations was significantly most abundant in the Dentro Lake. *Caiman crocodilus* was predominant in all sites, where its pattern of encounter rates increased in sequence: Rico Lake (2.5 ind/km), Coco River (4.82 ind/km), Dentro Lake (8.13 ind/km); whereas the pattern was the reversed for *M. niger*, decreasing in sequence: Rico Lake (1.59 ind/km), Coco River (0.33 ind/km), Dentro Lake (0.24 ind/km). Both species was basically composed by juveniles (SVL<70 cm for *C. crocodilus* - 91.5% of population; SVL<100 cm for *M. niger*: 63.7% of population) and distributed in different sites: Dentro Lake for *C. crocodilus* and Rico Lake for *M. niger*. The sex ratio was male-biased for both species in all study sites (*C. crocodilus* 2M:1F; *M. niger* 7M:1F). Males and juveniles presented more injuries, mainly on the region of the tail. Additionally, the data suggest that human presence may negatively influence *C. crocodilus* and *M. niger* abundances, and it was decreasing as human disturbance level increases. Regarding the caiman size/human disturbance relationship, we found smaller *C. crocodilus* individuals in high disturbance level areas, and larger individuals in low disturbance level areas; for M. niger, we found larger individuals in areas with high human disturbance level. Proximity to communities and accessibility to the Cantão State Park is possibly the causes of human disturbance. Our results provide important implications highlighting how wild crocodilians population respond to human disturbance.

Citotoxicity and Genotoxicity on *Caiman latirostris* (Broad-snouted caiman) Exposed to the Insecticide Chlorpyrifos and a Complex Pesticide Mixture Widely used in Extensive Agriculture

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Abstract

The aim of this study was to evaluate the cytotoxicity and genotoxicity induced by the insecticide Chlorpyrifos-(CPF) based formulation (Lorsban 48E® -48%) and a complex pesticide mixture consisiting of the insecticide Cypermethrin (Atanor® -CYP, 25%) + CPF + the herbicide Glyphosate (Roundup Full II® -GLY, 66.2%) on *C. latirostris* hatchlings. Sixty-four *C. latirostris* hatchlings, 20 days old, from four different clutches, were randomly distributed into four experimental groups (16 specimens per group with two replicates each): 1) a vehicle control (VC: 200 μl/L) treated with ethanol; 2-3) two groups exposed to different concentrations of CPF (CPF1: from 0.5 to 0.05 μg/L and CPF2: from 1 to 0.1 μg/L); and 4) a group treated with the complex mixture (M): CYP1 (from 0.5 to 0.05 μg/L) + CPF1 (from 0.5 to 0.05 μg/L) + RU1 (from 2.5 to 0.25 mg/L). Animals were maintained in plastics containers with the corresponding solutions (exposure by immersion) during two months, with a progressive decrease in all pesticide concentrations, in order to simulate their degradation in water through time, in natural conditions. After exposure, blood samples were taken to all animals for the determination of Neutral Red uptake (NRU) and MTT metabolism as cytotoxicity assays; while the Frequency of Micronuclei (FMN), Nuclear Abnormalities (NA) and DNA damage index (DI) through the Comet Assay (CA) were applied on erythrocytes as biomarkers of genotoxicity. In relation to cytotoxicity, NRU was significantly lower for group M respect to NC ($p= 0.043$), but no differences were observed in MTT metabolism. Genotoxicity results indicated a significant increase in the FMN in caimans exposed to both concentrations of CPF ($p<0.05$) and to the M ($p<0.01$), respect to the VC. Besides, CPF2 and M also induced a significantly higher DI respect to the VC ($p<0.05$). For the NAs we observed a significant higher frequency of Buds in the M and eccentric nuclei in CPF1 and CPF2 ($p<0.05$), compared to the VC. Together with our previous results on other different pesticides, these findings will help to estimate the ecological risk of *C. latirostris* populations under the natural conditions where they live. However, future studies on DNA damage are needed, considering more details for a better interpretation of the interactions among different pesticides in the complex mixtures widely used in soybean crops in Argentina and many other countries.

Nesting Behaviour of the Broad-snouted Caiman (*Caiman latirostris*)

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Abstract

The preferred habitat for the Broad-snouted caiman are heavily vegetated marshlands very difficult to access by humans, which explain the paucity of information on nesting behaviour. This information is necessary in the context of management programs and sustainable use that link local people through egg harvest programs, not only for the workers involved on the harvesting, but also for the researchers in the field. The observations of this work were carried out in a semi natural enclosure where animals breed every year with very little interaction with humans. It is located at Granja La Esmeralda Experimental Breeding Station in Santa Fe, Argentina. Two video cameras were installed at the nesting area which recorded activities of two nest 24 hours a day starting on December 2014 until March 2015, making a total of 93 days, where a total of 4464 videos of 30 minutes each were analyzed. Information on the weather conditions on a daily basis was gathered from the weather research center of Universidad Nacional del Litoral. An ethogram was made and the frequency of each activity was analyzed. We established the relationship between presence of *Caiman latirostris* at the nesting area and environmental variables. The observed behaviours were nest construction and maintenance (excavation, material incorporation, and compaction), vigilance (around the nest site, or at the nest) and aggressive social behaviours (confrontation, persecution, attacks). Vigilance activities are the most common, followed by the maintenance of the nest. The presence of female in the nesting area is related to environmental temperature and rainfall and varied over the breeding season. The activity in the nest decreases at high temperatures and heavy rains. In both nests multiple clutches occurred, which is one of the intraspecific interactions also registered in the wild.

Distribution and Diversity of Complement Genes in Crocodilians

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Abstract

The complement system plays an important role in the innate immune response in higher vertebrates. It is involved in cell lysis and initiation of phagocytosis by opsonisation of pathogens and induction engages the cells of the immune system to trigger processes leading to inflammation. The complement system consists of about thirty proteins grouped in five major gene families. These encode for distinct plasma proteins that react with each other forming three activation cascades (alternative, lectin and classical) which converge in a single terminal pathway. This system appears to be highly conserved in vertebrates. However there is a gap in the knowledge in regards to evolution and diversity of the complement system in crocodilians. To address this, we investigated a large number of complement system genes in the recent available genomes of three crocodilian species (*Alligator mississippiensis*, *Crocodylus porosus* and *Gavialis gangeticus*) and identified 28 genes. These genes were then compared to other vertebrates including reptiles, birds and mammals to further understand the extent of conservation and differentiation of the complement system among the three extant crocodilian families. We surveyed 25 exons, representing 7 genes, across 20 species of Alligatoridae and Crocodylidae. Phylogenetic analyses showed that crocodilian complement genes form orthologous clades across species suggesting that they have evolved independently from each other after speciation. As expected, a considerable level of sequence conservation among species was observed. However, we also found a relatively high frequency of DNA substitution in particular among species of Alligatoridae. No correlation between genetic diversity or allele distribution and species' habitat or geographic distribution was identified. These findings advance our knowledge of the evolution of this innate immune system and the immunogenetic resources generated here are now being used for population genetic and disease association studies.

Perivitelline Membrane-bound Sperm Detection for the Conservation and Management of Cuban Crocodiles (*Crocodylus rhombifer*)

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Abstract

Conservation measures for the critically endangered Cuban crocodile (*Crocodylus rhombifer*) include captive breeding programs, reintroduction in the wild, and habitat protection. A main objective of captive breeding is maximizing the reproductive output of the managed species. Advanced reproductive technologies (ARTs) can be used to enhance captive breeding programs and maintain genetic diversity. Perivitelline membrane-bound (PVM-bound) sperm detection has previously been demonstrated in avian and chelonian species as a useful technique for breeding management. In the absence of embryotic development, this technique confirms the presence of sperm trapped on the oocyte membrane and can be used to confirm breeding, male reproductive status, and pair compatibility. PVM-bound sperm were successfully detected in three clutches of Cuban crocodile eggs at the Smithsonian's National Zoological Park (NZP) for the first time in any crocodilian species. Oocyte membranes removed from Cuban crocodile eggs were stained with nucleic acid dye Hoechst 33342, and evaluated under fluorescence microscopy. Total sperm densities were assessed at 200x magnification. The results of this study showed significant differences in sperm densities between clutches. Additionally, there was not a significant difference between eggs that banded and those that did not band within each clutch. These results demonstrate the necessity to further examine eggs that do not develop, as fertility rates can be more accurately assessed. PVM-bound sperm detection could be a useful technique in assessing crocodilian fertility, as well as have potential uses in studies assessing sperm storage, artificial insemination and artificial incubation.

Introduction

The captive propagation of crocodilians is both beneficial to the conservation of these animals as well as the economy. Advanced reproductive technologies can often aid in improving captive breeding programs and ensure that genetic diversity remains high. Technological advances, such as perivitelline membrane (PVM) sperm detection (Bakst and Howarth 1977; Wishart 1987, 1997; Bramwell and Howarth 1992) can help animal managers investigate infertility as well as offspring mortality (Croyle *et al*. 2015, 2016). Utilized in birds (Bakst and Howarth 1977; Wishart 1987, 1997; Bramwell and Howarth 1992) and recently demonstrated in chelonians (Croyle *et al*. 2016), PVM-bound sperm detection may also prove useful in crocodilian propagation and management as the eggs of crocodilians and chelonians are similar to those of birds (Iverson and Ewert 1991).

In captivity, crocodilian egg fertility rates are generally assessed via banding, the process in which the vitelline membrane attaches to the inner shell membrane, creating an opaque spot atop the eggs shell (Webb *et al*. 1987). As the embryo develops this spot expands to the width of the egg, forming an opaque band within the first 7 days (Ferguson 1985). It is often assumed that eggs that do not band are infertile, even though eggs may have died from early embryotic mortality (EEM) before the vitelline membrane attached. Distinguishing between infertility and EEM is important when assessing low fecundity in captive breeding programs. Eggs that are truly infertile could be the result of pair incompatibility or an individual infertility, whereas eggs that die from EEM may be the result of animal husbandry, such as diet or incubation method.

Methods

In this study, PVM-bound sperm detection was used on three clutches of Cuban Crocodile, *Crocodylus rhombifer*, eggs at the Smithsonian's National Zoological Park. Clutch A was laid by a 36-year-old female housed with a single male; Clutch B was laid by a 36-year-old female who shares the enclosure with the 58-year-old female who laid Clutch C and a single male (Augustine and Watkins 2015). Eggs from Clutches A and B that banded were rotated two days after oviposition and several days thereafter to halt development as this would cause accelerated deterioration of membrane-bound sperm (Jensen 1969; Birkhead *et al*. 2008) and prohibit the use of these methods. Banded eggs from Clutch C were incubated, as this female is genetically valuable and producing hatchlings was a priority.

Eggs were opened and assessed for PVM-bound sperm periodically throughout incubation. For each egg, the PVM was removed, as previously described by Birkhead *et al*. (2008). In deteriorated eggs where the PVMs had lost integrity, as many pieces as possible were collected with a pair of forceps. The membrane was then stained with Hoechst 33342 (Life Technologies 3175 Staley Rd. Grand Island, NY 14072 USA) and microscopically evaluated with UV florescence at 200x magnifications.

A total of 79 eggs were laid in 2015. Clutch A contained 28 eggs, 71.4% of which banded, Clutch B contained 31 eggs, 75% of which banded and Clutch C contained 24 eggs, 41.7% of which were banded (only eggs that did not band were assessed). Of these, 10 were discarded from the data set: three oblong eggs that contained significantly more sperm than the other eggs and would have skewed the results, and 7 where less than 25% of the membrane was recovered. Of the 69 eggs assessed, 42 were banded and 27 were not.

Results and Discussion

This study found that PVM-bound sperm can be successfully detected in freshly-laid *C. rhombifer* eggs and those that have been incubating as long as 120 days; however, incubation time reduced the percent of membrane recovered, therefore reducing sperm detectability. Preliminary analysis found that banded eggs did not contain more sperm than eggs that did not band within each clutch; however, the eggs of the female (Clutch A) housed with a single male had more sperm than the eggs from two females (Clutch B and Clutch C) housed together with a single male (Augustine and Watkins 2015).

Determining if an egg is infertile or died from EEM may help determine whether genetic defects, incubation behaviour or environmental factors are the cause of death (Christensen 2001). Within each clutch of *C. rhombifer* eggs at NZP there was not a significant difference in sperm densities between banded eggs and those that did not band, suggesting that all of the eggs had enough sperm present for fertilization. Eggs that did not band may be the result of EEM, suggesting that husbandry related issues such as social grouping, incubation method or nutrition may be responsible for the lack of development. While some studies suggest housing a trio together may provide an ideal reproductive set-up for polygamous breeders such as *C. rhombifer*, restricted areas in captivity may also increase stress for resource competition (Seabacher and Griggs 1997). In American alligators, *Alligator mississippiensis*, high stocking densities were found to increase stress hormones and delay ovoposition resulting in eggs containing considerably advanced embryos leading to higher instances of early embryotic death (Ferguson 1985).

As human population growth and habitat degradation continue, ex-situ captive propagation will become a more vital component of species conservation, especially for *C. rhombifer* (Targarona *et al*. 2015). Advanced reproductive technologies such as PVM-bound sperm detection can help animal managers investigate the presence of sperm in the perivitelline membrane. As demonstrated in this study, eggs that do not band are not necessarily infertile. Although minimum sperm density for fertilization is not known, the eggs in this study that did not band had comparable levels of sperm as the eggs that did band.

Acknowledgements

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Effect of Nest Inundation on the Hatching Success of *Caiman latirostris* Eggs

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Abstract

Inundation is one of the main causes of poor hatching success in crocodilians, however there is little information about the tolerance of embryos to submergence and the role that eggshell structure has on embryo survival. The objective of this research was to determine the effect of inundation on hatching success of *Caiman latirostris* eggs flooded at two stages of embryonic development and whether eggshell surface roughness influences the tolerance of embryos to inundation. We used clutches from 10 nests, separated into two groups of five clutches; eggs of each clutch were divided into four treatments (nine eggs per treatment) as follows: 1. Non-inundated without scraping eggshell; 2. Non-inundated with scraping eggshell; 3. Inundated without scraping eggshell; 4. Inundated with scraping eggshell. A group of five clutches was inundated when each clutch reached day 30 of development (at 32°C± 0.5°C), whereas another group of five clutches was inundated when they reached day 60 of development. All inundated groups were immersed for a period of 10 hours. At day 30 of embryo development (at 32°C ± 0.5°C), inundation only reduced hatching success in scraped eggs. On the other hand, at day 60 of embryo development, inundation decreased hatching success in both groups (with or without scraping eggshell surfaces). We conclude that eggshell roughness mitigate the damage caused by early inundation. On the other hand, late inundation tend to be fatal independently of eggshell roughness.

Effect of Lipid Profile of *Caiman latirostris* Wild Female on Egg Fatty Acids Composition in Two Breeding Seasons

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Abstract

In oviparous reptiles, lipid content in tissues of females represents energy stored in the previous months to be invested in reproduction, which would be determined by the availability of food resources in environment. Energy that is destined to yolk, in particular as fatty acid (FA), and represents most of the investment maternal to the successful development of embryo. Yolk FA composition would be influenced by two main factors: maternal diet (particularly essential FA (C18:2, C18:3, C20:4) that animals cannot produce) and genetic (which regulates metabolic factors). The aim of this study was to assess effect of maternal tissue FA on egg FA composition of *Caiman latirostris* in two consecutive breeding seasons (2012-13 and 2013-14). We captured 8 wild females of *C. latirostris* (4 each breeding season) in Santa Fe Province. We extracted a sample of caudal muscle tissue (size 5-10 g) and collected 5 fertile eggs of each clutch (with development <10 days), all samples was stored at -20°C. Methyl esters of total lipids of the yolk and meat were analyzed by GC-MS. Using simple linear regressions, correlation between maternal meat FA and yolk FA composition of eggs were assessed to infer the energy that the female invests in her progeny. We evaluated by variance analysis differences between breeding seasons in FA content of maternal tissue and egg yolks. In female tissue and egg yolks the most abundant FA were C18:1, C16:0 and C18:2. In addition, C18:0 is abundant in yolk. We observed that high levels of FA C18:0 and C18:1 in meat, are positively correlated with high values of these same compounds in the egg. It is important to note that oleic acid (C18:1) is an important FA for embryonic development. On the other hand, in the first breeding season we recorded a lower percentage of polyunsaturated FA (PUFA) in the maternal tissue, and a higher percentage of essential FA C20:4 in yolk, which may indicate changes in the availability of food resources consumed by females, which would affect the FA composition stored and derivatives to egg. Our results, although preliminary, indicate that yolk essential FA such as C20:4 are affected by the seasons, which could reflect performance reproductive females in a given season, as this is an important component for embryonic development and hatching success. Finally, in order to understand the effect of the lipid profile of female on FA composition deposited in their eggs in one breeding season, we should take into account not only the diet, which is conditioned by the availability of resources, but also FA interconversions and female selective tissue incorporation. This should be evaluated in future studies.

The Decline of a Giant: Should We Be Worried About Madagascar's Crocodiles?

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Abstract

The Nile crocodile (*Crocodylus niloticus*) is the sole surviving member of Madagascar's once diverse megafauna. It is assumed to play the same vital role in the health of waterways on the island, as both an apex predator and a keystone species as it does on the African continent. Recent reports and a four year international ban (2010-2014) on exports suggest that Malagasy populations of wild crocodiles are in serious decline with illegal hunting and habitat loss identified as primary drivers (Ottley *et al*. 2008; DGF 2015). Here I present data from 5 years of seasonal monitoring in the northwest of the island, the results of a preliminary investigation in the relationship of crocodiles from Madagascar to their African mainland counterparts and the potential utility of captive crocodiles to act as genetic banks to aid conservation efforts. I discuss the results of these analyses in terms of phylogenetic placement of Madagascan *Crocodylus*, the potential for ranched and captive-bred stock to provide a viable option for invigorating or restocking wild populations and the apparent continuing decline of wild populations, post-international ban.

Novel Findings in Crocodilian Immunology

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Abstract

Research on crocodilian immunology have demonstrated interesting findings and promising perspectives. In recent years, the study of a significant number of genes coding for immune molecules have been identified in ectothermic vertebrates, and most of them tending to be cloned. Cytokines are a family regulatory molecules with a paracrine or autocrine hormone-like activity required to develop a immune response. They join to receptors in the surface of the cell with high affinity and trigger a set of protein synthesis, playing an important role in immune cell differentiation and activation that depends on the specific target cell type, and on the phase of the immune response. Interleukins are a large group of citokynes. Composition and bioactivity of these immunomodulatory proteins has been studied in many species but not in crocodilians. So, a detailed analysis on the identification and characterization of cytokines is very useful to understand the mechanism involved in the defense of these species. Also, they can be used as bioindicators to evaluate the individual and environmental health.

In order to detect cytokines, mRNA samples from spleen tissues of *Caiman latirostris* were obtained. We tried sets of primers designed from alignments of phylogenetically related species sequences, and others reported for avian and fish species. The expression of proinflamatories and antinflamatories cytokines (interleukin [IL] 1β and IL 4, respectively) were detected by reverse transcription PCR (RT-PCR) and real time PCR. These findings open a new window in the knowledge of the crocodilian immunology with the aims to identify more proteins of this group, and how they exert their activities in the organism and ecological context.

Phallus Development in the *Caiman latirostris*: Preliminary Results

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Abstract

In this study we provide a morphological description of *Caiman latirostris* phallus during their early ontogenesis. Caiman eggs were incubated at 33°C (male producing temperature), and samples were obtained at stages 24 and 26 (after the thermo-sensitive period) of embryonic development, at hatching time (or stage 28), and 24 months after hatching (juvenile). During the periods mentioned above, the phallus had an oval shape with a ventral and dorsal surface and two lateral edges; and a phallic sulcus running along its dorsal surface. In the gross anatomy two segments were recognized: the shaft and the glans (distal expansion like cuff). At stage 24, the sulcus was a stratified epithelium, flanked by irregular shaped cells and a profuse fundamental substance. Between stages 26 and 28, a ring of vascular elements distinguished the anlage of the corpora cavernosa from the corpus spongiosum; this latter immediately bordering the phallic sulcus. The corpora exhibited a network of collagen fibers loosely organized. Smooth muscle cells were differentiated adjacent to the phallic sulcus on the dorsal surface of the corpus spongiosum. Nerves were developing accompanying the anlage corpora organization. By juvenile period, the sulcus in the deep lumen was stratified ciliated epithelium with mucin cells different from earlier stages. The corpora cavernosa had turned into corpora fibrosa with smooth muscle bundles mixed among rich tissue collagen fibers. The muscle bundles in the corpora cavernosa had formed two orientations, radially (deeply) and longitudinally (superficial)-orientatated, while in the corpus spongiosum the muscle was longitudinally-orientated aligned with the sulcus. The glans contained a lacunar connective tissue, with large and irregular vascular spaces. Our analysis of caiman phallus development after the thermo-sensitive period, suggests that the glans development and the epithelium sulcus specializations are post-hatching process.

Community-Based Crocodile Conservation in the Sitatunga Valley Natural Reserve of Benin

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Abstract

The Sitatunga Valley Natural Reserve is one of the last sanctuaries of the Dwarf crocodile (*Osteoleamus tetraspis*) and the West African crocodile (*Crocodylus suchus*) in southern Benin. The Regional Centre for Research and Education for Integrated Development (CREDI-ONG), and NGO, which is technically and financially supported by the La ferme aux Crocodiles in Pierrelatte and SOS Crocodile Association in France, is engaged in the protection of the Natural Reserve in order to assist the local community with the conservation of their crocodiles. One of the challenges CREDI-ONG faces is to ultimately give responsibility to the Sitatunga Valley local community for crocodile conservation. Towards this goal, more than 400 hunters grouped in an association are involved in crocodile conservation. Annually, 60 youths from the club "Know and Protect Nature" and 2000 visitors are trained on crocodile ecology, habitat use, their importance in wetland ecosystems, and different methods for crocodile and nature conservation. In addition, exchange visits with the club members are organized in different regions of Benin to learn about the other African crocodile species and to sensitize the youth about crocodile conservation. We provide microcredits to hunters to support agricultural activities and livestock production, which, by providing alternative activities to poaching, has seen a reduced hunting pressure on wild crocodiles. Our efforts to date have helped the local Sitatunga Valley community become more aware of the importance of crocodiles and has motivated them as stewards for crocodile protection. While our efforts to date are already seeing changes, more awareness campaigns are needed to strengthen stakeholder involvement in crocodile conservation.

Introduction

The Republic of Benin in West Africa has three crocodile species (*Crocodylus suchus*, *Osteolaemus tetraspis* and *Mecistops cataphractus*) (Kpéra and Sinsin 2010; Kpera *et al*. 2011). The Sitatunga Valley Natural Reserve is one of the last sanctuaries of the Dwarf crocodile (*O. tetraspis*) and West African crocodile (*C. suchus*) in southern Benin. The Regional Centre for Research and Education for Integrated Development (CREDI-ONG), which is technically and financially supported by the La Ferme aux Crocodiles in Pierrelatte and SOS Crocodile Association in France, is engaged in the protection of the Natural Reserve in order to assist the local community with the conservation of their crocodiles. One of the challenges CREDI-ONG faces is to ultimately give responsibility to the Sitatunga Valley local community for crocodile conservation.

Methods

Area of intervention - the Sitatunga Valley Natural Reserve of Benin

CREDI-ONG began its crocodilian monitoring activities in the Sitatunga Valley (Fig. 1) in 2010. Thus, it consists in following the dynamics of crocodile populations in the right time to allow CREDI-ONG to evaluate the effects and impacts of its conservation actions on the territory. Such an activity that cannot be done outside of the community, we associate several actors (hunters) basing on the Brotherhood (hunters central process) of the Sitatunga Valley created in 2008 and currently has about 400 members, local elected officials, youth clubs Know and Protect Nature (CPN) youngs and visitors.

Indeed, it is to educate hunters of the brotherhood by making the extension of the current hunting code and identify hunters specialized in hunting crocodiles to integrate them after their training to the monitoring device. In addition to hunters, we have CPN's youngs association acting as ambassadors of the valley to the population in general and parents in particular. Park (on part of the Ramsar site in 1018) is a CREDI-ONG initiative started in 2007. It covers about 150 km^2 and aims to contribute to the conservation of characteristic marshy wetland ecosystems of the South Benin. Sitatunga Valley is mainly supplied with water by the Sô River valley and its tributaries. The Sô River is embedded in the nature reserve and is located between Kinto, Agué and Ahomey-Lokpo villages. It is located 65 km from the spring, and 25 km from the mouth (Guillou 2013). This valley has a high biological diversity and characteristic of South Benin. Animal diversity consists among other things of 51 mammal species (eg African civet, Genette, water guib and bushbuck, Walter's duiker, etc.), 182 species of birds (eg Astrilde of Niger, Little Egret, faced Whistling Duck, *Netapus auritus*, etc.) and 50 species of reptiles (eg *Lycophidion lateral*, *Dendroapsis viridis*, *Atractaspide dahomeyensis*, *O. tetraspis*, etc.).

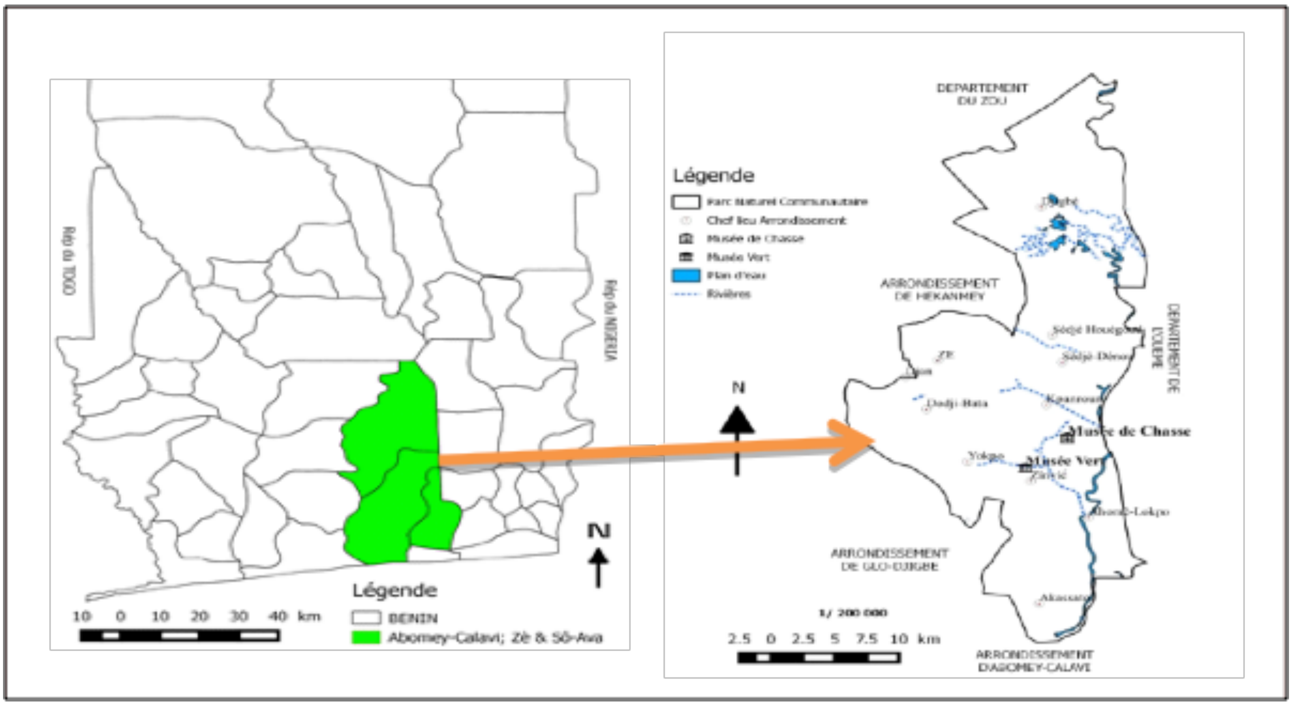

Figure 1. Location of the Sitatunga Valley Natural Reserve in Benin.

Club 1. Know and Protect Nature (CPN)

The "Know and Protected Nature Club" in the Sitatunga Valley Natural Reserve is an affiliation of the same club based in France. The club members consist of pupils and students living in villages in the vicinity of the reserve. Membership to the club is free. In 2016, the club consists of a hundred of members and two sessions: "Know" (theoretical) are indoor sessions whereas "Protect" (practical) are outdoor sessions. The program is essentially based on environmental concepts with a section on knowledge and sustainable crocodile conservation.

During the various meetings organized by CREDI-ONG with management bodies, accents are put on the conservation and protection of wildlife. In addition to the crocodilians monitoring, CREDI-ONG educates and draws local authorities attention on the issue of management and sustainable conservation of natural resources with particular emphasis on crocodilians.

Figure 2. Practical session on crocodiles with CPN club's youngs of the Sitatunga Valley.

Figure 3. Left: Projection session on the world of crocodilians for CPN club's youngs of the Sitatunga Valley by Samuel Martin. Right: Working session on the use of transponders on crocodiles in the valley.

Tourism and the different participations

The Sitatunga Valley Natural Reserve houses a Museum which is one of the most visited places. The museum contains a few individual crocodiles displayed for visitors. Our stand are opportunities for us to educate and inform the wider population. Through the activities that are carried out within the framework of the conservation of populations of crocodiles, the community helps us in gathering information about the crocodiles. The information give by different people who attend to our meetings (visitors and/or any other animation), allows hunters who capture to send us the crocodiles captured rather than selling them to other people.

Results and analysis

As far as crocodiles are concerned affects a lot of people. First, the people living in and around the Sitatunga valley, then those made up of learners, employees of all categories and expatriate tourists of the territory. The brotherhood of hunters today has over 400 members who are all aware of crocodiles and the various activities that we conduct on this animal population. They are also aware of the law that protects and regulates the management of wildlife and its habitats in Benin; good protection ensuring. Because in our national regulations, it is strictly prohibited to hunt crocodiles. Village chiefs, more than thirty, are the representatives of the State to the population, and in addition to information provided to hunters, we focalize on the space management because the bad land management can lead to the loss of ecosystems in which are the populations of crocodilians. More than 200 young pupils and students belonging to CPN clubs were educated, and had attended to the activities related to crocodiles.

Figure 4. Left: Volunteers (Joel and Vincent) Planète Urgence participant crocodile dropped in Sitatunga Valley. Centre and right: Student tours at Green Museum of Sitatunga Valley.

Since 2010 CREDI-ONG has hosted more than 5000 visitors to the Nature Park. These visitors, over 85% Beninese, are trained to recognize the different species of crocodiles and informed about their ecology and threats that hang over the two species of crocodiles in South Benin. Moreover, thanks to those people, information (reported presence and individuals caught) reach us from places other than our intervention field. More than a hundred visitors were associated to monitoring activities. In order to be efficient in collecting datum, an association of hunters has been made up. These hunters stand for our ambassador to the people for wide information about the activities carried out on animals. It would be difficult to comment on this aspect because the time is too short for an assessment. Nevertheless, we noticed a variation more or less growing of places of individuals capture. This, indeed, is a good sign because it shows that our information move far away. We can also evaluate the whole captures we receive to nearly 90% on the territory. The other indicator is related to the capture method we taught some specialized hunters in catching crocodiles which significantly reduces bad state in which animals were kept. A kind of animal micro-credit (allocation of 5 animal heads including one male and 4 female) was granted to a hunter as income generating activity. CPN club's youngs except the activities they carry out in the Sitatunga Valley benefitted from a special trip to the Pendjari which was dedicated to the African Slender-snouted crocodile (*M. cataphractus*) research. We could not say if the population of crocodiles has increased or not as any catch effort is not made. and in addiction the wetland ecosystem is difficult to reach.

Figure 5. Left: Visit youth CPN Valley in the Biosphere Reserve of Pendjari (2013); Centre: Exposition at the US Embassy in Benin (2013); Right: Don 'crocodile of West Africa by AGUIAH Family (2015).

The effectiveness of the methods used to raise awareness is lesser and could reach a significant level if we can increase the sensibilization and popularization of national regulations by publishing small magazines that describes the animal and its ecosystem and relays information emanating from young. This could be a means of communication linking the various young from different continents. With these means, we can reach a large number especially the large target. But, as hunting in the area is a secondary but destructive activity, it is necessary to guide and support the target (the hunters). So, the proliferation of livestock micro-credit for many beneficiary certainly diminish the threats to the population of crocodilians. It is also important to notify that not only they hunt but they also cultivate land (they do this as second activities). The animal micro-credit is indeed to provide a hunter with a herd of 5 individuals of animals including four females and one male an animal of their choice. They are supported with food and veterinarian over a year. This method could be more effective if we increase the number of beneficiary over a considerable period. Today, conservation and protection issue is unequivocal all people concern especially the young's because they have to ensure the future, and we should instille them the means and methods to keep in harmony with the nature. This is the reason why it is indisputable that the target to aim for is that of juvenile level. Nature sessions with young people from our CPN clubs are made on environmental issues with the specificities of a better knowledge of crocodile species and its ecosystem in order to share with them good conservation ways. So we get tourists know the species (especially students) what a crocodile is and our research on them. The different animals are submitted to tourism (Bathiono 2007).

The question of sharing experiences and improving that way of conservation take through visits to show the community the importance of the activities of conservation. His is the reason why it is very important to organize visits exchange for those involved in ecosystems and local authorities from other sites to show them another example of what is done by us, and especially to encourage creation of alternative activities to hunters so as to limit hunting frequency, and to buy at a fixed price specimens that are brought to us. So it is important to establish a permanent dialogue among all stakeholders to understand the merits of all the activities carried out in the direction of conservation. Obviously, no one can be above the law and it should be the real power. One of the things that hinder the harmonious participation in community management, protection and conservation is to deliver individuals who engage in this activity to men guarantor of regulations on wildlife and its ecosystem. Moreover, strict protection has done his proof by showing its limits which opened the way for close cooperation with the community. In the Sitatunga Valley, we have not identified as such dangers about crocodiles regarding the population. Indeed, the Dwarf crocodile is less aggressive and as its ecosystem is used by a minority. The only thing we note is the looting of fish by crocodiles in their hole. In reality, the area is favorable to fish farming. This explains the large number of people working in this domain. So, to satisfy their hunger, crocodiles penetrate into the "pond" and eat fishes that considerably reduce the fish stock. Considering the short study of Dwarf crocodile division in the valley, CREDI-ONG plans to reintroduce Dwarf crocodiles at one of these sites with the help of European zoos. We are to do this in collaboration with the La Ferme aux Crocodiles in Pierrelatte, France, and Leipzig Zoo in Germany.

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In Ovo Nile Crocodile Exposure to Aluminium

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Abstract

High levels of metals including Al, Fe and Mn have been observed in the sediment of rivers impacted by mining and other anthropogenic activities. Such rivers are in certain cases inhabited by Nile crocodiles, *Crocodylus niloticus*, and eggs are therefore laid and incubated in potentially contaminated sediment. Aluminium (Al) is one of the metals of concern due to the potential hazard posed to wildlife exposed to high dosages. The aims of this study were to: (1) assess the effect of Al exposure on *C. niloticus* hatching success and hatchling size; (2) evaluate the uptake and embryonic assimilation of aluminium in hatchlings. Eggs (22-24 days old) sourced from three clutches, were obtained from Lalele Crocodile Farm, Mookgopong, South Africa. The treatments included 3.3 g/L Al (pH 4), a pH control (pH 4) and a negative control (pH 7). The eggs were completely covered with vermiculite, dampened using the appropriate treatment, and incubated in a humidified incubator at 28°C until hatching. The endpoints recorded included survival, hatching success (ie the ability to break through the egg shell) and hatchling size. In addition, Al concentrations were quantified in the shells, shell membranes, chorions, femur bone, liver and kidney tissues of selected individuals using ICP-MS. No significant differences were observed in survival or hatching size among the treatments, whereas the proportion of individuals unable to break through egg shells was significantly higher in the Al treatment relative to the control. Our data therefore suggest a risk of compromised survival in *C. niloticus* hatchlings exposed to high doses of Al during development. Data on the concentrations of Al in the shells, shell membrane, chorion, femur bone, kidney and liver of Al exposed and control individuals will be presented.

Crocodilian Nuclear Factor Kappa B1

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Abstract

We deduced the amino acid sequence of the Nuclear Factor Kappa B1 (NF-ϰB1) protein from genomic data for the American alligator (*Alligator missisisppiensis*), the estuarine crocodile (*Crocodylus porosus*), and the Indian Gharial (*Gavialis gangeticus*). A 105 kDa protein, NF-ϰB1 exhibits complex post-translational processing, multiple mechanisms of activation, and acts as precursor for a p50, a Rel homology transcription factor which influences the transcription of key genes for developmental processes, apoptosis, and immune function. The amino acid sequences of the crocodilian proteins share very high sequence identity with each other (97.2 ± 0.7%), birds (81.0 ± 1.1%, N= 6), mammals (75.3 ± 1.6%, N= 4), reptiles (80.3 ± 5.1%, N= 2), and less identity with fish (55.5 ± 5.5%, N= 4) and one amphibian (66.1 ± 0.8%). The crocodilian protein has a well-conserved Rel homology domain, a nuclear localization signal, and a glycine-rich region which facilitates proteasome-mediated generation of p50. The Rel homology domain facilitates dimerization, DNA-binding, and nuclear translocation. In addition, 7 ankyrin repeats were located, which putatively allow for inhibition of transcriptional regulation by mediating interaction with Inhibitor kappa B. Other features include a death domain, and conserved serine residues, near the C-terminal end, which act as potential phosphorylation sites for activation of the proteolytic generation of p50. Western blot analysis showed both the 105 kDa precursor and the 50 kDa mature NFkB was expressed in the alligator liver. NFkB exhibited diffuse cytoplasmic distribution in alligator hepatocytes, but was primarily nuclear in infected animals. In addition, nuclear NFkB exhibited specific binding to the consensus NFkB promoter element.

Projet Faux Gavial

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Abstract

The critically endangered Slender-snouted crocodile, *Mecistops cataphractus*, was the most available bushmeat in the markets of Lambaréné, Gabon, in 2013, representing almost half of all bushmeat recorded in market surveys despite being legally protected under national law. In 2014, our team from the Gabonese non-profit OELO launched "Projet Faux Gavial" to raise awareness about the crocodile and reduce its commerce. We created an environmental education crocodile curriculum that has so far been presented to over 4000 students in 17 primary and secondary schools, launched a citizen science project to record crocodile sightings and bushmeat commerce using Whatsapp, created a student led nature club that performed outreach about crocodile conservation throughout the city, and created a an informative panel at the local market highlighting the protected status of the slender-snouted crocodile. We strengthened partnerships with local market vendors, local law enforcement, conservation partners, elected leaders, and religious leaders resulting in a signed decree by the Mayor of Lambaréné in 2015 to enforce protected species laws. In 2015, in partnership with the Catholic church and the city of Lambaréné, we unveiled a large Slender-snouted crocodile and manatee mural and community green space at the main intersection of town. By March of 2016, the Slender-snouted crocodile had not been observed in the local bushmeat market for a period of over 6 months for the first time in 4 years of surveys.

The Broad-snouted Caiman Population Recovery in Argentina: An Example of Genetics Conservation

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Abstract

"Proyecto Yacaré" started in 1990 Santa Fe, Argentina, because of the reduction of wild *Caiman latirostris* populations and it was implemented as management and monitoring plan. Although ranching system has a noteworthy success in terms of population recovery, there is scarce information about the estimation of population-genetic parameters. In particular, the consequence of the bottleneck phenomenon underwent by these populations has not been clearly described. In this work, we evaluated variability and genetic structure of broad-snouted caiman populations from Santa Fe over time, using microsatellites and sequence analysis of a mitochondrial DNA fragment (CytB). Population genetic parameters were compared among four sites and three different periods to assess the impact of management activities, and effective population size was estimate in order to detect bottleneck events. As a result, we observed an increase in genetic variability at nuclear level and low genetic variability in mitochondrial DNA lineages overtime. Variability estimates were similar among sites in each sampling period with no or low differentiation among them; therefore, these populations are, apparently, part of the same evolutionarily significant unit. The genetic background of each sampling site has changed overtime and populations are apparently not stable. Taking into account the expected heterozygosity and effective population size values, it can be assumed that bottleneck events indeed have existed in the past. Our results seem to suggest those management activities that increased the population size, also have contributed to maintain or increase genetic viability of the species. However, the information is still incomplete, and regular monitoring should continue in order to arrive at solid conclusions.

Presence of *Sebekia* sp. (Pentastomida) in Free-ranging *Caiman yacare* (Crocodylia, Alligatoridae) in Corrientes Province, Argentina

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Abstract

The pentastomes are a taxon of obligate endoparasites found into the respiratory system of terrestrial vertebrates. Within reptiles, they have been detected in snakes, lizards, turtles and crocodilians. In the respiratory tract of the definitive host, parasites must be capable of attaining sexual maturity. The objective of this work was to identify and characterize specimens of these parasites found in a wild individual of *Caiman yacare* from the Esteros del Iberá, an extensive wetland in Corrientes Province, in northeastern Argentina. Six specimens of the Family Sebekidae were extracted from the lung lobes, fixed in ethanol (70%) and mounted directly with lactophenol. Parasites were observed with magnifying glass and optical microscope. Morphological study found that all individuals were adult sexually mature females with two pairs of retractable hooks on each side of the mouth and lots of eggs inside. This is the first report on the presence of the genus *Sebekia* in *C. yacare* living in Argentina, and the second for Latin America, as *S. oxychepala* has been previously recorded in caimans from Corumbá, in Mato Grosso State, Brazil.

Long Submergences by Crocodylians and their Physiological Support: A Working Hypothesis

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Abstract

We present a working hypothesis about the significance of long submergences by crocodylians and their physiological support. Some authors have assumed that long dives made voluntarily rely on anaerobic support, an assumption probably influenced by the prevailing (but questionable) belief that crocs rely heavily on anaerobic metabolism for most of their activity. Long dives by mammals and birds too were thought to be supported anaerobically, but during the 1980s it became accepted that almost all their dives are aerobic; surfacing with high plasma lactate is unusual. Might the same model apply to crocodylians? Until the last decade there has been little information about the diving behaviour of free ranging crocodylians and it is difficult to make reliable generalisations about the behavioural circumstances in which dives of different lengths are made. However, recent data from *Crocodylus johnstoni* suggest that dives made actively, as in foraging, are short, whereas the longest dives are associated with 'resting'. In laboratory studies on *C. porosus*, neither short nor long voluntary dives showed an accumulation of plasma lactate. Calculated maximum aerobic dive times accommodate the longest voluntary field submergences recorded so far, assuming significant down-regulation of metabolic rate. We propose that, as in other diving vertebrates, even the long voluntary submergences by crocodylians are aerobic, a hypothesis consistent with their use of water for rest and refuge. More behavioural data from the wild are desirable, combined with end-dive plasma lactate (challenging!) whenever possible.

Introduction

We hope this paper will encourage researchers to collect a lot more data about the natural submergence patterns of undisturbed crocodylians, preferably free-living, and including some studies that include end-dive plasma lactate or pH. Recording submergence patterns has become much easier than it was previously because of the availability of suitable data loggers. Monitoring end-dive plasma lactate is much more challenging, although telemetry of pH may offer a way forward (but still challenging). Most crocodylian dives are short, most indeed within the scope that a trained human can manage. Many however are very much longer and it is these that are of particular interest.

Many questions remain about crocodylian diving and submergence, such as its significance, the patterns of diving and how it differs between species and between habitats, how long they can stay submerged, how deep they can dive and what physiological mechanisms make them possible. It has often been assumed that long periods of submergence are supported anaerobically. This paper compares recently published records of maximum free-range dive times with data on the physiology of diving from *Crocodylus porosus*, augmented by studies of other diving vertebrates. The comparison leads us to propose that long periods of submergence undertaken voluntarily are supported aerobically and that, as in other diving vertebrates, anaerobically supported dives are rare in nature. The full analysis is reported elsewhere (Grigg and Kirshner 2015) but we present an abbreviated version here in the hope of stimulating the collection of more field data relevant not only to this question but to broaden the understanding of diving and its significance across a wider range of species, seasons and habitats.

Prior to the last decade there was little information about the patterns of submergence shown by crocodylians living undisturbed in their natural habitat. Cott (1961) observed some captive Nile crocodiles, *C. niloticus*, and predicted that a 4 m crocodile would have a maximum dive time of about 2.25 hours. In the early 1970s Norbert Smith acquired some data incidentally from one American alligator, *Alligator mississippiensis*, in the wild, during a study of diving bradycardia. He found that, free-ranging and undisturbed, it surfaced every 5-7 minutes to breathe, showed little or no bradycardia during the dives but, if spooked, it remained under water for 20-30 minutes with the heart rate falling as low as 2 beats per minute (Smith *et al*. 1974). This is the earliest report of natural dive times from a free-ranging crocodylian. It showed that most voluntary dives are for only short periods and confirmed that they have a great capacity to stay longer. In 1981 some colleagues and I attempted to record diving by free-ranging Saltwater crocodiles, *C. porosus*, in a tidal estuary in Australia's Northern Territory. It was very frustrating; we had primitive dive recorders and got data from only one animal of the 7 out of 12 instrumented study animals that we recaptured. But that one yielded good data showing that almost all the dives were short, 1-3 minutes, which we interpreted as foraging dives, with one dive of 22 and one of 30 minutes. Furthermore, the daily pattern was influenced by the progression of the tidal cycle (Grigg *et al*. 1985).

In the last decade, however, with the availability of small dive loggers, four studies have added much more data, three of them on Australian freshwater crocodiles (*C. johnstoni*) and one on *C. porosus*. Seebacher *et al*. (2005) studied 5 *C. johnstoni* (4.0-26.5 kg) in a billabong at Lakefield National Park in North Queensland and found that most dives (about 55% of 423) lasted less than 15 minutes (most were probably much shorter, based on the subsequent studies). From laboratory studies of *C. porosus* smaller than all of these and at rest we know that resting dives of this length would be conducted aerobically, consuming less than 20% of the stored oxygen and without any accumulation of plasma lactate (Wright 1985a,b, 1987). Despite such a high proportion of the dives being short, the proportion of total time spent submerged was surprisingly high, 18-41%, the result of comparatively few longer dives; 15% of dives exceeded 45 minutes. Nearly 70% of all dives were in shallow water (<40 cm), from which it is easy to raise the snout to the surface for a breath.

The next two studies were also on *C. johnstoni*. Campbell *et al*. (2010a) logged about 6000 dives by 17 *C. johnstoni* (5-42 kg), also in Lakefield National Park, in winter. They were able to distinguish two different types of dives, 'active' dives which were mostly less than one minute and probably related to foraging, and inactive dives which they judged to be 'resting'. Resting dives averaged ~12 minutes, much longer than active dives and these were mostly in deeper water, with the animal at the bottom of the lagoon and surfacing briefly to breathe. Diagnosis of the short dives as 'active' and the longer dives as sedentary was possible by noting whether or not they were changing depths during the dive and because some of the study animals also had an attached telemetric device to record tail beat, an indication of swimming. They found that the smaller animals (5-7 kg) spent more time on the surface following a resting dive than larger animals and speculated that this may have been to clear an oxygen debt, whereas the larger animals did not need to. They concluded that dive times were dictated more by ecological factors than physiological constraints and wondered why the smaller ones endured an anaerobic debt in order to maintain similar dive durations to the larger ones, suggesting conspecific pressure as a possibility. Campbell *et al*. (2010b) logged a similar number of dives from animals at the same location in the following summer when body temperatures averaged about 5°C warmer. Among other things, they found that in both seasons crocodiles spent a similar proportion of the day submerged, but the median and maximum dive durations were shorter in summer, presumably because the oxygen store was used at a higher rate.

The fourth recent study in the last decade reported submergence behaviour in free-ranging *C. porosus*, collected while monitoring the movements of 27 large (2-5 m) acoustically tagged *C. porosus* over 60 km in the North Kennedy River, a tidal river on the west side of Cape York, North Queensland (Campbell *et al*. 2010c). Some animals were also fitted with dive loggers. The crocodiles made good use of tidal flows to facilitate their travel and when current direction was unfavourable they would either haul out onto the bank, or submerge 3-5 m to the bottom and await a change of tide. In submerged animals, periods between breaths averaged 18 minutes and some were as long as an hour (Hamish Campbell, pers. comm. 2011).

Collectively these recent studies have added greatly to our knowledge of submergence behaviour, particularly in one species of crocodilian in one habitat type. How typical that is remains to be seen, but *C. johnstoni*, in a billabong habitat anyway, confirmed previous findings that most undisturbed dives in the wild are very short, mostly a couple of minutes, and that much longer dives occur too. The new information was that the short dives are associated with swimming, the longer dives with inactivity and it is these that make up most of the time spent underwater each day. Quite reasonably, the interpretation has been made that the short, active dives are associated with foraging and the longer dives probably represent rest at the bottom (Campbell *et al*. 2010a,b). In support of that, Campbell *et al*. (2010b) noted that the 'active' dives coincided with times likely to be suitable for prey capture. The reason/s for long periods at the surface between short foraging dives is unknown. It seems unlikely that the longer surface times could be due to any need to 'pay back an oxygen debt' because most foraging dives would be short, speculative, unsuccessful, and end well before plasma lactate levels rise to a compromising extent, even from associated activity, unless of course there was a capture of prey that involved a prolonged struggle. Perhaps time at the surface is part of their 'sit and wait' foraging strategy.

The possibility of routine reliance on anaerobic metabolism to support resting dives (Campbell *et al*. 2010a,b) reflects a common assumption about crocodylian diving physiology and is worth further exploration. Firstly, most of the voluntary resting dives they reported (average ~12 minutes in the winter study) seem too short to require anaerobic support. For example, in laboratory studies of *C. porosus*, voluntary 'resting' dives by 1-3 kg animals for as long as 13 minutes showed no buildup of plasma lactate (Wright 1985a,b). On the other hand, the longest voluntary dives recorded in each study animal by Campbell and colleagues, 50-120 minutes, are much more likely candidates for needing anaerobic support and it is this possibility we will explore.

Secondly, although diving by birds and mammals was for many years thought to rely on anaerobic metabolism, it is now recognised as being supported aerobically except quite rarely. In a seminal study, Kooyman *et al*. (1980) found plasma lactate levels in Weddell seals still at resting levels after dives lasting up to about 25 minutes. Furthermore, 97% of their dives were less than 25 minutes - and they could make another dive immediately, without any need to rest at the surface. Following dives that exceeded 25 minutes, plasma lactates were elevated and the seals would haul out and lie on the ice recovering for longer than the length of the dive while plasma lactate slowly returned to normal, 'paying back the oxygen debt'. The same pattern has been found in other diving mammals and in birds too, making only occasional use of a capacity to go anaerobic and, when they do, needing longer at the surface to recover. This makes good functional sense. Diving aerobically rather than anaerobically is energetically advantageous, and for many species, foraging time can be maximised by making short dives repeatedly with little or no recovery periods in between. Kooyman *et al.* (1981) pointed out that a series of short, repetitive aerobic dives yields much more underwater foraging time than fewer, more prolonged dives with long recovery periods. They concluded that this is why 'prolonged dives are rare in nature'. This has become accepted as a generalisation applicable across diving mammals and birds (Butler and Jones 1997; Kooyman and Ponganis 1998; Hochachka 2000).

Vertebrates being generally conservative in their physiology, and with all this in mind, it seemed reasonable to ask whether crocodylians rely on anaerobic metabolism to support their long voluntary resting dives. Accordingly, we compared the longest dives recorded by each animal in each of the above studies to the calculated aerobic dive limit (cADL; Butler 2006). This was done for crocodiles over a suitable size range and at 25°C, a relevant temperature. Maximum dive times were either available already (Seebacher *et al.* 2005) or generously provided by Hamish Campbell and all are plotted in Figure 1.

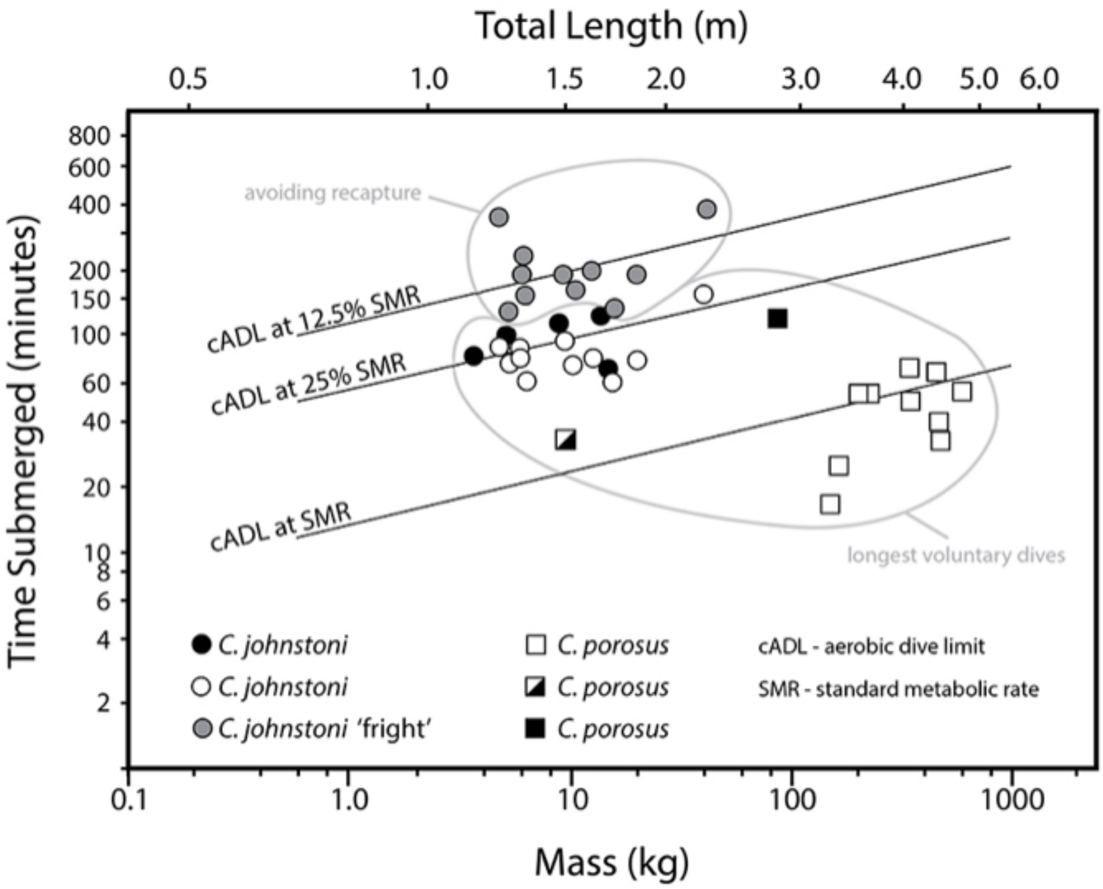

Figure 1. Longest voluntary submergences observed in the field (except as marked) in two species of Australian crocodylians, compared to predicted maximum aerobic submergence limits (cADL) calculated conservatively for *C. porosus* across a size range. Predicted limits were calculated at 25°C and, as discussed in the text, assuming use of 68% of the oxygen store with oxygen consumption maintained at pre-dive (resting) rates (lower line), at 75% reduction (middle line) and 87.5% reduction from pre-dive rates. *C. johnstoni* diving voluntarily (black circles) (Seebacher *et al.* 2005); *C. johnstoni* diving voluntarily (open circles) and with disturbance as the animals evaded recapture (shaded circles) (Campbell *et al.* 2010a); *C. porosus* diving voluntarily (open squares) (Hamish Campbell & Craig Franklin, pers. comm. 2011); *C. porosus* (black square) (Craig Franklin, pers. comm. 2009); *C. porosus* diving voluntarily (black & white square) (Grigg *et al.* 1985). Broad similarity across crocodylian species in metabolic rate, lung volumes and general physiology suggest that data gained on other species could usefully be compared with this graph.

Calculating aerobic dive limit, cADL

This depended heavily on the wealth of data on diving physiology on *C. porosus* assembled by Jonathan Wright during his PhD studies at the University of Sydney (Wright 1985a,b; 1986 a,b; 1987). Some background to the diving physiology of *C. porosus* is useful. Compared to mammals and birds, crocodylians have a low metabolic rate, dive with a substantial quantity of air in the lungs, have little myoglobin and, famously, have a pulmonary to systemic cardiovascular shunt (or pulmonary by-pass shunt, PBS). Like birds and mammals they lack cutaneous oxygen uptake. Their diving physiology has been described mainly from studies on juveniles, usually 1-3 kg. During short voluntary resting dives under Wright's laboratory conditions the animals sat quietly on the bottom of a shallow tank, surfacing to breathe from a respiratory hood. Submergences were typically for 1-6 minutes, during which oxygen consumption was not reduced, there was slight 'bradycardia' and, typically, they used 6-12% of the total oxygen store. At surfacing, lung oxygen was typically at 70 mmHg, compared to 123 mmHg at submergence. At the end of the longest, 13 minute dive, lung oxygen was 40 mmHg and the arterial blood about 75% saturated. Such dives left considerable room to extend the dives further aerobically. To encourage longer dives, Wright denied the animals access to the hood and, rather than immediately employing anaerobic metabolism, crocodiles reduced oxygen consumption (Wright 1987). Lung oxygen fell and so did heart rate, the slope of the decline in lung oxygen values being a good indicator of oxygen uptake. In such artificially prolonged dives, oxygen consumption did not decline until lung oxygen reached partial pressures of 40-50 mmHg, as if the fall in internal oxygen levels had provoked metabolic depression. The implication is that whereas crocodylians mostly dive with little or no bradycardia and little or no decrease on oxygen consumption, they can also extend their dive by progressively reducing heart rate and oxygen consumption during the dive. Plasma lactate was in the normal range after each of these dives. Wright found that he had to restrict access to air for much longer, 50-60 minutes, which was stressful, before increases in plasma lactate resulted. Even these small crocodiles apparently have a great capacity to conserve oxygen and extend their periods of submergence without needing to rely on anaerobic metabolism.

The ideal determination of aerobic dive limit would be as the longest dive possible under natural conditions without any buildup of plasma lactate. This would be the dive lactate threshold, DLT (Butler 2006). This is very difficult to collect in the field because of the difficulty of avoiding a capture (and activity) artifact, although monitoring plasma pH telemetrically could be instructive. Without information about a DLT, only speculative calculations of the aerobic dive limit (cADL) can be made, based on a series of reasonable assumptions. This involves dividing the useable oxygen available at the start of the dive by an estimate of the rate of oxygen consumption. But aerobic dives do not usually use all of the 'useable' oxygen; Wright (1987) showed that juvenile crocodiles routinely surface with substantial oxygen 'still in their tank'. Our estimates are therefore conservative because they are based on examples of actual oxygen usage in voluntary dives (whether cADL is a suitable term for this is debatable; maybe 'calculated voluntary aerobic dive limit', cvADL, would be more correct). Body size needs to be taken into account because metabolic rate and lung volume scale differently with mass and because metabolic rate varies with temperature, and dive time depends also on body temperature.

The oxygen store at submergence comprises what is held in the lungs, blood, and dissolved in the tissues. The calculations depend upon assumptions about some things that are difficult or impossible to measure, so the results must be regarded as indicative, not definitive. The details are in Grigg and Kirshner (in press); this is an abbreviated version. Lung gas, of course, is not all oxygen. Wright and Kirshner (1987) measured the volume of air in the lungs at submergence by plethysmography on 24 juvenile *C. porosus* over a size range from 271-3762 g at 23-25°C. From their work, a 1 kg *C. porosus* can be expected to submerge with a lung volume of 46.8 mL of which 7.86 mL would be oxygen. Scaling that up appropriately to accommodate increasing bone density with growth, to achieve the same slightly negative buoyancy, leads to a 500 kg crocodile diving with about 8.2 L and a 1000 kg crocodile with about 17.6 L of oxygen. This is based on the mammal/bird scaling exponent and, considering the bone density and osteoderm development of crocodylians, is likely to be an underestimate (Roger Seymour, pers. comm.). From hematological data, the blood oxygen store at submergence approximates 5.71 mL/kg of body mass and a small allowance must be made for oxygen dissolved in the tissues (these scale isometrically with mass). It is noteworthy that the relative contribution of the lungs to the oxygen store increases with body size from 56% in a 1 kg crocodile to 73% in a 1000 kg animal. Wright (1985a) found that the longest voluntary dive he observed used 68% of the oxygen store and that was without any reduction in metabolic rate. Because the longest voluntary dives by *C. johnstoni* were interpreted by Campbell *et al*. (2010a) as resting dives, resting oxygen consumption reported by Seymour *et al*. (2013) should be appropriate, converted to 25°C using a Q10 of 1.8 (Wright 1986b).

<u>Comparison of maximum observed long voluntary submergences with cADL</u>

Calculated aerobic dive lengths (cADL) over a wide range of body size are shown in Figure 1 and these can be compared with maximum observed submergences for each animal in the four most recent studies discussed above. These are conservative estimates because it is unlikely that 68% usage of the oxygen store is a maximum. Longer aerobic dives are achieved by reductions in metabolic rate, so cADLs are shown for 75% and 87.5% reductions in SMR as well.

The maximum voluntary submergences recorded from *C. johnstoni* can all be accommodated by a reduction in metabolic rate of about 75%. But are reductions of this magnitude reasonable? Wright's data imply reductions in oxygen consumption by about 60-90% of pre-dive levels. This can be compared with comparable drops of 60-85% in painted turtles, *Chrysemys picta*, although some of these were during forced dives (Jackson 1968). Similar reductions are consistent with the conclusion by Guppy and Withers (1999), in an extensive review of metabolic reductions in aestivating or hibernating animals, that 'metabolic depression to approx. 0.05-0.4 of rest is a common and remarkably consistent pattern for various non-cryptobiotic animals (eg molluscs, earthworms, crustaceans, fishes, amphibians, reptiles).' Such reductions parallel the reductions in heart rate seen commonly in diving reptiles quite well, so it seems reasonable to conclude, for the conservative calculations of cADL in Figure 1, that aerobic metabolic rate in crocodylians during prolonged voluntary dives may be reduced sufficiently to support these long dives aerobically. The reduction in metabolism during the dive is almost certainly a consequence of reduced perfusion of many of the organs and tissues by only partly oxygenated blood and a redistribution of blood from the skin, muscle and many of the other organs, as in diving mammals (Hochachka 1992, 2000). Redistribution of blood during prolonged dives has been observed in crocodylians too (Weinheimer *et al*. 1982) but, in striking contrast to mammals, operation of the pulmonary bypass shunt in crocodylians is likely to be effective in the down-regulation of aerobic metabolism (Grigg 1989, 1991; Axelsson and Franklin 2011). The longest dives of all were shown by animals evading capture (Fig. 1) and high plasma lactates were sampled from a couple of them when they were eventually caught (Campbell *et al*. 2010a). These are presumably examples of animals 'pulling out all stops' to remain hidden.

The lengths of voluntary, undisturbed dives by *C. porosus* are in striking contrast. All but one could be accomplished with little or no reduction in resting oxygen consumption, although several are close to the projected limit. It is probable that this is a reflection of different behaviour and not because *C. porosus* have so much less capacity than *C. johnstoni* for making long dives. The animals in the Campbell *et al*. (2010c) study were travelling, using the tidal currents in the river and waiting for them to reverse. Admittedly, the cADL lines in this figure are derived from *C. porosus* data, but physiological differences with *C. johnstoni* are unlikely to explain the difference.

The working hypothesis

Putting this series of observations together, it seems reasonable to conclude that quite long resting dives can be supported aerobically and that strategies to extend dive time aerobically, including metabolic depression, are employed before an animal resorts to anaerobic metabolism with its subsequent costs. The overall working hypothesis is that, like other diving vertebrates, crocodylians avoid the use of anaerobic pathways in support of periods of time submerged and that inactive dives of the type described by Campbell *et al*. (2010a,b) as 'resting' are almost always supported aerobically. This incorporates the interpretation of such dives as being for 'rest', which itself is a working hypothesis concerning the significance of such dives. This seems entirely reasonable but awaits further examination. We encourage further studies of all aspects of crocodylian diving, building the database with behavioural studies of a wider range of species and differences expressed in different habitats and in different seasons, as well as exploring whether or not our hypothesis about aerobic support of the longest voluntary submergences needs to be rejected, or whether crocodylians fit with the general pattern expressed by diving vertebrates.

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WORKING AND THEMATIC GROUP SUMMARIES

Public Education and Community Participation Thematic Group

The need to share information about the efforts being done to mobilize, motivate and sustain individual and collective action on crocodile conservation and management has to be addressed in order to help provide direction for other crocodile programmes and projects that will engage in public education and community participation activities. The identification of appropriate communication strategies and approaches for certain groups of audiences and certain situations often spell the difference between success and failure.

It is for this reason that a full session was devoted to Public Education and Community Participation (PECP) on the third day (23 May 2016) of the 24th Working Meeting of the IUCN-SSC Crocodile Specialist Group. Five papers were presented starting with an overview of the different programs and projects dealing with PECP in the different parts of the world and the presentation of four specific cases in the Philippines, Colombia, India, and Belize. Two PECP cases in Africa (Benin, and Gabon) were also included in the poster presentation.

PECP Overview

In various countries and targeting various species, individual and group efforts are being exerted to educate the public, develop positive attitudes and mobilize community participation in crocodile conservation. The first paper presented an overview of the outputs, cognitive and affective outcomes, and impacts of different crocodilian PECP campaigns reported from the different parts of the world. Fifteen (15) PECP cases were featured in the presentation primarily because these were the projects that were forwarded by members of the PECP Thematic group and other CSG members. In this presentation, the importance of documenting, monitoring and evaluating the impact of different PECP activities was discussed. A theory of change (Cureg *et al*. 2016) was presented as a basis for PECP campaigns to increase community support for and participation in crocodilian conservation. The lessons generated from different situations and experiences were presented in order that other projects can use these to help improve the effectiveness of PECP campaigns.

Philippines

Public education and community participation in the conservation of one the rarest crocodiles in the planet, the Philippine crocodile (*Crocodylus mindorensis*) in northern Philippines was discussed by Marites Gatan-Balbas of Mabuwaya Foundation. The paper described how the Communication, Education and Public Awareness (CEPA) campaign that aimed to increase support for, and participation in Philippine crocodile conservation has led to the establishment of 8 locally declared crocodile sanctuaries and an increase in Philippine crocodile population of less than non-hatchlings to more than 80 in 2015. A longitudinal study on the evaluation of the CEPA campaign in 2008 and 2013 was conducted which showed that a majority of the people in crocodile areas supported Philippine crocodile conservation (80%), compared to 20% only in control areas. It was concluded that CEPA campaigns and efforts to increase community participation in crocodile conservation remain very important to sustain community-based Philippine crocodile conservation program in northern Luzon.

Colombia

The use of spatial information to preserve crocodilians was explained by Sergio Balaguera-Reina who described the Crocs Geo-visor Initiative as a conservation tool. As reported, the two important factors used to assess the risk of extinction are range (its extension and variation through time) and habitat status (quality) of each species. However, precise range information is lacking for majority of species making it impossible to accurately measure the actual risk of extinction. Thus, the Crocs Geo-visor Initiative was created as a way to collect spatial data under established protocols with a primary goal of developing a curated spatial database for crocodylians around the world. A mobile application for data collection (Crocodylians of the World) was also developed in order to encourage participation of citizen-scientists and to be able to readily test data for uncertainty. It is a geographical/friendly/easy going database focused on gathering crocodiles' spatial information regarding sights, nests, and conflicts under a historical context. It was described as useful in sharing, easily, crucial data in conservation and management process.

India

One of the PECP programs being implemented in India which is being implemented by the Voluntary Nature Conservancy (VNC) was shared by Anirudhkumar Vasava. The implication of using citizen scientists to monitor Mugger crocodile population and threats in the conservation of crocodiles was presented. It argues that researchers can overcome the challenge of conducting long-term monitoring studies designed to survey large areas by utilizing the skills of volunteers, or what they call the citizen scientists. The VNC initiated the 'Charotar Crocodile Count'. It is a Citizen science based initiative, designed to involve people from various walks of life to come together for the conservation of this species. While helping to gain a better understanding of crocodile's status and distribution across the region, this voluntary based initiative also provide an opportunity to city dwellers an onsite experience of observing crocodiles and contribute to science and conservation. It was reported that in December 2013, VNC conducted its first volunteer-based crocodile count in Charotar and enlisted volunteers across the state to monitor crocodiles. The program is first of its kind in Gujarat and has generated a very useful data contributing significantly towards understanding Mugger's status and distribution in the Charotar region.

<u>Belize</u>

The last presentation for the PECP session was presented by Marisa Tellez who described the initiative of providing hands-on training to the next generation of crocodilian biologists. She described the Next Gen Croc Tours program on Cave Caulker, Belize in which high school students were provided the opportunity to lead nocturnal mangrove and croc tours. This was done in an effort to develop wildlife awareness and foster the new generation of crocodilian conservationists and biologists in Belize. Aside from educating locals and tourists about crocodiles and their environment, the tours include performing official, monthly nocturnal eyeshine to assist in the monitoring of the local population of *Crocodylus acutus* (American crocodile). It is hoped that with the creation of the eco-tourism project with student involvement, the young participants will be given a new appreciation for their local wildlife and environment connection with improving their scientific, leadership and communication skills, while simultaneously assisting the students financially in their education. As the students lead tours, and the money made goes towards paying off their school tuition.

<u>PECP Workshop</u>

Participants interested to share ideas and become part of the PECP thematic group were invited to attend the out of session workshop which was conducted immediately after the presentation of the PECP papers in the scientific session. The workshop started at 1800 and ended at 1856. Thirty (30) delegates composed of existing members of the PECP thematic group and other CSG members attended the meeting.

PECP Vice Chair Dr. Myrna C. Cureg provided background information on the thematic group (eg members, mission, vision and goals, and activities so far been implemented). The objective of the current workshop was to identify and future plans and activities for the PECP thematic group. Dr. Cureg mentioned several activities and solicited comments and further suggestions from the participants of the workshop. The following has been mentioned:

1. To assign key people representing different regions
2. To compile information on public education about crocodilians, such as articles, manuals and sample communication and educational materials
3. To put up resources online through the CSG website and/or other websites
4. To establish a circulation list of new information and progress
5. To maintain the existing yahoo group email
6. To create a google group email
7. To create a Facebook page
8. To create mailchimp email group
9. To create a link for PECP at the CSG website
10. To create a form in Survey Monkey as basis for the project descriptions to be featured in the CSG website

In the end, the group decided on the following:

<u>PECP Membership/Circulation List</u>

The event was set up to provide an opportunity for existing PECP Thematic Group members attending the CSG meeting to meet each other personally to develop familiarity with each other and plan for the future. It also opened up an opportunity to increase membership by inviting the attendees to join the PECP thematic group. It was agreed during the meeting that all the names in the attendance sheet will be added in the existing list of PECP members. Thus, the table below show the original members of the group (1-21) and the new members (22-47). The same list will be used as the distribution list for all information that will be shared in the group.

1. Myrna C. Cureg, Vice Chair, PECP Thematic Group, Isabela State University & Mabuwaya Foundation (Philippines)
2. Merlijn Van Weerd, Mabuwaya Foundation, Inc., Philippines
3. Chris Banks, Zoos Victoria, Australia
4. Jennifer Andringa, Walt Disney World, Animal Kingdom, USA
5. Oswald Braken Tisen, Sarawak Forestry Corporate Office, Kuching, Sarawak, Malaysia
6. Clara Lucia Sierra Diaz, Asocaiman, Colombia

7. Avishka Godahewa, Sri Lanka
8. Robert Carmichael, Wildlife Discovery Center, College of Lacke County Division of Biological Sciences, USA
9. Silvia Ten, CIBIOMA, Universidad Autónoma del Beni, Bolivia
10. Sergio A. Balaguera-Reina, Texas Tech University, USA
11. Gowri Mallapur, Madras Crocodile Bank Trust/Centre for Herpetology, India
12. Colin Stevenson, Crocodiles of the World, UK
13. Marisa Tellez, Dept of Ecology, Evolution and Marine Biology, UCSB, USA
14. Paolo Martelli, Ocean Park, Hong Kong
15. Tarun Nair, Gharial Conservation Alliance, India
16. Rainier Manalo, Crocodylus Porosus Philippines, Philippines
17. Nikhil Whitaker, Madras Crocodile Bank, India
18. Christine Lippai, South Africa
19. Marites G. Balbas, Mabuwaya Foundation, Inc., Philippines
20. Joni T. Acay, Mabuwaya Foundation, Inc., Philippines
21. Luis Pacheco, Instituto de Ecología, Universidad Mayor de San Andrés, Colombia
22. Joey Brown, San Diego Zoo, USA
23. Kim Lovich, San Diego Zoo, USA
24. Jen Brueggen, St Augustine, USA
25. John Brueggen, St Augustine, USA
26. Agata Staniewiez, Bristol University, UK
27. Alexander Meurer, DGHT, Germany
28. Kema Kema Juolicoel R., WWF India, Gabon
29. Emmanuel Amoah, KNUST, Ghana
30. Sebastian Brackbane, University Friburg, Germany
31. R.J. Rao, Jiwaji University, India
32. Ashley Pearcy, Wits University, Aarhus University, SANParks, Denmark
33. Jon Meisenback, Lincoln Park Zoo, USA
34. Joe Partyka, The Bear Park, Norway
35. Peter Watson, Crocodile Creek, SA, KZN South Africa
36. Anirudhkumar Vasava, Voluntary Nature Conservancy, India
37. Simon Pooley, Birkbeck College, University of Oxford, UK
38. Miriam Boucher, West Virginia University, USA
39. Corinna Browne, Queensland Wildlife Management, Australia
40. Matthew Brien, QLD Government, Australia
41. Adrian Sugiarto, Suryaraya, Indonesia
42. James Perran Ross, University of Florida, USA
43. Jeffrey Lang, Madras Crocodile Bank, India
44. Yusuke Fukuda, Parks and Wildlife Commission of the Northern Territory, Australia
45. Kent Vliet, University of Florida, USA
46. Georges Hedegbetan, CREDI-ONG, Benin
47. Vince Shacks, Okavango Crocodile Monitoring Program, Botswana

<u>Information Sharing</u>

It was decided by the group to start a simple medium of communication, that is the Google groups, and establish a link for PECP at the CSG website. The former will mainly be a means of continuous exchanging of ideas and connecting people together while the link at the CSG website serves as a centralized hub of different projects regarding public education and communication participation in crocodile conservation, to provide a list of projects, a summary of each project, links to external websites of the projects, and publicly available and downloadable information such as articles, reports or sample materials (eg posters, videos, photos, infographs, etc.). This will also be a means for other groups, such as Zoos and funders to have access to such information. It was emphasized the importance of indicating which ideas worked and which did not and the successes and challenges of each project. These will be important, especially for start-up projects, as a guide and can be modified to suit regional differences.

A form will be circulated amongst CSG members as basis for the information to be put up online.

Sergio Balaguera Reina and Colin Stevenson have volunteered to work together to create the link at the CSG website. Dr. Cureg shall circulate the form to CSG members. A timetable shall still have to be decided.

It was noted that the contents of the link shall have to be go through feed backing from several members (10-20 pax) from the PECP thematic group and the CSG Executive Committee (ie Tom Dacey and Charlie Manolis).

It was agreed that the google groups, link at the CSG website, and contributing information from each project shall be prioritized and future activities will be discussed via email.

Report prepared by: Myrna C. Cureg (Vice Chair, PECP), Merlijn van Weerd (PECP Scientific Session Chair) and Joni T. Acay (PECP Workshop Rapporteur)

Red List Working Group

On 25 May 2016, Perran Ross convened a session for those interested in being involved with Red List assessments. There were 8 attendees (see below) which doubles the previous number of members interested in the Red List (4).

Perran Ross, USA (pross@ufl.edu)
Sally Isberg, Australia (sally@crocresearch.com.au)
Robbie McLeod, Australia (robbymcleod@koorana.com.au)
Ashley Pearcy, Denmark/South Africa (Ashley.pearcy@gmail.com)
Mohammed Ezat, Egypt (Mohammed.ezat85@gmail.com)
Iri Gill, UK (Iri.gill@zsl.com)
Matt Brien, Australia (crocmatt@hotmail.com)
Sergio Balaguera-Reina, USA/Colombia (Sergio.balaguera-reina@ttu.edu)

Perran gave a brief overview of what the Red List is and why it is important for the CSG to be active in their assessments for IUCN. Perran briefed the group on the training that Sally Isberg, Robbie McLeod and Olivia Plume had completed during the year and some of the other attendees noted that they also have completed the training (eg Ashley Pearce, Sergio Balaguera-Reina). Others noted that they will begin the training upon returning home (eg Matt Brien). The group had a brief discussion regarding the recent taxonomy status of crocodilians with more species being defined. Perran's advice was to ignore these taxonomic splits unless they are recognised by the IUCN Taxonomy Committee rather than actively seeking separate Red List listings.

The one weakness that has been identified over the previous year relates to mapping as per Red List requirements. However, Sergio is very familiar with mapping and will work with the group to develop the maps as required. Robbie is also refreshing his knowledge in this area. It is hoped that Sergio and Robbie will impart their knowledge over time to other members of the group to further mitigate against this weakness.

Further on the mapping discussion, Sergio has developed an Android App for the public to record sightings of *Crocodylus intermedius* individuals and nests. It may be of future benefit to expand this model to other species globally to (a) encourage public interest in crocodilians, and (b) passively generate distribution maps for use in Red List assessments rather than just generating maps from published scientific literature which can often be biased towards particular river systems or, worse still, no official surveys conducted at all. Of course, screening of data would be essential and an Apple App would also need to be developed to ensure optimal utility. Along similar lines, Ashley Pearcy is creating a repository of geodata for her Post-doc which will also aid in Red List mapping.

Current status of assessments:

- *Alligator sinensis* - Perran has drafted and is ready for review [Action item].

- *Crocodylus suchus* - At the 3rd Regional West and Central Africa Meeting held in Abidjan, Côte d'Ivoire in December, 2015, a workshop was conducted, similar to the *C. niloticus* workshop at this meeting. Perran will begin drafting the assessment and has identified appropriate assessors (Action item].

- *Crocodylus johnstoni* - The assessment is currently under review. Matt Brien will also be asked to review and given his current posting in Queensland may be able to provide some insight into the current population status given the lack of survey data since the late 1990s. Sally to send Sergio recent published literature on *C. johnstoni* for a map to be generated [Action item].

- *Crocodylus mindorensis* - Assessment has been reviewed by Grahame Webb and has been accepted by the Red List but is awaiting a map. Perran will send Sergio points to generate this map [Action item].

Immediate priorities [Action items]:

- *Crocodylus niloticu*s - Given the workshop on 23 May 2016 (see report for further details), Sally will begin drafting the *C. niloticus* assessment with the assistance of Robbie McLeod, Ashley Pearcy and Mohammed Ezat. Richard Ferguson will be contacted for additional data particularly from the range states that were not represented at Monday's workshop.

- *Crocodylus intermedius* - Sergio and colleagues are in the process of publishing a paper on the status of the Orinoco crocodile. This will be modified into a formal Red List assessment by Sergio Balaguera-Reina.

- *Crocodylus porosus* - A team volunteered to begin drafting the assessment for Saltwater crocodiles; Matt Brien, Sally

Isberg, Robbie McLeod, Rainier Manalo and Adrien Surguiato.

- *Caiman crocodilus* - Perran will work with Alvaro and Alejandro to begin this assessment. It was discussed to pre-prepare participants to bring data to the 26th Working Meeting in Argentina to allow a workshop similar to the *C. niloticus* workshop on Monday.

Other items discussed were:

- Using Regional and Working meetings to run Red List workshops of local species is a good way of getting a collective group of appropriately knowledgeable people together to begin drafting assessments. However, we need to be more organised and give people notice so they can come prepared with the appropriate data/reports.

- The generation interval for crocodiles is often assumed to be 25 years. This is based on valid survival assumptions but Caroline Pollock from the IUCN Red List has suggested that we publish a paper to affirm these assumptions and can reference it within our assessments. This does not mean we cannot use a different generation interval for difference species given differences in life history. Perran will action this.

Report prepared by: Sally Isberg, Robbie McLeod and Perran Ross

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West African crocodiles (*Crocodylus suchus*). Photograph: Matthew Shirley.

Veterinary Science Group

1. Attendance

   Paolo Martelli, Cathy Shilton, Charles Caraguel, Luis Sigler, Silke Pfitzer, Csaba Geczy, Samuel Martin, Jaelle Rowland, Paul Reilly, Danny Govender, Chris Foggin, Bruce Douglas, Gerry Swan, Gowri Mallapur, Jan Myburgh, Eric Langelet, David Huchzermeyer, Susan Van Der Woude, Bed Bahadur Khadka

2. Business arising from previous meeting:

   - Presentations from previous Veterinary Science Workshop in Cambodia [*Action: Paolo and Cathy will add a Table of Contents and page numbers to their presentations from the workshop - Done (with Charlie Manolis for uploading to website)*].

   - A survey of the target group, eg a year after the workshop would be very informative for determining the true value of the workshop. [*Action: Lonnie advised at Cambodian Veterinary Science group meeting that he will try to do this with the local Cambodian farmers. Not done, and likely won't happen*.]

   - Additions to the website [*Action: Gowri will look into editing the video of the workshop at the Madras Crocodile Bank from a few years ago, and this could perhaps be used. In progress*.]

   - Health assessment guidelines for translocation of crocodiles [*Action: Gowri and Paolo will draft something up to send around. Done. To be reviewed at this meeting - see below. Action: Perran will check whether we can get material from the reintroduction group. Done, material was distributed to group drafting guidelines*.]

   - Most important crocodilian conditions list [*Action: Cathy will put together the spreadsheet and organize to send it around. Done. See item 7 below.*]

3. Proposed appointment of Gowri Malapur as CSG Vet Group Secretary

   It is envisioned that this is likely to improve scope and efficiency of vet group activities and administration. Proposed, seconded and supported unanimously.

4. Review of the pre-meeting Veterinary Workshop

   A questionnaire was distributed to participants during the CS meeting.

   - Responses are overall positive and strongly support continuing and expanding the veterinary workshops. Some improvements or critics were made. The venue (Kruger Park slaughter room) was ideal for the hands-on sessions abut acoustics and projections were not ideal. Some commented that foreign accents are difficult to deal with, this is true and in an international group where everybody is foreign to the others and where communicating in broken English it may well be unavoidable (?) but to be kept in mind.
   - Most respondents felt the topics were relevant, and some felt it was the best workshop ever
   - A suggestion was made to allow broader audience for the theory and keep the restricted format for the practical sessions only.
   - The style and focus of the workshop likely depends on the country where the meeting will be hosted
     - Paolo advised please think about suggestions for the CSG Vet Workshop in Argentina to capitalize on the strengths of that meeting in an academic setting. Possible topics involved emerging diseases, genetics, molecular diagnostics,
     - Possibly more on skin diseases? Was suggested as a topic for the Kruger workshop, but rejected as not being directly conservation related so not a primary focus for the CSG. Also, any skin research knowledge is generally considered confidential.
     - Are the workshops supposed to be aimed at teaching participants, techniques, or more collaborative, with vets across regions forming a consensus on diseases, and skin terminology, etc.
     - Argentina were invited to come with their own suggestions based on need. Paolo to follow up on this with Pablo Siroski and Alejandro Larriera.
     - One suggestion was to include a session for presentation of short case histories among participants for informal comment and information sharing.
     - Transport of crocodilians of different ages and in different conditions was also seen as a useful topic to include in future workshops.

- Over time we aim to create a library of topics that can serve as modules for future workshops to help alleviate the workload that it is to prepare and deliver workshops.

5. Updating/expansion of the Veterinary Science Group e-mailing lists, membership and expertise register.
   - The expertise register was sent around for attendees to enter/remove their names if desired.
   - Attendees were invited to provide their e-mail if they would like to be added to the Veterinary Science Group e-mailing list [*Action: Add these people to the list and then sent around an e-mail to the expanded list to solicit ideas as to other people to ask to join the e-mail list*]

6. Health assessment guidelines for crocodile releases.
   - Gowri and Paolo have headed up drafting up some guidelines, to be reviewed/commented at this meeting (sent as an addendum with the Agenda).
   - Paolo and Gowri both have tried out the guidelines and will give feedback, see also at end of draft guidelines an example of a form used by Paolo for an FFI project [*Action: Tidy up draft and incorporate current ideas. Then sent around to the vet group for comment. Action Gowri and Paolo*]
   - One method from India (Gharials) is to emphasize on clinical history to counteract the challenges in running diagnostics (ie the animals have to be historically healthy, be behaving appropriately, eating, selected originally for visually assessed healthy animals). Also age/size criteria.
   - The comment was made that biosecurity will be difficult to enter into the equation since testing is either not available or not practical. A certain risk has to be accepted. Eg some amelioration can be achieved with eg fasting so that the croc doesn't take a gut full of parasites/bacteria, etc., from the captive environment.
   - Pre-release quarantine and use of sentinel animals would also be practical methods to include in the document with a view to ameliorate the possibility of release of pathogens along with the crocodile.

7. List of most important diseases of farmed crocodilians
   - This was initiated by CSG Chair Grahame Webb.
   - Cathy worked with Grahame on this. A simple survey was put together as a 10 column Excel sheet to fill out and it was decided that initially, the survey was sent out just to the CSG Veterinary Science Group e-mail list.
   - It was sent out to this list, along with a few others, totaling 26 people.
   - Response rate was poor (7 respondents). See Appendix for initial collation of results. (This document has also been forwarded to the CSG Executive to fulfill the agenda item requesting this for the Steering Committee meeting).
   - Upon review of the mailing list, it was apparent that the Veterinary Science Group list doesn't actually have many people on it that would be able to respond re diseases important to farms.
   - Also, we don't have good coverage/knowledge of who to ask in some countries (eg SE Asia, many of the South American countries).
   - The current plan is to send out the survey again using an expanded list through:
     i. General expansion of the Veterinary Science Group e-mail list (see item 5 above).
     ii. Targetting specific people that could be good responders, whether they are in the CSG or not, including non-vets.
   - *Action: Gowri (Secretary) to assist with continuing to increase response rate over the next year.*

8. Welfare of farmed crocodiles. The criteria will only become more stringent, how to engage all farmers proactively.
   - There was a meeting organised by HCP re humane euthanasia after the South African Crocodile Farmers' Association AGM on Wednesday. A few veterinary members of the CSG have been formally invited to this meeting.
   - Charles Caraguel summarized the point of the workshop and advised that people that are servicing the industry and providing advice would be welcome (1600 h on Wednsday in the room used for the Steering Committee).
   - Some background provided is that the meeting is not part of the CSG meeting, but CSG members that are very interested may attend, but realizing that space may be limited, and seating organized so that the focus is on members of the South African Farmers' Association.
   - The meeting was very informative and well run. As in all other industry the focus is initially placed on slaughter techniques.

9. Review of website- additions/changes, future directions (see Appendix re current contents)
   - Possible re-arrangement of Veterinary Science group page (to something other than just a list of resources)
   - Other suggestions requested
     - Possibly a filtering system by author/topic.
     - Copyright issue was raised. Currently, there is no copyrighted material on the website, all contributors are alerted to that.
     - We all agree that a comprehensive bibliograophy of all matters crocodilians would be wonderful and to get around the copy rights issue a suggestion was made that an Endnote database of relevant veterinary references for crocodilians could be added, and then the person would need to find the reference themselves. Possibly provide the list as a searchable database using freeware.

- *Action: enquire of Kent Vliet has a current list that he keeps updated, so perhaps he could provide a list in some searchable format. Cathy/Paolo to approach Kent re this.*

10. Other business
    - A guideline on how to recognize visual signs of stress was suggested, in terms of animal welfare, was suggested.
      - Jan Myburg advised that their group is starting to concentrate on stress, developing better assays, etc. (eg faecal corticosterone measurement), so in the next few years, there will be a lot more information
    - Major issues that need addressing are over-use of antimicrobials on croc farms and another issue is waste management on crocodile farms.
      - The recently released Best Management Practices for Crocodile Farming only deals with this superficially.
      - *Action: Gerry Swan volunteered to review the document and summarize existing information to propose to add to the BMP manual. (To be sent around to the vet group for comment once compiled).*
    - There is a crocodile farm veterinarian job in northern Australia being advertised. [*Action: Paolo to send around advert to the Veterinary Science Group for dispersal*].

Addendum: Draft of crocodilian health assessment for release protocol and form (sent with this Agenda as separate document)

Appendix: Summary of Most Important Conditions of Farmed Crocodilians, as of 15 May 2016

- Survey sent to 25 people (CSG Vet Group plus a few others closely allied with Veterinary Science Group)
- Survey was an Excel sheet with the following columns (headings in blue were drop-down lists): Name of reporter, Role with crocodilians, County, Province/region, Species, Age, Condition, Reason for importance, Level of importance, Comment
- 7 respondents: Australia (3), South Africa (2), Cambodia (1), PNG and Bangladesh (1)
- Respondents produced a total of 58 entries, 27 of which were rated as "most" or "very" important:
  - Septicaemia in various forms (6)
  - Skin issues (lucencies, poxvirus, dermatitis) (6)
  - Eye issues (conjunctivitis, red eye, Chlamydia) (5)
  - Runting, poor yolk absorption/infection, embryo death (2 for each)
  - Fungal infection, Salmonella in meat, herpesvirus, dilation of pupil (1 of each)

Age Class	Entries	Condition	Entries
Hatchlings			
1-4 months	15	Eye issues (conjunctivitis/hepatitis, conjunctivitis, "red eye", dilated pupil)	5
		Septicaemia (various forms)	3
		Other misc. single entries	7
Juveniles (4-12 mth)	15	Septicaemia (various forms)	3
		Runting	3
		Conjunctivitis/hepatitis	2
		Poxvirus	2
		Other misc. single entries	5
Juveniles (2-4 y)	12	Skin related (lucencies, pix, scars, wrinkles, ulcers, infectious)	9
		Other misc. single entries	3
Hatchlings (<1 mth)	5	Retained/infected yolk sac	3
		Other misc. single entries	2
Juveniles (1-2 y)	5	Misc. single entries	5
Eggs/embryos	3	Embryo death	2
		Misc. single entry	1
Sub-adults (> 4 y)	2	Misc. single entries	2
Breeders	1	Misc. single entry	1

Appendix: Veterinary Science Group Website Resources (www.iucncsg.org/pages/Resources-of-Vet-Science-Group.html)

1.1. Step by Step Guide and Reporting Form for the Necropsy of Crocodilians (English) (Dr. Paolo Martelli and Dr. Fritz Huchzermeyer)

1.2. Guia Paso por Paso y Formato de Reporte para la Necropsia de Crocodilianos (Español) (Dr. Paolo Martelli and Dr. Fritz Huchzermeyer; translated by Luis Sigler)

1.3. Manuel Operatoire et Formulaire pour la Realisation d'Autposies de Crocodiliens (Francais) (Dr. Paolo Martelli and Dr. Fritz Huchzermeye; translated by Dr. Samuel Martin)

2. Literature relating to crocodilian immobilization (compiled by Dr. Annabelle Olsson)

3. Manual for Parasite Collection and Preservation (Dr. Marisa Tellez)

4. A Checklist of Host-Parasite Interactions of the Order Crocodylia (Dr. Marisa Tellez; University of California Press, Berkeley). Dr. Tellez has also provided a Crocodile-Parasite Data Sheet (Excel format) that summarises current knowledge on crocodile-parasite interactions for different species. Literature cited in this document are available in "A Checklist of Host-Parasite Interactions of the Order Crocodylia".

5. Link to the Histopathology site (Spectrum WebViewer) Click here for a list of available tissues.

6. Crocodilian Laboratory Diagnostics and Necropsy Reference List (compiled by Dr. Cathy Shilton)

7. Crocodilian Clinical Laboratory Diagnostics Overview: Haematology and Clinical Biochemistry (Dr. Cathy Shilton)

8. Crocodile Necropsy: Procedure and Appearance of Tissues (Saltwater Crocodile *Crocodylus porosus*) (Dr. Cathy Shilton)

9. Crocodile Necropsy: Examples of Lesions in Farmed Saltwater (*Crocodylus porosus*) and Freshwater (*C. johnstoni*) Crocodiles (Dr. Cathy Shilton

10. Biosecurity: A Brief Introduction (Dr. Cathy Shilton)

11. Necropsy Recognition of Disease in Hatchlings (Dr. Cathy Shilton)

12. Diagnosing Disease in Live Crocodiles and Treatment Guidelines (Dr. Paolo Martelli)

13. Euthanasia/Killing Methods for Crocodilians

14. Sampling Tips for Various Types of Ancillary Necropsy Testing (from CSG Veterinary Workshop, 2016)

15. Immobilization of Crocodiles (Dr. Silke Pfitzer, Dr. Samuel Martin and Dr. Paolo Martelli) (from CSG Veterinary Workshop, 2016)

16. Necropsy Basics and Taking the Best Samples (Dr. Cathy Shilton) (from CSG Veterinary Workshop, 2016).

17. Live Blood and Tissue Sampling Techniques (from CSG Veterinary Workshop, 2016).

18. Ultrasound - a Useful Tool for Health Assessment in Crocodilians (Dr. Paolo Martelli and Dr. Brian Chin Wing Kot) (from CSG Veterinary Workshop, 2016).

19. Transmitter Attachment and Monitoring - Appropriate Techniques and Technologies (Dr. Xander Combrink, Dr. Jonathan Warner and Dr. Danie Pienaar) (from CSG Veterinary Workshop, 2016).

20. Marking: Tags, Natural Tail Marks and Scute Clipping (Dr. Xander Combrink, Dr. Jonathan Warner and Dr. Danie Pienaar) (from CSG Veterinary Workshop, 2016).

21. Parasite Drawings: The following drawings, drawn by Dr. Ralph Appy, were submitted by Dr. Marisa Tellez.

Future Leaders Working Group

Report of activities during the 24th CSG Working Meeting (18-19, 23 May 2016)

The Future Leaders Working Group (FLWG) of the CSG made its debut at the Working Meeting in Skukuza, South Africa. To best launch this initiative we held two main events. Prior to the principal working meeting, on 18-19 May, we held a 2-day leadership workshop which brought together 8 FLWG members (Matthew Shirley, Sally Isberg, Matt Brien, Sergio Balaguera-Reina, Laura Porras Murrillo, Rainier Manolo, Marisa Tellez, Pablo Siroski) with 9 senior CSG leaders (Tom Dacey, Grahame Webb, Charlie Manolis, Kent Vliet, Perran Ross, Alvaro Velasco, John Caldwell, Allan Woodward, Phil Wilkinson) to discuss the past, present and future of the CSG. We additionally held a side event the evening of 23 May, open to all FLWG members, and any individual interested in participating in the FLWG. Here we summarize these events and discussions that took place on future directions of the FLWG.

FLWG Leadership Workshop

This workshop was suggested by the future leaders interested in the continuity and future of the CSG and approved by the Executive Committee. They identified the need to understand the mechanics of the group and the past, present, and future of its operational goals to begin the process of taking leadership roles over time. Over the course of the workshop we broke out into small discussion groups and identified the following:

Strengths of the CSG

- Diversity of membership (eg age, gender, expertise)
- Culture
  - Accessibility of networking
  - Welcoming environment
  - Openness of distinguished and expert members to mentor the future generation
  - Commitment to moving forward in crocodile conservation and political management
- Innovation, flexibility and adaptive management
- Institutional memory
  - Conservation and management of other wildlife. Can use that experience to improve tasks or management of crocodiles)
  - History of the CSG
  - How the politics of the IUCN and CITES work
- Credibility, Confidence
- Communication and networking
  - Collaborative amongst each other
  - Easily accessible for communication

Weaknesses of the CSG

- Financial unsustainability, need for financial support
  - Need to consider hiring on a CFO, development officer, or foundation director?
  - Need to consider running like a business. Sell items, not necessarily crocodile-related.
  - Need to continue to develop IACS for long-term sustainability.
- Inability to force compliance from member countries
- Lack of diversity of regional membership
  - For example, there are a lot of US members, but not many Central American or West/Central African members
- Lack of continuity of communication

Central to the discussions during this workshop were how best to implement the FLWG to ensure the transference of the capacity and biopolitical acumen. One idea presented was for the CSG Executive to, where appropriate, farm out tasks such as letter writing, communications, reporting, missions, etc., to members of the FLWG. Products produced by FLWG members would predominantly be drafts and such to be reviewed by senior leaders before being presented to their final audience. This would have the duel purpose of reducing the Executive workload, though it would require considerable commitment from the Executive to implement.

A second proposal was the need to ensure aspiring leader presence at CITES Conferences of Parties, Animal Committee meetings, and Steering Committee meetings, as well as IUCN events like the World Conservation Congress that happens every 4 years. While everyone acknowledged this would be a critical step, it will obviously require a certain level of financing that may or may not always be available.

FLWG Side Event (23 May)

The core FLWG group had a joint meeting with the full membership, as well as other working meeting participants who were considering joining the initiative, to discuss the mission, expectations and future of the FLWG. In total, 43 people attended the meeting. Matt Shirley opened the meeting with a brief background of the creation of the FLWG, its mission, and gave a brief summary of the FLWG workshop that occurred the previous week with distinguished members of the CSG. The principal points discussed during the meeting among the distinguished members and FLWG were as follows:

- Provide representation and funding that allows FLWG members to attend IUCN or CITES meetings
- Continual mentorship: osmosis of institutional memory and training in CITES and IUCN politics
  - Future leaders must reach out to distinguished members and vice versa
- Set up some continuous funding source (business, non-profit, etc.)
  - Letters of support for travel funds to assist in FLWG travel
- Encourage or emphasize work in non-commercial species among all CSG members

During the second portion of the meeting, we opened the floor to interested members of the FLWG to discuss their expectations and the steps needed to be taken to ensure the success of this group. The following is a summary of proposed ideas and suggestions:

- As a background source on how the IUCN and CITES works, to read Belly of the Beast
- Set up a Facebook, or other discussion/social network, page as a source to communicate and keep updated with projects and ideas. *Pablo Siroski volunteered to create FLWG Facebook Page, which is now established and ready to launch for its debut among FLWG members and interested individuals.*
- It was suggested that overall, CSG lags behind in utilizing technology to foster a network of communication amongst each other, such as not having a strong on-line presence on Facebook or other web outlets. Thus, besides a Facebook Page, a FLWG page on the CSG website would be to created as another means of resources and communication amongst each other.
- To understand the diverse backgrounds of FLWG members, Matt Brien agreed to create a form (similar to the PECP group form) that can assist members in connecting with others with similar interests, etc. *In addition to that, a page listing people's region of origin as well as background/expertise should be created (similar to the Veterinary Science Group's page). This project has not been delegated at the moment.*
- For future meetings, we need to connect with the Industry Vice Chair to inquire his input on the industry's expectations of the future of the CSG.

One subject that was not discussed due to time, was the possibility of funding 1 or 2 students who were awarded the SRAS grant to a CSG meeting. We will be circulating the discussion on how to implement such funding among the interested parties.

We additionally discussed the issues of the Junior CSG and ultimately feel that this does not fall under the interests of the FLWG. We recommend that this group is nestled within the PECP group of the CSG.

We are in the process of evaluating how to expand our membership and ensure that the FLWG successfully reaches its goals for all levels of group membership. And, in addition, we are strategizing how to tackle the issue of FLWG participation for non-CSG members.

Zoos Group

1. The Zoos Thematic Group meeting had difficulties with space availability which hindered our meeting. The room reserved for the meeting was occupied, and we ended up using the main meeting hall but this became too noisy to continue due to the poster session. The meeting was thus foreshortened.

2. That said, the meeting was very well attended, with 28 participants, from 10 countries, and representing perhaps 10% of all of the working meeting participants.

3. We continue to add zoo professionals to the CSG membership and this has greatly increased the communication, cooperation and coordination of crocodilian efforts between the CSG and zoological institutions and associations.

4. As we attempted to do in the Symposium "The impact of zoos on crocodilian conservation" held at the 2014 meeting in Lake Charles, we need to continue to document the financial contributions zoos make to crocodilian conservation.

5. We again discussed the fact that we have quite unequal representation of zoos and zoo thematic group membership across geographic regions. We have very weak representation in Africa, and are also relatively weak in Asia and Latin America. The group is asking for suggestions for other zoo contacts to build a greater range of communication and to highlight what more zoos are doing for crocodiles.

6. We recognized the passing of our valued colleague Ralf Sommerlad. Ralf acted as an unofficial but highly valued advisor to the Vice Chair of the Zoos Thematic Group for many years. Fabian Schmidt (Zoo Zurich) has stepped forward to fill this role in representing European zoos and colleagues.

7. Colin Stevenson (Crocodiles of the World) discussed World Tomistoma Day, to be held on 5 August, in honor of Ralf Sommerlad's birthday. The goal is to get as many zoos involved as possible and to raise awareness for Tomistoma, and to hold fundraisers.

8. Julian Medrano (Vivarium del Caribe) asked if there is a database of all the individuals of all the species of crocodilians in zoos and other institutions? The answer is Yes and No. There is ZIMS (www.zims.isis.org), a private company that provides animal record software for many zoos. Recently changed its name to Species360 (www.species360.org). Not all zoos are members. Member institutions are mostly from American Association of Zoos and Aquariums (AZA), the Australian association, and the European Association of Zoos and Aquariums (EAZA). The southern hemisphere's representation is low and the collection is not entirely global.

9. We continue to experience difficulty in moving animals between different countries, because of permits and limitations. This hinders many cooperative breeding programs, for example, Fabian Schmidt with the three species of *Osteolaemus*.

10. The CSG Newsletter is released four times a year, and it would be ideal if zoos can contribute more under the zoo by-line.

Prepared by: Kent A. Vliet

IUCN/Species Survival Commission

The Species Survival Commission (SSC) is one of six volunteer commissions of the IUCN, a union of sovereign states, government agencies and non-government organisations. IUCN aims to mobilize the communities working for biodiversity conservation, sustainable development and poverty reduction in common efforts to halt biodiversity loss and apply nature-based solutions to global challenges. This is achieved through three key priorities areas of work: valuing and conserving nature, fair and effective governance of nature's use and nature-based solutions to global challenges.

The current mission of the SSC is to continue to play a leading role in enabling IUCN to be the world's most authoritative voice on behalf of global biodiversity conservation and the sustainability of natural resource use. As a volunteer network comprised of some 7500 scientists, field researchers, government officials and conservation leaders from 188 countries, the SSC membership is an unmatched source of biological information about biological diversity and its conservation. SSC members provide technical and scientific counsel for conservation projects throughout the world and serves as resources to governments, international conventions, and conservation organisations and support the implementation of multilateral environmental agreements.

Working in close association with IUCN's Global Species Programme, SSC's major role is to provide information to IUCN on biodiversity conservation, the inherent value of species, their role in ecosystem health and functioning, the provision of ecosystem services, and their support to human livelihoods. This information is fed into The IUCN Red List of Threatened Species.

IUCN

SSC
Species Survival Commission