# CROCODILE

# **SPECIALIST**

# GROUP

# NEWSLETTER

VOLUME 36 No. 4 • OCTOBER 2017 - DECEMBER 2017



IUCN • Species Survival Commission

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IUCN - Species Survival Commission

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COVER PHOTOGRAPH: West Nile crocodile (*Crocodylus suchus*) in Awash National Park, Ethiopia (see pages 24-27). Photograph: Ludwig Siege.

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**Big Bull Crocs!** (\$15,000 or more annually or in aggregate donations)

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# Editorial

A close friend, Akira Saikyo, passed away on 7 November 2017, at 87 years of age. He was a very influential member of the Japanese leather industry, and played a vital role in helping the industry adjust to post-CITES regulations, often with CSG involvement. He was the Chair of Toyo Hikaku Tanning Co., Ltd., and participated in many CSG working meetings and CITES COPs (from CoP5, Buenos Aires 1985 to CoP13, Bangkok 2004) representing the Japanese reptile leather industry. He supported the establishment of the Singapore Reptile Skin Trade Association (SRSTA) and the Asian Conservation and Sustainable Use Group (ACSUG), and was a key player in Japan's program that developed crocodile farming in the Philippines. He devoted the last 40 years of his life to promoting conservation and sustainable use of wildlife. Saikyo-san was a wise and skilled leader, much respected inside and outside Japan. See obituary on page 4.

With the assistance of the IUCN-SSC, the CSG has entered into a collaboration with Albuquerque Biopark on the development of "CSG Conservation Priorities for World Crocodilians'. This document will be based upon the current review of CSG Species Action Plans and will draw heavily on the CSG membership to document conservation species priorities at national, regional and international levels. The goal is for the CSG to develop an evidence-based process, for prioritising actions, that in turn can be used by members seeking project support from organisations and agencies.

I recently provided a Foreword to "Muggers of Vadodara", conceived and compiled by Raju Vyas (CSG Regional Vice Chair, India) and supported by the Voluntary Nature

Conservancy. The book covers the urban Mugger crocodiles (*Crocodylus palustris*) in Vadodara City, Gujarat, India, and makes very interesting reading. It is now available, and details are on the homepage of the CSG website (www.iucncsg.org).

Simon Pooley has produced two posters entitled 'Don't get eaten by a croc' which are aimed at locals (especially children) and visitors to areas of South Africa, Swaziland and India (Gujarat) where humans live alongside crocodiles. They are intended to raise awareness in areas where attacks have occurred, so saving lives, human and crocodilian. Details are on the homepage of the CSG website (www.iucncsg.org).

Matthew Shirley (Chair, Future Leaders Working Group) attended the 69th CITES Standing Committee meeting, in Geneva, Switzerland (27 November-1 December 2017). Matters of interest to the CSG included Traceability, and an intersessional working group was established with a specific mandate [SC69 Sum. 6 (Rev. 1)].

The 26th CSG Working Meeting (Santa Fe, Argentina; 7-10 May 2017) is now only 4 months away. It promises to be a wonderful meeting and I urge everyone interested in crocodilian conservation, management and research to attend. The camaraderie that develops at our working meetings is a truly important source of motivation for everyone. Details on registration, accommodation, etc., are available on the meeting website (https://www.25wmcsgsantafe.com)

Professor Grahame Webb, CSG Chair.

#### CSG Student Research Assistance Scheme

The Student Research Assistance Scheme (SRAS) provided funding to 6 students in the October-December 2017 quarter, and 2 further applications are currently under review.

- 1. João Emílio Almeida-Júnior (Brazil): Population assessment of Broad-snouted caiman (*Caiman latirostris*) in an agricultural landscape in the Upper Paranapanema River, São Paulo State, Brazil.
- 2. Agata Staniewicz (UK): Acoustic communication and the use of acoustic tracking methods in population assessment of forest-dwelling crocodiles.
- 3. Shanta Bahadur Magar (Nepal): Population status, distribution and conservation issues of Gharial (*Gavialis gangeticus*) in the Rapti River, Chitwan National Park, Nepal.
- 4. Camdon Bland (South Africa): Effects of protein source fed in an isocaloric and isonitrogenous diet on the growth response of commercial crocodiles (*Crocodylus niloticus*).
- 5. Saul Ortega Oliveros (Venezuela): Optimization and reduction of costs in Orinoco crocodile (*Crocodylus intermedius*) hatcheries in Hato Masaguaral.
- 6. Jessica Castellanos-Labarcena (Cuba): Mitogenomics for the conservation of the genus *Crocodylus* (Crocodylia: Crocodylidae) in Cuba.

Tom Dacey, CSG Executive Officer, (csg@wmi.com.au).

### **Obituary**

### Akira Saikyo (1930-2017)



Akira Saikyo, aged 87, passed away on 7 November 2017. He was Chair of Toyo Hikaku Tanning Co., Ltd., an advisor to the Japan Reptile Leather Industries Association (JRA), and a former Vice-Chair of the Japan Leather and Leather Goods Industries Association (JLIA).

Akira participated in numerous CSG Working Meetings and CITES Conferences of the Parties [CoP5 (Buenos Aires, 1985) to CoP13 (Bangkok, 2004)], representing the Japanese reptile leather industry. He also supported the establishment of the Singapore Reptile Skin Trade Association (SRSTA) in 1987. He devoted the last 40 years of his life to promoting conservation and the sustainable use of wildlife.

In the 1980s, as a top leader of the Japanese reptile leather industry, Akira seriously recognized the fact that proactive contribution to conservation and sustainable use of wildlife was essential for their future. His strong initiative moved the Japanese Government into action. In 1987, the Crocodile Farming Institute, currently known as Palawan Wildlife Rescue and Conservation Center (PWRCC), was founded in Puerto Princesa (Palawan, Philippines) and the Republic of the Philippines-Japan Crocodile Farming Institute Project started. Its two goals were to conserve Saltwater crocodiles (Crocodylus porosus) and Philippine crocodiles (C. mindorensis) in the Philippines and to promote socio-economic well-being of local communities through commercial crocodile farming. It was the first Japanese Official Development Assisstance (ODA) project which clearly stated wildlife conservation as its goal.

PWRCC and people of the Philippines kept their effort to achieve both goals of the project, even after the Japanese cooperation ended in 1994. In 2000 PWRCC started distributing captive-bred *C. porosus* hatchlings of to qualified private companies known as "Cooperators" for commercial crocodile farming. In July 2009, 50 *C. mindorensis* from PWRCC were reintroduced into Dicatian Lake in the Northern Sierra Madre Natural Park on Luzon by the Mabuwaya Foundation. In March 2013, 36 *C. mindorensis*  were reintroduced into Paghongawan Marsh within the Siargao Island Protected Landscape and Seascape, Province of Surigao del Norte by Crocodylus Porosus Philippines Inc. (CPPI).



Figure 1. Ms. Veronica D. De Guzman, then Director of PWRCC, and the late Mr. Saikyo, in February 2014. Photograph: Koichi Fujishiro.

From October 1986 to September 1989, Akira and Jack Cox supported sustainable development of the Indonesian crocodile leather industry through an FAO program called Crocodile Industry Development on Sustainable Basis in Irian Jaya (current West Papua, Indonesia). The program aimed at establishing a crocodile ranching and farming industry in Irian Jaya through activities such as monitoring of the wild crocodile population, providing husbandry techniques, and marketing of crocodile products.

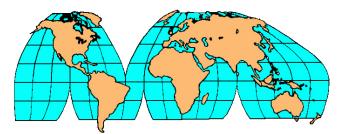
In 1992, Asian wildlife industries established the Asian Conservation and Sustainable Use Group (ACSUG) as an association of academics and industries, at the initiative of Prof, Harry Messel and Akira. ACSUG aimed at promoting the idea of "conservation through sustainable use" by supporting scientific monitoring and research of wildlife in the region. ACSUG consisted of members from Australia, Hong Kong, Indonesia, Japan, Papua New Guinea, Singapore and Thailand, and advisors such as Prof. Harry Messel, Dr. Choo Hoo Giam and Prof. Grahame Webb.



Akira was a strong and thoughtful leader with wide perspective and foresight. He was always surrounded and loved.

Tadatsugu Tanabe (Chair, Japan Reptile Leather Industries Association).

# **Regional Reports**



# **Europe**

# Denmark

SECOND EUROPEAN CROC NETWORKING MEETING. The second European Croc Networking Meeting (ECNM) was held at Krokodille Zoo in Eskilstrup, Denmark, on 6-8 October 2017. This year, there were 60 participants from 13 different countries, surpassing last year's 54 from 10. This was in spite of the ECNM having to compete with large herpetology, conservation, husbandry and leather events in Europe on the same weekend.

Eleven speakers covered topics in husbandry, physiology, sociopolitical complications in crocodilian conservation, and others. We try to cover a wide range of topics and keep the meeting casual to encourage networking between and within the research, husbandry and industry communities. To further facilitate this, and for what the ECNM has become known, we hosted a crocodile speed dating session, allowing for direct interaction between most of the participants. Rene Hedegaard also gave participants a private tour of his zoo where he showcased his conservation efforts and plans for expansion.

From last year's meeting we were able to arrange for some internships and a graduate project. We look forward to see what happens as a result of this ECNM, where everyone felt much more comfortable to look for students, ask for samples and seek advice on their projects, zoos and companies - the goal of this meeting! In 2018 the host of the ECNM will be La Planète des Crocodiles in Civaux, France, for a whole new region and an additional batch of people keen to work with crocodilians.



As always, if you have a project or company where you are willing and able to have volunteers or interns, please reach out to me to promote your project at our meetings and on the

ECNM website (https://ecnm2017dk.wordpress.com/). The idea is to provide information on as many opportunities to gain experience with any aspect of working with crocodilians to the meeting's participants.

Ashley Pearcy (ashley.pearcy@gmail.com).

# Norway

NEW CROCODILIAN DISPLAY AT BJORNEPARKEN, NORWAY. During Easter of 2016 Bjørneparken (Bear Park) in Flå, Norway, unveiled a brand-new exhibit "Krokodilleland" (= Crocodile Land) during the grand seasonal opening. Crocodile Land is a 500 m<sup>2</sup> temporary exhibit on display from 2016 throughout the 2018 season (ending in November). The future plans for the Reptile Department and the crocodilians will be completed and announced during the 2018 fiscal year.

Crocodile Land is the brain child of The Bear Park's director Kees Ekeli and Krokodille Zoo (Denmark) director René Hedegaard. The exhibit was built during the winter of 2015-2016 and houses 25 crocodiles representing 5 species of crocodilian (*Crocodylus acutus*, *C. novaeguineae*, *C. rhombifer*, *Caiman latirostris*, *Alligator mississipiensis*). In the exhibit you will also find several species of snakes, a crocodile monitor lizard and Aldabra tortoises, all of which are also on loan from Krokodille Zoo.

The reception has been overwhelmingly positive with visitors. Norwegians have a limited relationship with reptiles, with only 5 native species mostly residing in the lower latitudes of the country. Seeing these unusual and impressive animals up close clearly creates excitement and joy for our visitors. To increase the impact we have daily snake handling as well as crocodile feeding and species presentations. All are part of an effort to improve the reputation of crocodilians and snakes among Norwegians, who rarely encounter reptiles in the wild. The Bear Park-Krokodille Zoo partnership is based on ideological grounds as well as financial ones. All of the animals have been loaned to The Bear Park in exchange for a donation plan where the park pledges to donate 0.5 Norwegian Kroners (approx. \$US0.06) from every admission to benefit crocodilian research and conservation (including the CSG).





The Bear Park greatly appreciates the partnership that has developed with René Hedegaard, and hope our donations will help further the work being done by the CSG.

Joe Kristoffer Partyka, *Bjørneparken*, *Flå*, *Norway* (www. bjorneparken.no).

# Latin America and the Caribbean

## **Mexico**

THE LEGENDARY "PAPILLON"- AN AMERICAN CROCODILE (*CROCODYLUS ACUTUS*) ICON FOR TABASCO STATE, MEXICO. In 1964 Otto Wolter Peralta of German origin and who lived in Comalcalco, Tabasco, Mexico, captured a 3-m long crocodile with fishing nets in the "La Machona" Lagoon, in the pond of "San Felipe". Due to its size and characteristics, the crocodile was offered to Chapultepec Zoo in Mexico City, but it could not be accepted at that time due to a lack of appropriate enclosures. Mr. Wolter contacted the Governor of Tabasco, Carlos Alberto Madrazo Becerra, and the poet Carlos Pellicer Cámara, who managed to transfer it immediately to Parque Museo "La Venta" in Villahermosa, Tabasco, Mexico.

This crocodile was creating its own legend because it escaped on two occasions from the Park to the "Las Ilusiones" Lagoon. It was recaptured each time, but on the second occasion a local fishermen shot at it with a shotgun that caused the loss of its right eye (the pellet was found during necropsy, inside the eye socket). The crocodile remained in captivity for more than 51 years until 21 January 2014, when he was found dead in his pond at 1400 h.

"Papillon" is considered one of the largest specimens of C. *acutus* reported in captivity and the longest known for Mexico. At capture he was estimated to be around 3 m long and approximately 30 years old - making him around 81 years of age when he died.

When Papillon's death was reported, B. Gabriela Mari Vázquez contacted Jerónimo Domínguez Laso, President of the Civil Association COMAFFAS, requesting his support to carry out the review of the corpse, its physical evaluation, support in carrying out the official necropsy and perform the necessary actions with the support of specialist Pedro López González to preserve this Titan for posterity. Traveling from Chiapas to Tabasco to arrive at 0700 h on 22 January 2014 to begin review of the corpse, which due to the lack of freezer space was wrapped in plastic bags and covered in ice.

Papillon was marked with the single caudal scale cut (600) in 2003 by Jerónimo Domínguez Laso to obtain biological material for DNA analysis - which confirmed a hybrid specimen (Pacheco-Sierra *et al.* 2016). There had been speculation that Papillon was a female, but this was ruled out. He was identified as an American crocodile (*C. acutus*), although Tabasco State is not currently considered in the area of distribution for the species - only for *C. moreletii*. Prior to performing the necropsy, a series of morphometric measurements were recorded:

Nuchal plates	4-4-2
Dorsal osteoderms	16
Double caudal scales	18
Single caudal scales	17
Nasal width	15 cm
Interorbital width	12 cm
Width of external nares	6 cm
Length of right leg	30 cm
Width of right leg	17 cm
Length of right hand	18 cm
Width of right hand	11 cm
Total dorsal length	4.26 m
Total ventral length	4.30 m
Snout-vent length	2.25 m
Cloaca-tail length	2.05 m
Weight	500 kg
Width of abdomen at chest height	0.60 m
Total cranial length	0.60 m
Snout length	0.40 m
Cranial width	0.37 m
Snout width	0.25 m
Maxillary width	0.22 m
Cranial diameter (snout level)	0.80 m
Cranial diameter (mandible level)	1.03 m
Diameter at neck height	1.17 m

Approximately 150 measurements were taken to assist with



the taxidermy of Papillon's body. The official necropsy was performed by Jerónimo Domínguez Laso and DVM Juan José Carrera Flores (Clinical Manager of Parque Museo "La Venta"). Death was caused by bronchial respiratory problems, as the lungs were in an advanced degenerative state and collapsed, full of granulomas and tumors; the other organs were in apparent healthy conditions.

Taxidermy took approximately 20 full days and more than 300 hours of physical work of the team COMAFFAS AC Chiapas, concluding in an exceptional work that was installed in a special room with the name "Sala Papillón" of Parque Museo "La Venta", inaugurated by the State Governor Arturo Núñez on 17 February 2014. The exhibition has immortalized this unique specimen ("El Papillón") as an icon for the people of Tabasco and Mexico.

A video is available at: https://www.youtube.com/ watch?v=gFV28ttDuog.

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Pacheco-Sierra, G., Gompert, Z., Domínguez-Laso, J. and Vázquez-Domínguez, E. (2016). Genetic and morphological evidence of a geographically widespread hybrid zone between two crocodile species, *Crocodylus* acutus and *Crocodylus moreletii*. Molecular Ecology 25(14): 3484-3498.

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Jerónimo Domínguez Laso (*President, COMAFFAS AC, Chiapas, Mexico; jeroxdl@yahoo.com.mx*), Pedro López González (*Taxidermy Specialist, Collaborator of COMAFFAS AC*) and Berenice García Reyes (*Technical Secretary, COMAFFAS AC, Chiapas, Mexico*).

# Venezuela

NATIONAL CENSUS OF ORINOCO CROCODILE (*CROCODYLUS INTERMEDIUS*) IN VENEZUELA. With the financial support of the CSG, CrocFest and Río Verde (Venezuela), in November 2016 members of the Crocodile Specialist Group of Venezuela (GECV) began fieldwork on the National Census of Orinoco Crocodile in Venezuela, that will continue until the end of the 2018 dry season.

The main goal of this project is to establish and update the current status of *Crocodylus intermedius* in the country, through population censuses in localities in which its presence is actually known, areas where specimens have been released

through the captive breeding program, and other localities reported from bibliographic references in the past but where the presence of the species has not been corroborated in years. This knowledge will allow us to prioritize and start new conservation efforts.

The localities selected to visit were based on the national censuses performed by Godshalk and Sosa (1978) and Thorbjarnarson and Hernandez (1992), and the recent assessment of Balaguera-Reina *et al.* (2017) who analyzed priority areas for conservation of the species.

Based on these sources we selected 27 rivers to monitor population status, and by November 2017 we had carried out surveys in 10 of them. In these areas the presence/absence was confirmed, and the abundance and size structure of the population was estimated, as well as a general characterization of the habitat where crocodiles were observed. In some localities, depending on the date, an additional survey of nest and hatchling pods was performed to estimate the female reproductive population.

The main problems facing the development of this project have been the difficulties in obtaining food supplies for field trips, little/no availability of 4-wheel drive vehicles for rent, and serious public security concerns in some areas. This last factor has affected carrying out of surveys in tributaries of main rivers in border areas between Colombia and Venezuela, such as the Capanaparo and Arauca.

Alvaro Velasco (Fauna Silvestre productos y servicios; velascocaiman@gmail.com), Omar Hernández (FUDECI) and Ricardo Babarro (Crocodile Specialist Group of Venezuela).

SECOND VENEZUELAN SYMPOSIUM ON ECOLOGY AND CONSERVATION OF CROCODYLIA. On 29 November 2017, within the celebration of the first centenary of the Academy of Physical, Mathematical and Natural Sciences of Venezuela, the "Second Venezuelan Symposium on Ecology and Conservation of Crocodylia" was held in Caracas.

Fifty-one participants, including researchers from Colombia and Venezuela, participated in the symposium, sharing the latest unpublished research on the conservation of the Orinoco (*Crocodylus intermedius*) and American (*C. acutus*) crocodiles. Twenty-two original works and current research advances were presented, relating to the history of commercial hunting, conservation efforts, captive breeding, bacteriology, ecology and habitat use, professional and technical training, conflicts, and environmental education of local communities living with these species.

Two topics were particularly novel, from the traditional Venezuelan viewpoint, within a comprehensive crocodile conservation policy in Venezuela. The first was the promotion by D.O.C. Restaurant (http://www.doc-restaurant.com/) of the market of gourmet dishes based on fresh meat of wild *Caiman* 

*crocodilus*, opposed to the traditional and generalized use of meat preserved in salt, which ostensibly reduces its value as an economic resource. The second was the program of tourist trips carried out by Rio Verde, a publishing and audiovisual company (http://www.rioverde.com.ve), in order to publicize the conservation program of the Orinoco crocodile as an emblematic species of the Llanos bringing direct economic benefits to local people who cohabitate with this critically threatened species.

All of the presentations will be published in the next Bulletin of the Academy of Physical, Mathematical and Natural Sciences (in Spanish with English abstracts) and will be available in PDF format.

At the end of the symposium the Academy appointed the Crocodile Specialist Group of Venezuela as a member of its Technical Advisory Commission in Environment, in recognition of its work of more than 30 years in the conservation and sustainable use of crocodilians in Venezuela.



Crocodile Specialist Group of Venezuela (gecvenezuela@ gmail.com).

## Cuba

A DECADE OF WORK ON THE CONSERVATION OF THE CRITICALLY ENDANGERED CUBAN CROCODILE (*CROCODYLUS RHOMBIFER*) AT THE MOST IMPORTANT CUBAN CAPTIVE BREEDING FACILITY. The captive breeding of *Crocodylus rhombifer* is a key strategy for the conservation of this endemic and critically endangered species in Cuba. Among the three captive breeding facilities operated by the Cuban National Enterprise for the Protection of Flora and Fauna (ENPFF), the Zapata Swamp Crocodile Breeding Farm (ZSCBF) stands out as the most important in the country. Here we update the information on the management of the captive population at the ZSCBF and its contribution to Cuban crocodile conservation; precisely a decade after the interchange accomplished with members of the CSG in Zapata Swamp. The goals at that time were to know the conservation status of C. *rhombifer* and to look for alternatives to support Cuban researchers on conservation topics. In that respect, we would like to take this opportunity to summarize a decade of work on the conservation of C. *rhombifer*, an icon of the Cuban herpetofauna (Fig. 1).



Figure 1. Cuban crocodile, Crocodylus rhombifer.

Over the last few years, our efforts at ZSCBF have focused on understanding the biology of *C. rhombifer*. Our studies have been developed as part of broader collaborations with national and international institutions and involving both undergraduate and postgraduate students. The results generated so far have been published in peer-reviewed scientific journals (eg Purón Guzmeli *et al.* 2014; Morera *et al.* 2015; Milián-García *et al.* 2014, 2016).



Figure 2. Reintroduction of C. rhombifer in Zapata Swamp.

Also, and more importantly, the information obtained has been the basis of the reintroduction program of C. *rhombifer* (Fig. 2) and project entitled "Increase of the Genetic Diversity in the Zapata Swamp Captive Breeding Facility and the Refuge of Fauna 'Canales de Hanábana'". Likewise, the results of the aforementioned study on the reproductive behaviour of *C. rhombifer* and the genetic characterization of the genus *Crocodylus* (Crocodilya: Crocodylidae) in Cuba have been integrated into the conservation management plans of the facility. In that respect, it represents the first on-island captive breeding facility operated by the ENPFF integrating genetic information into their management plans.

Considering that the management of the population in captivity is one of our main efforts, we have the responsibility to maintain a healthy population based on the operation plans and these are modified according to the needs of the ENPFF. These plans include long-term strategies of the replacement, sacrifice and reintroduction/recovery into the wild. At present, the size of the captive *C. rhombifer* population at ZSCBF is around 4910 individuals [485 hatchlings, 4108 juveniles, 91 sub-adults, 226 adults (191F, 35M)]. This population size is possible thanks to the incubation of an average of 69 nests per year and a survival rate of 10 hatchlings per nest.

Currently, one of the key goals of the work in which we spend more time is an increase of the present distribution area of the Cuban crocodile. In 2016, a multidisciplinary group comprising specialists from different institutions and after several years of work, agreed to carry on the first reintroduction/recovery of the Cuban crocodile into the wild in Zapata Swamp (Fig. 2). We are currently evaluating all the information we have on the distribution and habitat selection of the released group and we hope to use this knowledge for future releases. In addition to this, individuals bred at ZSCBF are used to reinforce the population at the Cayo Potrero Captive Breeding Facility. The latter is the second most important captive breeding facility for C. rhombifer. Also, captive individuals from ZSCBF are used to increase the populations of zoos and aquariums in Cuba and more recently to found a new remote captive facility- "The Gabina" - in Granma Province.

Monitoring of the wild populations is also an important line of work. These surveys are generally difficult to accomplish with the desired regularity due to the complexity and difficult access to the habitat, as well as the lack of resources for field expeditions. However, thanks to internal and offshore financing, we have been able to monitor crocodile populations in different areas of Zapata Swamp, to reach basic goals like determining the number of individuals there, as well as exploring key ecology aspects that have an impact on the selection and use of habitat. An example is the project entitled "Evaluation of the Potential Impacts of the Climatic Change on the Biodiversity and Development of Strategies of Adaptation in Two Regions of Fragile Ecosystems of Cuba", which has financed the sampling of an extensive zone of the southern coast of Zapata Swamp.

Looking at the future, our work will focus on aspects related to habitat selection of the genus *Crocodylus* in Zapata Swamp (effect of interspecific competition), sex determination (temperature effect) in *C. rhombifer*, ecology of reproduction (fertility and energy involved), physical condition, and conservation genomics of C. *rhombifer* in Cuba. In this sense, the recent V International Workshop of Cuban Crocodiles, was an important space to develop ideas about future collaborations in research on crocodile conservation in Cuba.

#### Acknowledgments

We are deeply grateful to the crocodile specialists and workers of the Zapata Swamp Captive Breeding Facility, for their valuable contribution for more than 55 years to the conservation of the Cuban crocodile. Special thanks to all the students involved along these years in the conservation projects of Cuban crocodiles.

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# **East and Southeast Asia**

# Indonesia

SUMATRA CROCODILE WORKSHOP. A training workshop on the medical management, conflict management and conservation of crocodiles with special focus on *Crocodylus porosus* and *Tomistoma schlegelii* was held in Medan, Indonesia, on 24-27 November 2017. The workshop was organized by BKSDA Aceh together with the Center for Wildlife Studies at the Veterinary Faculty of the Syiah Kuala University Banda Aceh. The CSG's Veterinary Science Group and Tomistoma Task Force (CSG-TTF) worked together along with the workshop organizers to recruit presenters and establish the lecture and practical sessions.

Funding for the workshop was provided by Syiah Kuala University Veterinary Faculty's Center for Wildlife Studies and the CSG-TTF, with support from the Phoenix Herpetological Society, Singapore Zoological Gardens and Krokodýlí ZOO Protivíně. The classroom and practical sessions of the workshop were presented by Dr. Paolo Martelli, Bruce Shwedick, Joe Wasilewski, Dr. Adam Britton, Dadang Suryana, S. Hut. T. and Dr. Christopher Stremme. Lectures and practical sessions were conducted in both English and Bahasa Indonesian.

The presentations included an overview of the crocodilians, an introduction to the CSG, the Tomistoma Task Force and the CSG's Student Research Assistance Scheme (SRAS), introduction to Human-Crocodile Conflict and Human-Crocodile Conflict management within Indonesia, an example of crocodile habitat management and introduction to field techniques for monitoring wild crocodile populations, fundamentals of crocodile biology, fundamentals of captive management, crocodile handling (manual and chemical restraint), diseases, clinical techniques and sampling for health assessment, nutritional, metabolic and infectious disease diagnosis, prevention and treatment. Participants were provided an opportunity to share case histories, experiences and for Q&A. The two days of lectures concluded with an introduction to the CSG Step by Step Guide and Reporting Form for the Necropsy of Crocodilians.

The workshop participants represented the following organizations: BKSDA Aceh; BKSDA Kalimantan Barat; BKSDA Kalimantan Timur; BKSDA Nusa Tenggara Timur; BKSDA Sumatera Utara; Yayasan Inisiasi Alam Rehabilitasi Indonesia; Veterinary Faculty Syiah Kuala University Banda Aceh; Berbak National Park; Danau Sentarum National Park; People's Resources and Conservation Foundation; Orangutan Information Center; Sumatra Orangutan Conservation Program; and, PT Pal Crocodile Farm.

Over the following two days, practical training was conducted at Taman Buaya Asam Kumbang Medan (Medan Crocodile Park) with the assistance of Joey Heung, a veterinary nurse from Hong Kong Ocean Park and Medan Crocodile Park staff members. The workshop's 18 participants were divided into four groups and each group participated in the capture and handling of 4 *C. porosus* ranging from 1.8 to 2.2 m in length, each group under the direct supervision of at least one of the workshop presenters. The workshop participants were given instruction in techniques for external physical examination, collecting morphometric data, sex determination and collecting blood samples. Two female Tomistoma, 2.8 m in length, were also handled in order for the participants to repeat the above mentioned procedures with *C. porosus* (Fig. 1). A demonstration of ultrasound techniques (Fig. 2) was also performed and then practiced by participants on both *T. schlegelii* and *C. porosus*. Tail scute clipping and fitting of a GPS unit was also demonstrated.



Figure 1. Participants practising crocodile capture techniques with supervision and assistance from Dadang Suryana. Photograph: Adam Britton.



Figure 2. Drs. Paolo Martelli and Christopher Stremme demonstrating ultrasound procedure on a female Tomistoma. Photograph: Adam Britton.

The practical sessions concluded with participants examining juvenile crocodiles, observing techniques for the humane euthanasia of crocodilians and in performing necropsy procedures on four 1.5 m crocodiles (2 *C. porosus* and 2 *C. novaeguineae*) provided by PT Pal Crocodile Farm while using the CSG Step by Step Necropsy Guide and Reporting Form during the entire process.

Following the workshop two field site visits were conducted at Way Kambas and Berbak National Parks which will assist with the update of the CSG's *Tomistoma schlegelii* Conservation Action Plan. Field reports are in preparation.

The first direct result of the workshop was the creation of an Indonesian crocodile discussion group by the participants, to maintain contact and for networking purposes.

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# South Asia and Iran

### India

GHARIALAND MUGGER IN UPSTREAM TRIBUTARIES OF THE CHAMBAL RIVER, NORTH INDIA. The Gharial Gavialis gangeticus, endemic to the Indian subcontinent, has been classified as Critically Endangered by IUCN on the basis of a precipitous decline in distribution and abundance, as well as only a remnant global population (IUCN 2007). The National Chambal Sanctuary (NCS) in India holds the largest population of Gharial, estimated at >80% of the remaining world's wild Gharial, with much smaller populations in Katerniaghat Wildlife Sanctuary on the Girwa River, in Chitwan National Park (Nepal) on the Narayani River and on the contiguous Gandak River in India, and in Corbett National Park on the Ramganga River. Nesting has been documented in all of these remaining populations, including those in reservoirs (Katerniaghat and Corbett), but >90% of annual nesting recorded globally occurs in the NCS population (Lang 2016). After an initial and slow recovery in the late 1970s-early 1990s, The NCS population has undergone two sharp declines, one in the decade prior to 2005, and the other in 2007-2008 (Sharma and Basu 2004; IUCN 2007; Stevenson 2015), followed presently by evidence of a robust recovery of the only open-river, selfsustaining Gharial population still extant.

The Chambal River is the last remaining unpolluted tributary river in the greater Ganges drainage system, relative to all others (Hussain *et al.* 2011). However, the extent of Gharial distribution and abundance in the upstream tributaries (Parvati, Banas and Kali Sindh) has remained undocumented. The objective of this study was to document Gharial in these upstream Chambal tributaries, prompted in part by anecdotal reports and previous rescue efforts in the Banas River where seasonal flow has been disrupted. The surveys were carried out in 2015-2017, with each survey focused on a specific objective.

#### Study Area

The Chambal is a clear and fast-flowing river that originates from the Vindhya hill range, in central India, lying between 24°55' and 26°50' N, 75°34' and 79°18' E. It flows northeast and joins the Yamuna River to form part of greater Gangetic drainage system. It averages 400 m in width, and 1-26 m in depth (Hussain and Choudhury 1990). A 618-km stretch of the Chambal River, between Jawahar Sagar Dam and Panchhnada, which is protected under NCS, receives water from the mainstream and additionally from three major tributaries, the Parvati, Kali Sindh and Banas Rivers (Hussain 1999), where the main study area lies. The area is within the semi-arid zone of northwestern India at the border of Madhya Pradesh and Rajasthan.

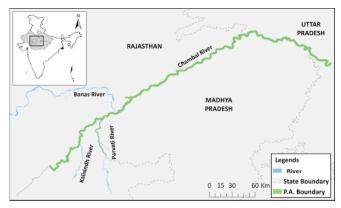


Figure 1. The Banas, Kalisindh, and Parvati Rivers flow into the Chambal River in its upstream (lower left on this map); river flow is from lower left to upper right. The NCS includes 618 km between Jawahar Sagar Dam and Panchhnada (on Yamuna, 10 km downstream from Chambal confluence). On the Parvati River, ~60 km upstream from Chambal confluence is protected; the other two rivers are outside the NCS.

- a. The Parvati River (~159 km in length) originates in the northern slopes of the Vindhyan hills in Madhya Pradesh and enters Rajasthan near Chatarpura village in Baran District, where it forms the boundary between Madhya Pradesh and Rajasthan for about 18 km, then flows for about 83 km in Rajasthan before again forming the boundary between Madhya Pradesh and Rajasthan for a length of about 58 km up to Pali village in Kota District, where it joins the Chambal at 25° 54' N, 76° 33' E (Sharma 1979). It is a continuous flowing river having a unique ravine ecosystem consisting of sand banks, rocks and boulders spread along the banks in entire survey stretch. It is heavily interspersed by marsh beds forming a diversified habitat for variety of flora (Singh and Shrivastava 2007; Chorghe *et al.* 2011).
- b. The Kali Sindh River (~145 km) originates from Bagli (District Dewas) in Madhya Pradesh and is fed by the Ahu, Niwaj and Parwan Rivers. The river enters in Rajasthan in Binda village and flows down further to the north through Baran and Jhalawar Districts. The river then joins the Chambal River at 25° 32' N, 76°16' E to the downstream of Kota District in Rajasthan. The total distance covered by the Kali Sindh River in Rajasthan is 145 km (Sharma 1979). The banks of the Kali Sindh River are primarily dry pebbles, coarse gravel and rocks; consequently, the banks are hard, rocky and barren. Small interspersed sandy portions are usually invaded by thin bushy growth of inferior species and regeneration is negligible.

c. The Banas River (~512 km) originates in the Khamnor Hills of the Aravalli Range, about 5 km from Kumbhalgarh in Rajsamand District. It flows northeast through the Mewar region of Rajasthan, and meets the Chambal at 25° 54' N76° 16' E, near the village of Rameshwar in Sawai Madhopur District (Sharma 1979). The Banas River has dried out since the Bisalpur Dam was completed in 1999. It flows only when surplus water is released from the dam and during the lean period it is reduced to a few deep pools with little or no flow between them.

The Chambal valley of Parvati and Banas confluences is ravine thorn forest and eroded riverbanks and adjacent ravine lands have sparse ground cover (Champion and Seth 1968). Climatically the region lies within semi-arid and sub-humid region of India. Moderate rainfall, high temperatures, dry summers and cold winters are the main features of climate. The landscape either is flattened for agriculture purpose or is invaded by thickets of *Prosopis juliflora* (Sharma 1979).

#### Methodology

The 2015 survey was conducted on all three tributaries, to assess the potential of each tributary for the presence of either Gharial and/or Mugger. In winter (December-February), animals were detected basking, and in summer (June) nests and/or hatchlings were evident. Survey routes were plotted on digital maps of each river. The Parvati and Kali Sindh Rivers were surveyed on foot, and targeted surveys were conducted for the Banas River in the remaining pools. Individuals at the Chambal confluences were excluded, since these areas are included in annual surveys on the main Chambal channel (see Discussion). The downstream stretches of each tributary (Parvati 67 km, Kali Sindh 24 km, Banas 53 km) were surveyed.

Visual observations were aided by Olympus  $10 \times 50$  field binoculars. Garmin eTrex units were used to record GPS coordinates for each sighting. The sex of observed Gharial was also recorded in the survey, classifying them as 'males' based on a clear protuberance at the tip of the snout, females/ sub-adults for large individuals lacking such a protuberance, and juveniles for smaller size classes. The intensity of human activities along the river banks was recorded as well throughout, notably net fishing, water extraction and sand mining.

#### <u>Results</u>

In brief, Gharial and Mugger were present only in the main channel of the Parvati River at any appreciable distance upstream from the confluences of all three rivers. In addition, breeding adults, nests and hatchlings were observed upstream in the Parvati, but nothing comparable was found in the other rivers. The Banas River has records of seasonally isolated individuals; the Kali Sindh River lacked Gharial and had Mugger only a short distance upstream from its confluence with the Chambal. Consequently, the Kali Sindh was excluded after the initial survey, and the Banas was monitored through rescue operations and local reports.

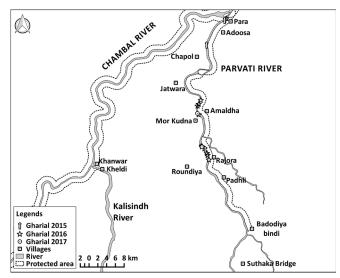


Figure 2. Locations of Gharials encountered on the Parvati River during surveys in 2015, 2016, and 2017. Gharials were observed (Table 1) from the Chambal confluence up to 41 km upstream, but not in the most upstream 26 km section of the 67 km survey upstream. The surveyed Parvati River segment is shown within the dashed lines; Gharial were found up to Roundiya, within the upstream boundary of the NCS at ~60 km.

In the initial 2015 survey along the Parvati River, 14 Gharial were encountered between Pada Ghat and Koth. On the Banas, only one Gharial was observed near Banas bridge, 14 km from the Chambal confluence; and no Gharial were found on the Kali Sindh River. In 2016, 5 Gharials were reported in the Banas River near Aamlideh - 1 was rescued and 4 were noticed alongside by the the Rajasthan Forest Department.

Based on these findings, we focused primarily on the Parvati

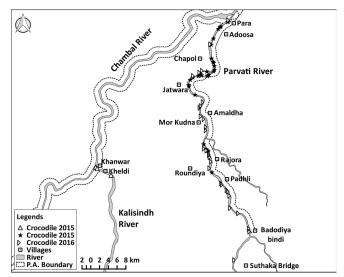


Figure 3. Locations of Muggers encountered in the Parvati and Kalisindh Rivers during surveys in 2015 and 2016. Muggers occurred near the confluence of the Kalisindh River (triangles, 2015), but not upstream. In the Parvati River, Muggers were found at localities along the entire 67 km survey (stars in 2015; right triangle in 2016), from the Chambal confluence upstream. The NCS boundary is at ~60 km, at Badodiya bindi (Table 1).

River in subsequent surveys. In 2016, 29 Gharial (1 male and 28 females/sub-adults) were observed from Pada Ghat to Rondi (~40 km). The presence of a male Gharial at Jind Baba along the Parvati River (~ 38 km upstream; within the NCS) prompted us to investigate signs of nesting. In June 2017 another survey was conducted at Jind Baba and Mor Kudna area (~30 km upstream), in which 1 male and 3 females/ sub-adults along with 203 hatchlings were spotted at Jind Baba (Fig. 4); in addition, 2 females/sub-adults along with

Table 1. Gharial and Mugger encounters, as well as disturbance, on a 67-km stretch of the the Parvati River, 2015-2017. The boundaries of the National Chambal Sanctuary on this river are from ~60 km upstream to the Chambal confluence above localities FK and RG. Hatchlings were detected in 2017 at upstream localities MK (30 km) and JB (38 km) at nest sites. PG= Pada ghat; GD= Gudla; CG= Chapol ghata; GR= Gauri; AD= Amaldha ghata; FG= Fara ghata; MK= Mor kudna; JB= Jind baba; KH= Koth area; FK= Fusod killa; RG= Rajora ghata; RD= Rondi; PJ= Padli joja ka Jind Baba; SB= Suthaka Bridge.

Locations	PG	GD	CG	GR	AD	FG	MK	JB	KH	FK	RG	RD	PJ	SB	Totals
Distance (km)	1	1	8	26	27	28	30	38	38	40	40	41	45	67	67
Gharial counts															
February 2015	3	3	2	0	0	0	0	0	6	0	0	0	0	0	14
February 2016	2	0	0	3	7	2	3	5	1	3	1	2	0	0	29
June 2017					0		44	207							251
Mugger counts															
February 2015	2	1	5	9	2	1	13	17	5	1	8	5	11	3	83
February 2016	2	0	4	7	5	2	1	12	2	3	4	3	13	8	66
Disturbance															
Net fishing	12	10	13	10	0	0	1	0	0	0	0	5	7	13	71
Sand mining	0	0	0	0	0	0	0	2	0	0	0	3	0	1	6
Water pumps	3	15	19	9	13	3	16	13	0	2	3	7	27	34	164

42 hatchlings were sighted at the Mor Kudna area. Based on an average clutch size of 35-45 eggs/nest, these observations indicate the presence of 4-6 nests at Jind Baba, and possibly an additional 1-2 nests at Mor Kudna. The upstream boundary of the NCS on the Parvati is at ~60 km, so the nesting areas lie within the NCS. Gharial distribution and abundance on the Parvati River are shown on Figure 2 and in Table 1.

Mugger distribution and abundance was noted during the Parvati surveys, and is shown in Table 1 and Figure 3. In comparison with Gharial, Mugger numbers were higher, 83 in 2015 and 66 in 2016, than those of Gharial, and the species was distributed throughout the survey section. Concentrations were highest in the midstream area in both years, where Gharial were also most evident in both years. Size class information for Mugger was not recorded, but incidental observation suggest that small sub-adults and juveniles (<2 m total length) were the most frequently encountered Muggers.

Human activities along the Parvati River were noted (Table 1). These consisted of net fishing, sand mining and water extraction. Fishing and agriculture activities were concentrated near the Chambal-Parvati confluence, diminished in the midstream survey section, then resumed in intensity upstream. In marked contrast to the main Chambal channel, sand mining was infrequent throughout the survey area; sand mining along the Chambal channel has intensified significantly in recent years, and poses a major threat to main stream nesting banks. Clearly, water extraction is the main resource removal activity on the Parvati at present, and occurred throughout the river course surveyed, even near the two sites where nesting occurred in 2017. As is evident in Table 1, Gharial presence during breeding and nesting in 2017 coincided in general with the absence of fishing activity, where sand mining was not detected, and where water extraction varied by site.

#### Discussion

The Parvati River lies within the NCS where it reaches its confluence with the Chambal River, and a ~60 km stretch from the confluence upstream is protected. Previously, no records of Gharial using this stretch were available. The present study firmly establishes that this protected stretch of the Parvati River is an important additional segment of Gharial habitat within the NCS, because Gharial utilize this section for breeding, nesting, and hatchling habitats. Human activities are common along this stretch of river, particularly net fishing and water extraction, and pose potential threats to Gharial along the protected Parvati River section, as well as the continuing threats of these activities in the mainstream Chambal. Throughout its entire length inhabited by Gharial, from above Pali to the Yamuna confluence, and the adjacent protected areas upstream and downstream from that point on the Yamuna, these threats are combined in recent years with industrial level sand mining, particularly on the Madhya Pradesh and Rajasthan riversides, upstream.

During seasonally low water periods, the Parvati and the Kali Sindh Rivers are the primary sources of water for the

mainstream Chambal. Three large storage dams (Gandhi Sagar, Jawahar Sagar, and Rana Pratap Sagar) and the Kota Barrage have severely limited water discharge into the mainstream Chambal, with zero water discharge at times during the lean season (Hussain *et al.* 2011). Thus, in addition to its role as a major source of water for the Chambal, the Parvati River lower stretches provide suitable additional river habitats for Gharial.

In contrast, the other two rivers surveyed do not appear to be suitable Gharial habitat. On the Banas River, the Bisalpur Dam completed in 1999 has reduced the river to a few disjointed deep pools, and the river only flows during the monsoon when the dam gates are open. Occasionally, Gharial move upriver during this time, only to be stranded once water levels recede (Taigor 2014). On the Kali Sindh River, the riversides are predominantly rocky, with few sandy areas. Lying outside the NCS boundary, the river has many anthropogenic disturbances in addition to the suboptimal riverside habitat, and consequently harbors few Gharial beyond its confluence with the Chambal. An outlying record exists for the Kuno Wildlife Sanctuary in Madhya Pradesh for a single animal at a distance of 45 km from the confluence of the Kuno River in 2010 (S. Taigor, pers. comm.).



Figure 4. View of Paravati River at the Jind Baba locality (JB on Table 1), looking upstream from the west bank, in June 2017. The open sandy area (center of image) was the nest site where more than 200 hatchlings were detected on the shoreline below. Based on this count, there were likely 4-6 nests at Jind Baba.

Gharials prefer clean, fast flowing rivers with ample sand deposits, especially around sections with deep water (Hussain 2009, 1999; Katdare *et al.* 2011; Stevenson 2015). Recent studies have shown that Gharials undergo seasonal long distance travels, from feeding areas near major confluences during the monsoon, to their basking, breeding, and nesting areas upstream during the post monsoon, winter, and pre monsoon periods (Lang and Whitaker 2010). Female Gharials prefer to nest in colonies on particular sandy stretches that provide high sand banks adjacent to deep water (Lang and Kumar 2013, 2016).

Although preferred areas tend to be consistent over decades,

the exact location of colonial sites, and the numbers of nests per site vary annually, depending on the local river topography produced during the previous monsoon. Traditional river activities by people tend not to deter Gharial from using nearby river habitats, but Gharial generally avoid areas subject to frequent disturbances, especially those where sand mining and net fishing are concentrated. Lateral connectivity in the river is essential because it allows Gharial to seasonally adjust their basking, breeding, and nesting activities to areas where disturbances are minimal (Lang and Kumar 2013, 2016).

Not only does our study highlight the importance of the upstream segments of the NCS, as suitable Gharial habitats and important water sources, but the Gharial counts of breeding adults and nests from the Parvati surveys add to the emerging picture of how many Gharial actually live in the Chambal River, largely within the confines of the NCS. In 2017, independent surveys by the Madhya Pradesh Forest Department and the Gharial Ecology Project, using different methodologies (boat counts vs. stationary counts, respectively), estimated that the mainstream Chambal River, from Pali upstream in Rajasthan to the Yamuna confluence (a river length distance of 415 km), contains a Gharial population >1250 individuals, with conservative counts of 617-761 mature adults (ie 65-121 males and 491-696 females; MPFD 2017; GEP 2017).

In addition, above Pali to the Kota Barrage on the mainstream Chambal, 11 adults were tallied in the top 143 km of the NCS (H. Meena, pers. comm. 2017) We estimate an additional 10-15 mature adults inhabit the stretches of the Parvati and Banas in the areas surveyed in this study. Thus, the total population in the Chambal likely exceeds 1300 non-hatchling Gharial of all sizes, adding an additional 20+ mature adults to those already counted in the Chambal mainstream.

Importantly, our observations in the Parvati add 4-6 nests to the estimated nest tally in 2017 on the Chambal, based on combined surveys on the upstream (WII 2017) and downstream (GEP 2017) main channel of the Chambal. The nesting observed in 2017 was 411 nests along the 415 km stretch of Chambal from Pali (upstream) to Kasaua (downstream) plus 4-6 nests on the Parvati, resulting in a total of ~415+ nests overall, all of these lying within the NCS boundaries.

The fact that Mugger occurred in the lower Parvati that was surveyed may be indicative of the levels of disturbance noted. Some authors have speculated that there has been a gradual increase in Mugger numbers in the NCS, and have suggested that an apparent correlated trend for a decrease in Gharial abundance may be causative (Sharma and Singh 2015). Mugger-Gharial interactions are poorly understood presently and desire further study.

The NCS has well documented issues regarding habitat degradation, in the form of sand mining, fishing, stone mining, river side agriculture, and water extraction projects (Nair 2017). Despite being administrated by three states,

illegal sand mining, fishing and agriculture have shown few signs of subsiding, and sand mining especially has intensified in recent years to an industrial scale, with tractors and trolleys plying 24/7 almost year-round, during all but the 2-3 monsoon months when the river is in full flood. Backhoes are now common on riverbanks (Lang and Kumar 2016).

Recent considerations of river interlinking, particularly on the Parvati and Kali Sindh, need to take into account the devasting effects such projects would have on Gharial specifically, and on the water flow regimes upon which the Chambal fauna and flora depend. All of these anthropogenic activities threaten the entire ecosystem of the NCS, particularly the lateral connectivity of the river at present and the relatively unpolluted quality of the Chambal water.

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EMERGENCE OF A NEW POTENTIAL THREAT TO THE MUGGER (CROCODYLUS PALUSTRIS) POPULATION OF GUJARAT STATE, INDIA. The Mugger crocodile (Crocodylus palustris) is one of most widely distributed species across West Asia, including Iran, Pakistan, India, Bangladesh, Bhutan, Nepal and Sri Lanka (Whitaker and Andrews 2003). Due to their adaptability, Muggers inhabit a variety of natural as well as urban habitats, ranging from large rivers, lakes and ponds to small puddles, village tanks and roadside trenches. During the early 1960 the C. palustris population was depleted across its entire distribution, mainly as a result of a range of threats, including hunting, habitat destruction, fragmentation, and transformation, pollution and increased anthropogenic activities (Da Silva and Lenin 2010). In India the species is legally protected under the Indian Wildlife (Protection) Act as a Schedule-I species, and it is listed as Vulnerable by the IUCN.

Two noteworthy incidences recorded from Gujarat State confirmed an additional threat to the species. On both occasions we came across dead Muggers with missing tails. The first case was noted on 30 July 2015, and involved an adult Mugger (>90 cm in length without tail) on the edge of Chhasiya Talaw (22°27'49.40"N, 73°31'1.63"E), Pavagadh, Halol, Panchmahal. The waterbody where the dead Mugger was found is located at an altitude of 600 m asl, along side Pavagadh Hill. The tail was missing, along with some other internal organs, and the animal was stained with multiple red colored vermilion tilaks along with rice seeds - these are typically used for Hindu rites and rituals. These observations suggested that the animal was possibly worshipped and sacrificed in an unknown religious custom, in which the tail and other viscera were collected and taken away.

The second case was noted on 21 November 2017. A dead adult Mugger was found on the banks of Ajasar Talaw (23°19'25.39"N; 69°37'36.16"E), Ghosa Village, Nr. Bhuj, Kutchh District. This village pond was known to contain three Muggers. The crocodile was over 150 cm in length without the "missing" tail, (possibly about 60 cm long). The reasons why the animal was presumably killed and part of

the tail (containing the single tail scutes) taken are unknown. However, Forest Department staff were able to recover tail vertebrae from the culprit (Fig. 1).



Figure 1. (above) Adult Mugger found missing posterior of tail at Ajasar Talaw; (below) tail vertebrae confiscated by the Forest Department.

Both cases indicate the use of Mugger's tail for superstitious practices or aphrodisiacs. Crocodilians, snakes, lizards, turtles and tortoises serve as important sources of protein for humans around the world, and the consumption of reptile meat is often intertwined with cultural or medicinal beliefs (Klemens and Thorbjarnarson 1995). Reptiles, amongst all other animal species, are most frequently used in traditional folk medicine. Their role in folk practices relates to healing and/or prevention of illnesses in different social-cultural contexts throughout the world (Alves *et al.* 2008). A review has revealed that 165 reptile species belonging to 104 genera and 30 families are used in traditional folk medicine, including 11 species of crocodilian.

WCCB (2011) and Pushpangadan *et al.* (2014) mentioned that Mugger meat, fat and eggs are used in India for Ayurvedic and Unani medicines. Direct mortality of crocodiles for the collection of tail (and other body parts) has been recorded in Nile crocodiles and West Nile crocodiles (*C. niloticus*, *C. suchus*). According to Ikram (2010) and Pooley (2016) many African groups and tribes hunted and trapped crocodiles for food, and they also used body parts for artifacts, leather work, medicines or black-magic, as aphrodisiacs and for their musk. We are aware of the range of threats to Mugger populations in Gujarat, including water pollution, habitat encroachment, development on the river banks (pseudoscientific and unethical developments by urban planners), pet trade and the recently emerging threat of vehicular traffic (Vyas 2010, 2011). These cases indicate another potential threat to Muggers - their use in superstitious rituals and possible medicinal use.

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# **Science**



### **Recent Publications**

Hernán Ciocana, H., Imhof, A., Parachú Marcó, M.V., Isberg, S.R., Siroski, P.A. and Larriera, A. (2017). Increasing photoperiod enhances growth in captive hatchling *Caiman latirostris*. Aquaculture (https://doi.org/10.1016/j.aquaculture.2017.10.002).

Abstract: Captive crocodilians reduce their intake of food during cold periods, even when kept under controlled conditions and fed ad libitum. These manifestations may be due to photoperiod length which is known to influence circadian rhythm. To test this hypothesis, 72 Caiman latirostris hatchlings from three wild-harvested nests, were reared under controlled conditions of temperature and humidity with varying hours of light exposure. Three treatments were performed: A) increasing photoperiod to 16 h light; B) decreasing photoperiod to 8 h light; C) and the control treatment with constant 12 h light/12 h dark. Caiman growth was measured as both weight and linear measures (snout-vent length and total length). Individuals from the increased photoperiod treatment group (16 h) showed a significant increase in all growth parameters compared to other treatments (control = 12 h; reduced photoperiod = 8 h). There was no difference in stress (plasma corticosterone levels) between the treatment groups ensuring that the additional growth benefit was not antagonistic to welfare. From this, it can be postulated that photoperiod, along with availability of temperature, is another important factor which influences the growth of C. latirostris.

Amavet, P.S., Rueda, E.C., Vilardi, J.C., Siroski, P., Larriera, A. and Saidman, B.O. (2017). The broad-snouted caiman population recovery in Argentina. A case of genetics conservation. Amphibia-Reptilia (doi: 10.1163/15685381-00003123).

Abstract: Caiman latirostris wild populations have suffered a drastic reduction in the past, and for that reason, a management and monitoring plan was applied since 1990 in Santa Fe, Argentina, in order to achieve population recovery. Although ranching system has a noteworthy success in terms of population size recovering, there is no information about the estimation of population genetic parameters. In particular, the consequence of the bottleneck underwent by these populations has not been assessed. We evaluated variability and genetic structure of C. latirostris populations from Santa Fe through time, using microsatellites and mitochondrial DNA. Population genetic parameters were compared among four sites and three different periods to assess the impact of management activities, and effective population size was estimated in order to detect bottleneck events. We observed an increase in microsatellite variability and low genetic variability in mitochondrial lineages through time. Variability estimates are similar among sites in each sampling period; and there is scarce differentiation among them. The genetic background of each sampling site has changed through time; we assume this fact may be due to entry of individuals of different origin, through management and repopulation activities. Moreover, taking into account the expected heterozygosity and effective population size values, it can be assumed that bottleneck events indeed have occurred in the recent past. Our results suggest that, in addition to increasing population size, genetic variability of the species has been maintained. However, the information is still incomplete, and regular monitoring should continue in order to arrive to solid conclusions.

Vyas, R. and Stevenson, C. (2017). Review and analysis of human and Mugger crocodile conflict in Gujarat, India from 1960 to 2013. Journal of Threatened Taxa 9(12): 11016-11024.

Abstract: Human-Crocodile Conflict (HCC) occurs to varying degrees around the world, and with a number of crocodilian species (CrocBITE 2013). The Mugger or Marsh Crocodile Crocodylus palustris found in Gujarat State is the crocodilian species responsible for conflict with local people. This paper is a compilation of HCC occurring in various parts of Gujarat from 1960 to 2013. A total of 64 crocodile attacks were recorded: 44 (24 fatal and 20 non-fatal) on males, and 20 (9 fatal and 11 non-fatal) on females. By region 52 HCC were recorded in central Guiarat; five in Saurashtra, four in the northern region and three in Kutch; no crocodile attacks were recorded in southern Gujarat. Of the two major river systems in central Gujarat, 41 attacks occurred within the Vishwamitri-Dhadhar River System and 11 in the Narmada system. Most crocodile attacks happened between the months of April and September, peaking in May with 14 attacks. These months are the peak breeding season for the species in Gujarat. The most obvious contributors to HCC are lack of basic facilities in rural areas, poverty, illiteracy and the presence of adult animals close to human settlements and activities. Other contributing factors are lack of preventive measures by the forest department, absence of protocols for Mugger crocodile rescue, and haphazard release of problematic animals.

Fitri, W.-N., Wahid, H., Rinalfi, P.T., Rosnina, Y., Raj, D., Donny, Y., Qayyum, L. and Malek, A.A.A. (2018). Digital massage for semen collection, evaluation and extension in Malaysian estuarine crocodile (*Crocodylus porosus*). Aquaculture 483: 169-172.

Abstract: Study in semen of the estuarine crocodiles is limited partly due to the lack of knowledge on the semen collection method and their reproductive biology. Therefore, this study was conducted to determine the possibility of semen collection via digital manipulation of the penis. Four matured male crocodiles kept in the same enclosure were physically restrained for semen collection. The collection was done in January 2015 during crocodiles mating season. Mean (± SEM) seminal volume, mass movement, pH, sperm concentration, general motility, forward movement, rotating and vibrating movements measured were  $0.55 \pm 0.12$  ml,  $2.75 \pm 0.48$ ,  $7.13 \pm 0.24$ , 396.88  $\pm 43.41 \times 106$  sperm/ml, 45  $\pm 17.56\%$ , 37.13  $\pm$  5.12%, 9.56  $\pm$  5.38% and 53.13  $\pm$  1.92% respectively. The most important sperm abnormalities that requires further investigation is the cytoplasmic droplets which was  $28.25 \pm 2.29\%$ . Sperm motility trend was best preserved in extender made up of 0.01 M PBS with 5% egg yolk. Sperm stored 4°C in this extender had survived longer than the other two extender which was 11 days. There is a unique trend in the spike of spermatozoa motility post 48 h in chilled condition however the cause of such pattern is still unknown. Semen collection by digital manipulation of the penis was adequate in obtaining semen and the semen characteristics of the estuarine crocodile are described.

Moore, R.L., Isberg, S.R., Shilton, C.M. and Milic, N.L. (2017). Impact of poxvirus lesions on saltwater crocodile (*Crocodylus porosus*) skins. Veterinary Microbiology 211: 29-35.

Abstract: Cutaneous poxvirus infections are common in several crocodilian species and are of importance in crocodile farming due to their potential impact on the tanned hide. To confirm poxvirus infection and understand the impact on saltwater crocodile (*Crocodylus porosus*) skin, 14 animals from different age groups (5 hatchlings, 5 yearlings and 4 grow-outs) were selected based on a criterion of 10 poxvirus-like lesions per animal. One lesion on each animal was extruded for genetic analysis and transmission electron microscopy. Both methods confirmed poxvirus so the remainder of lesions were re-examined every 6 weeks over a 24-week study period. Each lesion went through 4 distinct phases: early active, active, expulsion and healing. To understand how these lesions impact on the final skin product, one crocodile from each age group was euthanised and the lesions examined. Using standard skin grading

techniques (light-table), the early phase (early active - expulsion) lesions were all translucent and would lead to downgrading of the skin or, at worst, rendering them unsaleable. At the later stages of healing, the translucency reduces. Histological examination of the phases confirm that the basement membrane is not breached by the infection further indicating that poxvirus lesions, given enough time, will eventually have no detrimental effect on skin quality. This is obviously dependent upon no more lesions developing in the interim.

Santos, X., , Navarro, S., Campos, J.C., Sanpera, C. and Brito, J.C. (2017). Stable isotopes uncover trophic ecology of the West African crocodile (*Crocodylus suchus*). Journal of Arid Environments (https://doi.org/10.1016/j.jaridenv.2017.09.008).

Abstract: Stable isotope analysis is a widespread tool in ecological studies of diet composition and habitat use. In deserts, freshwater environments constitute threatened local hotspots of biodiversity. In these environments, stable isotopes may help to describe trophic ecology of top-predators. We examined stable carbon ( $\delta$ 13C) and nitrogen ( $\delta 15N$ ) isotopes from scute keratin samples of 33 Crocodylus suchus and muscle samples from 39 potential prey collected in Southern Mauritania. Isotope ratios were compared among crocodiles according to size (non-adult and adult), and habitat (rock pools and floodplains). There was a significant interaction effect of habitat and size on crocodile  $\delta$ 13C values. Whereas  $\delta 13C$  was similar for all crocodiles collected in rock pools, adults had lower signatures than non-adults in seasonal floodplains. δ15N indicated an ontogenetic dietary shift with adult crocodiles foraging on prey from higher trophic level. Standard ellipse areas showed wider isotopic niches for adult than non-adult crocodiles, and within adults, for those from floodplains than those from rock pools. These environments are small, seasonal, overexploited for livestock watering, and polluted. They support very small and isolated crocodile populations. This study is aimed to provide conservation authorities with baseline information to strictly protect water-bodies where these predators subsist in arid environments.

Rodgers, E.M. and Franklin, C.E. (2017). Physiological mechanisms constraining ectotherm fright-dive performance at elevated temperatures. Journal of Experimental Biology 220: 3556-3564.

Abstract: Survival of air-breathing, diving ectotherms is dependent on their capacity to optimise the time available for obligate underwater activities, such as predator avoidance. Submergence times are thermally sensitive, with dive durations significantly reduced by increases in water temperature, deeming these animals particularly vulnerable to the effects of climate change. The physiological mechanisms underlying this compromised performance are unclear but are hypothesised to be linked to increased oxygen demand and a reduced capacity for metabolic depression at elevated temperatures. Here, we investigated how water temperature (both acute and chronic exposures) affected the physiology of juvenile estuarine crocodiles (Crocodylus porosus) performing predator avoidance dives (ie fright-dives). Diving oxygen consumption, 'fright' bradycardia, haematocrit and haemoglobin (indicators of blood oxygen carrying capacity) were assessed at two test temperatures, reflective of different climate change scenarios (ie current summer water temperatures, 28°C, and 'high' climate warming, 34°C). Diving oxygen consumption rate increased threefold between 28 and 34°C (Q10= 7.4). The capacity to depress oxygen demand was reduced at elevated temperatures, with animals lowering oxygen demand from surface levels by 52.9  $\pm$  27.8% and 27.8  $\pm$  16.5% (means  $\pm$ s.e.m.) at 28°C and 34°C, respectively. Resting and post-fright-dive haematocrit and haemoglobin were thermally insensitive. Together these findings suggest decrements in fright-dive performance at elevated temperatures stem from increased oxygen demand coupled with a reduced capacity for metabolic depression.

Lio, G., Agnolin, F.L., Martinelli, A.G., Ezcurra, M.D. and Novas, F.E. (2017). New specimen of the enigmatic, Late Cretaceous crocodyliform *Neuquensuchus universitas* sheds light on the anatomy of the species. Cretaceous Research (https://doi. org/10.1016/j.cretres.2017.09.014).

Abstract: Neuquensuchus universitas is an enigmatic crocodyliform known by a single, incomplete postcranial skeleton collected in the Upper Cretaceous (Santonian) Bajo de la Carpa Formation, Neuquén province, Patagonia, Argentina. Here we describe a second specimen of *N. universitas* that adds important features related to the neck and limb anatomy. The new specimen allows recognizing several convergent features with dinosaurs, including a roughly S-shaped neck in lateral view and extremely gracile limb bones. In addition, the anatomy of the neural arch of the available cervical and dorsal vertebrae possesses a unique combination of traits not seen in any other crocodylomorph, including a laterally concave, fan-shaped prezygodiapophyseal lamina that overlaps most of the anterior half of the base of the neural arch and centrum in lateral view and a conspicuous, horizontal prezygopostzygapophyseal lamina. The phylogenetic analysis conducted here found an unresolved position for Neuquensuchus among early branching non-mesoeucrocodylian crocodyliforms.

Shirley, M.H. and Austin, J.D. (2017). Did Late Pleistocene climate change result in parallel genetic structure and demographic bottlenecks in sympatric Central African crocodiles, *Mecistops* and *Osteolaemus*? Molecular Ecology (doi: 10.1111/mec.14378).

Abstract: The mid-Holocene has had profound demographic impacts on wildlife on the African continent, though there is little known about the impacts on species from Central Africa. Understanding the impacts of climate change on co-distributed species can enhance our understanding of ecosystem dynamics and for formulating restoration objectives. We took a multi-genome comparative approach to examine the phylogeographic structure of two poorly known Central African crocodile species - Mecistops sp. aff. cataphractus and Osteolaemus tetraspis. In addition, we conducted coalescent-based demographic reconstructions to test the hypothesis that population decline was driven by climate change since the Last Glacial Maximum, versus more recent anthropogenic pressures. Using a hierarchical Bayesian model to reconstruct demographic history, we show that both species had dramatic declines (>97%) in effective population size in the period following the Last Glacial Maximum 1500-18,000 YBP. Identification of genetic structuring showed both species have similar regional structure corresponding to major geological features (ie hydrologic basin), and that small observed differences between them are best explained by the differences in their ecology and the likely impact that climate change had on their habitat needs. Our results support our hypothesis that climatic effects, presumably on forest and wetland habitat, had a congruent negative impact on both species.

English, L.T. (2017). Variation in crocodilian dorsal scute organization and geometry with a discussion of possible functional implications. Journal of Morphology (doi: 10.1002/jmor.20760).

Abstract: Dermal ossifications, including osteoderms, are present in many vertebrates and are frequently interpreted as a defense against predators. Nevertheless, osteoderms remain ubiquitous in adult crocodilians while being absent in hatchlings, even though adults rarely experience predation. In other biological systems, increased variation, particularly fluctuating asymmetry, have proven useful for identifying biological structures likely to have evolved under relaxed selection, which in turn may inform their function. Therefore, using the keratinous scutes as proxies for the underlying osteoderm morphology, I investigated the average intraspecific variability of geometry and fluctuating asymmetry in dorsal scutes in five species of crocodilians. I first tested for differences in variability of scute length and width, then for differences in bilateral

fluctuating asymmetry of scute number, before finally investigating scute distribution patterns for each species compared to hypothetical rectangular and hexagonal scute arrangements. The American crocodile, Crocodylus acutus, shows significantly more asymmetry than other species, which is consistent with relaxed selection on osteoderms in this species. A suspected decrease in intraspecific aggression within C. acutus, in conjunction with the inferred relaxed selection, suggests that, in general, crocodilian osteoderms function primarily as defensive armor in aggressive encounters with conspecifics. The smooth-fronted caiman, Paleosuchus trigonatus, exhibits increased variation in scute dimensions linked to the mediolateral offset of osteoderms in adjacent rows, possibly resulting in a more rigid carapace. Unfortunately, comparative data on crocodilian behavior, physiology, and development is extremely limited and restricts the ability to explore other potential explanations for the patterns observed, highlighting the need for more research on rare and cryptic crocodylians.

Tabassum, R. (2017). Molecular cloning and 3D structure of first cytochrome P450 from CYP3A subfamily in saltwater crocodile (*Crocodylus porosus*). Biochemical and Biophysical Research Communications (https://doi.org/10.1016/j.bbrc.2017.10.079).

Abstract: Cytochrome P450s (CYPs) play critical role in oxidative metabolism of numerous xenobiotics and endogenous compounds. The first CYP3A subfamily member in saltwater crocodile has been cloned and modelled for three-dimensional (3D) structure. The full-length cDNA was obtained employing reverse transcription polymerase chain reaction (RT-PCR) strategy and rapid amplification of cDNA ends (RACE). The cDNA sequence of 1659 nucleotides includes 132 nucleotides from 5' untranslated region (UTR), an open reading frame of 1527 nucleotides encoding 509 amino acids designated as CYP3A163. The alignment of CYP3A163 sequence with CYP3A subfamily across the lineages exhibit the loss of one residue in birds and 7 residues in mammals in comparison to reptiles suggesting the adaptation processes during evolution. The amino acid identity of CYP3A163 with Alligator mississippiensis CYP3A77 and Homo sapiens CYP3A4 is 91% and 62% respectively. The 3D structure of CYP3A163 modelled using human CYP3A4 structure as a template with Phyre2 software, represents high similarity with its functionally important motifs and catalytic domain. Both sequence and structure of CYP3A163 display the common and conserved features of CYP3A subfamily. Overall, this study provides primary molecular and structural data of CYP3A163 required to investigate the xenobiotic metabolism in saltwater crocodiles.

Boucher, M. (2017). Bioacoustics and Behavior of American Crocodiles in Belize. MSc thesis, West Virginia University, Morgantown, West Virginia, USA.

Abstract: American crocodiles (Crocodylus acutus) are large-bodied crocodylians broadly distributed throughout coastal and lowland wetlands in the Americas. Despite their being large, charismatic megafauna, the study of American crocodile ecology is still lacking in many areas. As such we assessed the behavior and bioacoustics of American crocodiles in Belize to address the paucity of data regarding these two facets of American crocodile ecology. We conducted behavioral observations from three sites in the coastal zone of Belize. We categorized American crocodile behavior and recorded activity duration for observed behaviors. In conjunction with behavioral data, we also assessed study sites to quantify the intensity of anthropogenic impact. Our results determined that American crocodiles spent the highest proportional time performing maintenance activities to fulfill basic biological needs. However, the proportion of social and agonistic activities differed between sites, and was greater at sites with higher human disturbance. The results from this project establish activity-budgets for American crocodiles in Belize as well as indicate adverse behavioral responses to anthropogenic impact which should be further considered in management decision making, as should bioacoustics. American

crocodiles, like most crocodylian species, have a repertoire of acoustic signals used to communicate intraspecifically and in interaction with their environment. Of the acoustic calls produced, distress calls play an important role in crocodile ecology, particularly for juvenile American crocodiles. The distress call is produced to elicit a defense response from nearby conspecifics, enhancing the survivorship of young American crocodiles. We recorded American crocodile distress calls from three sites in the coastal zone of Belize. We recorded from captured hatchling, juvenile, subadult, and adult American crocodiles. We measured temporal and spectral parameters of the calls to describe the call structure of this species for each size class. We compared call parameters among size classes and determined that call structure remained similar among size classes but call parameters differed. We found that hatchling and juvenile distress calls were highly modulated and higher in frequency, whereas sub-adult and adult calls were longer in duration, lower in frequency, and had less modulation. We determined that call parameters could be used to successfully classify 82.4% of individuals into the correct size class. We also recorded call production by captured individuals as not every capture resulted in successful acoustic recording. Proportion of calls produced by individuals differed by size class and site. We found that American crocodiles at sites with high anthropogenic impact produced distress calls at a higher proportion. Our results indicate that anthropogenic activity in crocodile habitat may be impacting the acoustic ecology of American crocodiles in Belize. The study of crocodylians, in this case American crocodiles, benefits from the ability to effectively discern individuals in the field. Conventional identification techniques are to physically alter captured crocodiles by clipping the upright caudal scutes. However, this technique is difficult to observe accurately in the field and has no passive marking alternative. In compliment to the behavioral and acoustical study of American crocodiles in Belize, we implemented novel marking techniques through tail spot pattern coding and visual tagging. We used existing tail spot pattern coding methodology developed for Nile crocodiles (C. niloticus), and adapted this technique to create a second method. The original coding protocol, numeric code, was 84% successful in differentiating tail spot patterns from American crocodiles and sympatric Morelet's crocodiles (C. moreletii). The second method, the additive code, integrated irregular scale groups and vertical caudal scutes into the original coding system. We were 99% successful in differentiating individual crocodiles using the additive code. In conjunction with tail spot coding, we applied flexible, self-piercing, plastic tags to tail scutes to mark sub-adult and adult American crocodiles. We used the tail tags to verify spot pattern recapture and facilitate individual identification for behavioral observation. The use of these tags is beneficial to behavioral studies and short-term population monitoring and offer an opportunity to augment current marking techniques. We determined that spot pattern coding and visual tagging are effective means to individually identify crocodiles in Belize and are a tool that can be easily implemented by current managers as well as integrated into community-based citizen science initiatives.

Madawala, M., De Silva, A., Botejue, M. and Karunarathna S. (2017). Sri Lanka's wetlands: Habitats of the last surviving dinosaurs are threatened. Wetlands News Bulletin: 18-23.

Fuentes-Castro, B.E., Reyes-García, J.G., Valenzuela-Vargas, M.T. and Martínez-Gómez, F. (2017). Histopathology of murine toxoplasmosis under treatment with dialyzable leukocyte extract. Mem. Inst. Oswaldo Cruz. 112(11): 741-747.

<u>Abstract</u>: Dialyzable leukocyte extracts (DLEs) contain molecules smaller than 10 kDa with biological activity in receptor organisms. Primarily, they participate in the regulation of the Th1 immune response, which is essential for the control of several intracellular infections, such as toxoplasmosis. This disease is associated with congenital infection, encephalitis or systemic infections in immunocompromised individuals. The clinical course of this infection fundamentally depends on a well-regulated immune response and timely treatment with the appropriate drugs. The aim of this study was to evaluate the effect of treatment with a leukocyte extract, derived from crocodile (Crocodylus moreletii) lymphoid tissue, on the histopathology and brain parasite load in NIH mice that had been infected with cysts of Toxoplasma gondii (ME-49 strain). The treatment was applied during the acute and chronic stages of the infection. Histopathological changes were evaluated in the ileum, liver and spleen at one, four and eight weeks after infection and in the brain at week 8. The parasite load was evaluated by counting the cysts of T. gondii found in the brain. Compared to the control mouse group, the mice infected with T. gondii and under treatment with DLE showed less tissue damage, mainly at the intestinal, splenic and hepatic levels. In addition, a greater percentage of survival was observed, and there was a considerable reduction in the parasite load in the brain. The results suggest that DLE derived from crocodile is a potential adjunctive therapy in the conventional treatment of toxoplasmosis.

Junker, K. and Mutafchiev, Y. (2017). *Typhlophoros kwenae* n. sp. (Nematoda: Ascaridida: Heterocheilidae), a gastric parasite from the Nile crocodile *Crocodylus niloticus* Laurenti (Reptilia: Crocodylidae) in South Africa. Systematic Parasitology (https://doi. org/10.1007/s11230-017-9757-x).

Abstract: Based on light and scanning electron microscopical observations, Typhlophoros kwenae n. sp. (Heterocheilidae), a new nematode parasite, is described from the stomach of the Nile crocodile Crocodylus niloticus Laurenti in South Africa. In having three lips with well-developed posterior prolongations and prominent interlabial longitudinal cuticular ridges, four pairs of precloacal papillae and complex spicules divided into handle and broad alate blade in males, as well as the position of the vulva near mid-body in females, the specimens conform to the generic diagnosis of Typhlophoros von Linstow, 1906. They can, however, be distinguished from the two previously described congeners, T. lamellaris von Linstow, 1906 and T. spratti Sprent, 1999, by the number of complete interlabial ridges, the length of spicules in males and the position of the vulva as well as the length of the tail in females. This is the first record of the genus Typhlophoros from the Afrotropical Realm.

Rice, B. (2017). Illegal Wildlife Hunting and Trade in Southern Belize: An Assessment of Impacts and Drivers. Capstone Collection. 3057. (http://digitalcollections.sit.edu/capstones/3057).

Abstract: The use of wildlife as a resource is a common practice in all countries around the world, however, illegal activities are contributing to various environmental and social altercations amongst the involved communities and individuals, both directly and indirectly. This has led to the generalized global narrative on illegal wildlife hunting and trade as a "good vs. bad" convention. Although legal frameworks are in place to manage hunting and trade sustainably, governments and organizations often find themselves struggling to protect wildlife from illegal hunters, often facing dangerous situations thus the establishment of militarized conservation units. To date, most of the focus is on the African continent and Southeast Asia, with less attention on other biodiverse locations, such as Central and South America. Information about illegal wildlife hunting and trade is increasing in Central and South America but the data is still lacking in both qualitative and quantitative analysis. Frameworks such as Conflict Sensitive Conservation and Conservation Conflict Transformation have been developed to address the complex factors impacting wildlife conservation. In Belize, previous studies have examined the legal and social aspect of wildlife hunting and trade, but there remains a void of information regarding the activities. Herein, this study explored some of the causation and subsequent results of illegal hunting and trade in Southern Belize through semistructured interviews with conservation practitioners and hunters; 10 stakeholders from Stann Creek and Toledo districts in Southern

Belize were interviewed. The findings reveal that all participants think hunting in Belize is unsustainable, while five participants cited enforcement as the biggest thing needed to reduce this activity, three cited more farming support and two cited education; additionally, three participants mentioned that starting a gibnut ranching program could help reduce the pressure on wild populations. Eight participants addressed livelihood or the need for additional income as the main motivation for hunting and trading illegally, while hunting for identity was second and protein sources was third. Interestingly, all participants discussed the hunting of Paca (Cuniculus paca), known as gibnut in Belize, as the main hunted species, suggesting that animals that have a legal hunting season are hunted illegally more than other species that are considered non-huntable. This finding is different from other illegal hunting studies that focus on animals that are completely off-limits for hunting. Eight participants mentioned crocodiles (Crocodylus acutus or Crocodylus moreletii) as the species that is hunted the most that does not have a hunting season; participants did not specify a species. Parrots, namely the endangered Yellow Head Parrot (Amazona oratrix), were the species mentioned the most when asked about animals captured for the pet trade. Lastly, recommendations are provided for short, medium and long term initiatives to address this issue from both a technical and behavioural standpoint.

Clarac, F., De Buffrenil, V., Cubo, J. and Quilhac, A. (2017). Vascularization in ornamented osteoderms: Physiological implications in ectothermy and amphibious lifestyle in the Crocodylomorphs? Anat. Rec. (doi: 10.1002/ar.23695).

Abstract: Vascularization in the core of crocodylian osteoderms, and in their superficial pits has been hypothesized to be a key feature involved in physiological thermoregulation and/or acidosis buffering during anoxia (apnea). However, up to now, there have been no quantitative data showing that the inner, or superficial, blood supply of the osteoderms is greater than that occurring in neighboring dermal tissues. We provide such data: our results clearly indicate that the vascular networks in both the osteoderms and the pits forming their superficial ornamentation are denser than in the overlying dermis. These results support previous physiological assumptions and indicate that vascularization in pseudosuchian (crocodylians and close relatives) ornamented osteoderms could be part of a broad eco-physiological adaptation towards ectothermy and aquatic ambush predation acquired by the crocodylomorphs during their post-Triassic evolution. Moreover, regressions demonstrate that the number of enclosed vessels is correlated with the sectional area of the cavities housing them (superficial pits and inner cavities). These regressions can be used to infer the degree of vascularization on dry and fossilized osteoderms and thus document the evolution of the putative function of the osteoderms in the Pseudosuchia.

Abstract: Zooarchaeologists have long relied on linear traces and pits found on the surfaces of ancient bones to infer ancient hominid behaviors such as slicing, chopping, and percussive actions during butchery of mammal carcasses. However, such claims about Plio-Pleistocene hominids rely mostly on very small assemblages of bony remains. Furthermore, recent experiments on trampling animals and biting crocodiles have shown each to be capable of producing mimics of such marks. This equifinality - the creation of similar products by different processes - makes deciphering early archaeological bone assemblages difficult. Bone modifications among Ethiopian Plio-Pleistocene hominid and faunal remains at Asa Issie, Maka, Hadar, and Bouri were reassessed in light of these findings. The results show that crocodiles were important modifiers of these bone assemblages. The relative roles of hominids, mammalian carnivores, and crocodiles in the formation of Oldowan zooarchaeological assemblages will only be accurately revealed by better bounding

Sahle, Y., El Zaatari, S. and White, T.D. (2017). Hominid butchers and biting crocodiles in the African Plio-Pleistocene. PNAS (www. pnas.org/cgi/doi/10.1073/pnas.1716317114).

equifinality. Critical analysis within a consilience-based approach is identified as the pathway forward. More experimental studies and increased archaeological fieldwork aimed at generating adequate samples are now required.

Moleon, M.S., Parachu Marco, M.V., Pietrobon, E.O., Jahn, G.A., Beldomenico, P.M. and Siroski, P.A. (2017). Corticosterone levels and immunological indices in stressed juvenile broad-snouted caimans. Journal of Zoology (doi:10.1111/jzo.12513).

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

Kahui, V., Moyle, B. and Brunell, A.M. (2017). Alligator conservation and hunting efficiency. Natural Resource Modeling (doi: 10.1111/nrm.12155).

Abstract: The successful conservation of Florida's alligator rests, among other things, on a recreational harvest program that provides seasonal, nontransferable hunting permits. We develop an economic hunting model adapted from evolutionary ecology to show how the incentives under Florida's program still allow for efficient behavior. Hunters can select the length for kill from a given lengthfrequency distribution and pursue alligators for revenue from hide and meat. We exploit extensive data sets on harvest reports, alligator population estimates, permit costs, and annual prices for wild hides and meat across 33 alligator management units in Florida between 2000 and 2015. We show that an increase in the abundance of bulls increases the minimum length of alligator for kill, while an increase in prices and cost of foraging time pushes hunters to accept smaller alligators for harvest. Understanding the drivers of human hunting behavior is essential for effective wildlife management. Recommendations for Managers: Effective wildlife management relies on the understanding of both animal and human dynamics. Alligator population levels are actively monitored but there is limited understanding of hunter behavior. Our research shows that recreational alligator hunters in Florida respond to abundance levels of bulls, and prices and cost of foraging time by adjusting the smallest length of alligator for harvest. Management of harvest quotas should actively consider behavioral responses of hunters.

De Celis, A., Narváez, I. and Ortega, F. (2017). Pelvic and femoral anatomy of the Allodaposuchidae (Crocodyliformes, Eusuchia) from the Late Cretaceous of Lo Hueco (Cuenca, Spain). J. Iber. Geol. (https://doi.org/10.1007/s41513-017-0044-0).

<u>Abstract</u>: The fossil record of postcranial remains assigned to Allodaposuchidae is currently sparse. However, the Late Cretaceous paleontological site of Lo Hueco (Cuenca, Spain), from where two new taxa of allodaposuchid have been described, has yielded numerous postcranial remains assignable to this clade. Among them, the large amount of pelvic and femoral material is notable, providing the opportunity to study these allodaposuchid elements and assess their morphological similarity with other eusuchian remains. The comparison with extant crocodylians was accomplished using traditional morphometric techniques, whereas the comparison with other fossils and establishment of morphotypes was done using morphological criteria. The results of the cluster and principal components analyses show morphological differences between extant crocodylians and allodaposuchids from Lo Hueco, allowing the segregation of these lineages. The similarities found between the pelvic and femoral remains from Lo Hueco, and those referred to Allodaposuchus precedens and other putative allodaposuchids from the Iberian Peninsula, allows referral of these remains to allodaposuchids. The differences found among the femoral and pelvic remains of Lo Hueco enables us to recognize two morphotypes per each element. This study allows a better understanding of allodaposuchid postcranial elements that were previously poorly known. The ilia, ischia and femora from Lo Hueco allodaposuchids are distinct from those of other crocodylian lineages. Finally, the fact that there are two morphotypes per each element at Lo Hueco is congruent with the presence of two different allodaposuchids at the site.

Ponce, D.A., Cerda, I.A., Desojo, J.B., and Nesbitt, S.J. 2017. The osteoderm microstructure in doswelliids and proterochampsids and its implications for palaeobiology of stem archosaurs. Acta Palaeontologica Polonica 62(4) (doi:https://doi.org/10.4202/app.00381.2017).

Abstract: Osteoderms are common in most archosauriform lineages, including basal forms, such as doswelliids and proterochampsids. In this survey, osteoderms of the doswelliids Doswellia kaltenbachi and Vancleavea campi, and proterochampsid Chanaresuchus bonapartei are examined to infer their palaeobiology, such as histogenesis, age estimation at death, development of external sculpturing, and palaeoecology. Doswelliid osteoderms have a trilaminar structure: two cortices of compact bone (external and basal) that enclose an internal core of cancellous bone. In contrast, Chanaresuchus bonapartei osteoderms are composed of entirely compact bone. The external ornamentation of Doswellia kaltenbachi is primarily formed and maintained by preferential bone growth. Conversely, a complex pattern of resorption and redeposition process is inferred in Archeopelta arborensis and Tarjadia ruthae. Vancleavea campi exhibits the highest degree of variation among doswelliids in its histogenesis (metaplasia), density and arrangement of vascularization and lack of sculpturing. The relatively high degree of compactness in the osteoderms of all the examined taxa is congruent with an aquatic or semi-aquatic lifestyle. In general, the osteoderm histology of doswelliids more closely resembles that of phytosaurs and pseudosuchians than that of proterochampsids.

Evans, L.J., Davies, A.B., Goossens, B. and Asner, G.P. (2017). Riparian vegetation structure and the hunting behavior of adult estuarine crocodiles. PLoS ONE 12(10): e0184804.

Abstract: Riparian ecosystems are amongst the most biodiverse tropical habitats. They are important, and essential, ecological corridors, linking remnant forest fragments. In this study, we hypothesised that crocodile's actively select nocturnal resting locations based on increased macaque predation potential. We examined the importance of riparian vegetation structure in the maintenance of crocodile hunting behaviours. Using airborne Light Detection and Ranging (LiDAR) and GPS telemetry on animal movement, we identified the repeated use of nocturnal resting sites by adult estuarine crocodiles (*Crocodylus porosus*) throughout the fragmented Lower Kinabatangan Wildlife Sanctuary in Sabah, Malaysia. Crocodile resting locations were found to resemble, in terms of habitat characteristics, the sleeping sites of long-tailed macaque; positioned in an attempt to avoid predation by terrestrial

predators. We found individual crocodiles were actively selecting overhanging vegetation and that the protrusion of trees from the tree line was key to site selection by crocodiles, as well as influencing both the presence and group size of sleeping macaques. Although these findings are correlational, they have broad management implications, with the suggestion that riparian corridor maintenance and quality can have implications beyond that of terrestrial fauna. We further place our findings in the context of the wider ecosystem and the maintenance of trophic interactions, and discuss how future habitat management has the potential to mitigate human-wildlife conflict.

Du Preez, M., Govender, D., Kylin, H. and Bouwman, H. (2018). Metallic elements in Nile crocodile eggs from the Kruger National Park, South Africa. Ecotoxicology and Environmental Safety 148: 930-941.

Abstract: The Nile crocodile is the largest predator on the African continent. Recent mass mortalities in the Kruger National Park (KNP) raised concerns about possible influence of pollution. We analysed eggs and their eggshells collected from nests inside the KNP and from a crocodile farm for metallic elements. We found that mercury, selenium, and copper occurred at levels of concern. Eggshells had very high concentrations of iron. Apart from toxicological implications associated with elevated concentrations in eggs, we found iron possibly contributing towards thicker eggshells. Thicker shells may act as a barrier to gas and water exchange, as well as possibly increasing the effort required for the hatchling to emerge from tightly packed shells under sand. Pollutants are transported into the KNP via rivers, and possibly via air. Mercury and copper pollution are waste-, industrial- and mining-related; ecotoxicological concern should therefore be extended to all areas where the four African crocodile species occur. Reptiles are under-represented in ecotoxicological literature in general, and especially from Africa. We know of only one previous report on metals and metalloids in crocodile eggs from Africa (Zimbabwe), published 30 years ago. Reduced fitness, endocrine disruption and effects on behaviour are other possible sub-lethal effects associated with metallic elements that may only become apparent decades later in a long-lived species such as the Nile crocodile. In the face of habitat destruction, pollution, human population increases, and climate change, further research is needed regarding pollutant concentrations and effects in all African reptiles. The rivers that carry water from outside the park sustain its aquatic life, but also transport pollutants into the KNP. Therefore, improved source mitigation remains an important task and responsibility for all involved.

Buah-Kwofie, A., Humphries, M.S., Combrink, X. and Myburgh, J.G. (2018). Accumulation of organochlorine pesticides in fat tissue of wild Nile crocodiles (*Crocodylus niloticus*) from iSimangaliso Wetland Park, South Africa. Chemosphere 195: 463-471.

Abstract: Nile crocodiles (Crocodylus niloticus) are important apex predators in many tropical and subtropical aquatic habitats throughout much of sub-Saharan Africa. In South Africa, large crocodile populations inhabit lakes and wetlands that are impacted by organochlorine pesticides (OCPs). Despite the continued use of these compounds and their potential adverse effects on key wildlife populations in southern Africa, limited ecotoxicoloigcal data exist. In this study, we examined the accumulation of OCPs in fat tissues of live, wild Nile crocodiles from iSimangaliso Wetland Park, a region of significant biological importance. All samples (n=15) contained multiple contaminants in highly elevated concentrations, with total residue burdens varying between 3600 and 8000 ng g<sup>-1</sup> ww. DDT and its metabolites were the dominant compounds detected in most samples, with  $\Sigma$ DDT concentrations ranging between 520 and 3100 ng g<sup>-1</sup> ww. Elevated levels of other OCPs were also detected, including lindane (67-410 ng g<sup>-1</sup> ww), aldrin (150-620 ng g<sup>-1</sup> ww) and heptachlor (170-860 ng g<sup>-1</sup> ww). Our findings show that crocodiles are exposed to OCPs throughout their range

within iSimangaliso Wetland Park and contain some of the highest concentrations ever recorded in crocodilian tissue. Results indicate the need for a greater understanding of the impacts of OCP exposure and toxicological responses in crocodiles from iSimangaliso, and in Nile crocodile populations in general. The novel surgical technique described in this study provides an effective method for assessing relationships between contaminant body burdens and their potential reproductive and developmental consequences in crocodilians.

Williamson, S.A., Evans, R.G., Manolis, S.C., Webb, G.J., Reina, R.D. (2018). Ecological and evolutionary significance of a lack of capacity for extended developmental arrest in crocodilian eggs. R. Soc. open sci. 4: 171439.

Abstract: Hypoxia within the oviducts maintains embryonic arrest in turtles at the pre-ovipositional stage, which expands the timeframe over which nesting can occur without compromising embryo survival. The arrest can be extended post-oviposition through incubation of eggs in hypoxia. We determined whether crocodilian embryos have this same capacity. We also tested whether increased oxygen availability during incubation alters hatching success. We incubated freshly laid saltwater crocodile (Crocodylus porosus) eggs (N= 83) at 32°C in one of five treatments; control (normoxia; 21% O<sub>2</sub>), 3-day and 6-day hypoxia (1% O<sub>2</sub>), or 3-day and 6-day hyperoxia (42% O<sub>2</sub>). Incubation (approx. 82 days) was then completed in normoxia. There was a significant effect of treatment on survival of embryos through to hatching (p<0.001). The hypoxic treatments resulted in almost no hatching (6.7% and 0% survival for the 3- and 6-day treatments, respectively), while the hyperoxic and control treatments resulted in normal to high hatching success (86.6%, 100% and 64.2% for the control, 3- and 6-day hyperoxic treatments, respectively). Unlike turtles, hypoxic incubation of crocodile eggs failed to delay development. Our results provide the first experimental evidence that, unlike turtles, crocodiles do not exhibit embryonic arrest when incubated under hypoxic conditions immediately following oviposition. An absence of embryonic arrest is of ecological and evolutionary significance, as it implies that crocodilians lack an ability to avoid adverse environmental conditions through delayed nesting and that, unlike turtles, embryonic arrest may not be a potential explanation for the lack of viviparity in the Order Crocodylia.

Tang, K.-Y., Wang, X., Wan, Q.-H. and Fang, S.-G. (2018). A crucial role of paralogous  $\beta$ -defensin genes in the Chinese alligator innate immune system revealed by the first determination of a Crocodilia defensin cluster. Developmental & Comparative Immunology 81: 193-203.

<u>Abstract</u>: The  $\beta$ -defensin, one of the antimicrobial peptides (AMPs), is a significant component of the innate immune with a broad range of antimicrobial activities. Differing from the widely-studied mammals and birds, limited information about β-defensins has been reported in reptiles, especially in crocodilians. As a same ancient species as dinosaurs and the most endangered species of 23 crocodilians, the survival of Chinese alligator (Alligator sinensis) means a powerful immune system and possible involvement of AMPs in its immune resistance. In this study, we identified 20 novel Alligator sinensis  $\beta$ -defensin genes (AsBDs) from a 390 kb region using bioinformatic and experimental approaches, and successfully distinguished six orthologous AsBDs to birds and nine paralogous AsBDs undergoing gene duplication events. The amino acid alignment shows that the AsBD paralogs, like  $\alpha$ -defensions, encode a significantly longer pro-piece comparing with the orthologs. The calculation of nonsynonymous  $(d_{\rm N})$  and synonymous  $(d_{\rm S})$  substitutions in the mature peptide reveals that the AsBD paralogs experience a significantly higher selective pressure  $(d_N/d_s)$  than the orthologs, but a similar evolutionary force to  $\alpha$ -defensins. The gene expression result indicates that the AsBD paralogs have a significantly higher expression level than the orthologos in gastrointestinal tract where the host is vulnerable to enteric pathogenic bacteria, as observed

in  $\alpha$ -defensins. These three pieces of evidence demonstrate that the *AsBD* paralogs do play an important role in maintaining long-term survival of this endangered reptile. Thus, this survey of *AsBDs* on the genomic structure, evolutionary characteristics, and expression pattern provides a genetic and immunological foundation for further investigating their antimicrobial function and alternative antibiotics potentiality.

Lewis, C.F. (2017). How does land use and water quality affect the population distribution of Morelet's crocodiles (*Crocodylus moreletii*) in northern Guatemala? Integrated Masters of Ecology,

Abstract: At the frontier of agricultural expansion in Central America is the Maya Biosphere Reserve (MBR), the largest area of tropical forest remaining in Central America, located in northern Guatemala. Despite allocation of zones within the MBR to promote sustainable development by allowing only certain zones to be converted into agriculture, the reserve is at risk due to a lack of enforcement of such rules. The Morelet's crocodile (Crocodylus moreletti), listed as CITES Appendix I in Guatemala, can be found throughout the MBR. However, population status data for Guatemala is outdated with a lack of knowledge available on how this species is responding to increased agricultural activity. This study aimed to firstly evaluate the population status in the Laguna del Tigre National Park, a protected area within the MBR that has suffered much unregulated agricultural expansion. And secondly, how the population is responding to changes in land use by measuring water quality along the river and using GIS to evaluate land cover. Using the scores from principal component analysis for water and land cover in a linear regression was done to see if either could predict relative crocodile density. The results showed that density of crocodiles has increased greatly from the last reported dataset in 1999, and is greatest in areas of river with less agriculture surrounding it with a preference for sibal marsh habitat. Although signs of agrichemicals in the water are null and that the Morelet's population is healthy, the study shows a need to protect the marsh habitats in the national park and increased law enforcement to prevent further agricultural expansion.

Chang, M.S., Ghulam Sarwar Gachal, G.S. and Memon, K.H. (2017). Captive breeding and conservation status of Marsh Crocodiles (*Crocodylus palustris*) in New Jatoi Farm Naushehroferoze, Sindh, Pakistan. Biological Forum - An International Journal 9(2): 217-223.

Abstract: A study on captive breeding and conservation status of Marsh Crocodiles (Crocodylus palustris) in New Jatoi Farm Naushehroferoze was undertaken during the years 2011-2015. To achieve this objective the study monitored the current population status, feeding, reproductive mode and measurements of hatchling, juvenile, adult and size of eggs. The observation revealed that the total numbers of Marsh Crocodiles were counted 133 in the New Jatoi Farm, among them 20 males and 25 females in Pond-1 were adults, 44 were juveniles in Pond-2, and 76 hatchlings in Pond-3 were counted. With a view to study the breeding behavior, 22 adult females were observed to lay the eggs per female/per year. However, the breeding habitat of NJF for Crocodiles as observed in subsequent years was found productive and the number of eggs per clutch was recorded as in F-01. 23  $\pm$  2.73, F-02. 25.8  $\pm$  3.42, F-03. 25  $\pm$  3.16,  $F\text{-}04.\ 25.8 \pm 2.86, F\text{-}05.\ 26.2 \pm 3.70, F\text{-}06.\ 23.4 \pm 4.92, F\text{-}07.\ 25.8 \pm 2.86, F\text{-}07.\ 25.86, F\text{-}07.\ 2$  $3.27, F-08. 25.2 \pm 4.54, F-09. 26.2 \pm 2.77, F-10. 26.6 \pm 2.88, F-11.$ 25.6 ± 3.04, F-12. 24.4 ± 4.27, F-13. 26.6 ± 3.04, F-14. 26.4 ± 3.91, F-15. 24.6 ± 4.27, F-16. 25.6 ± 2.96, F-17. 25.4 ± 3.36, F-18. 24.6  $\pm$  3.64, F-19. 26.2  $\pm$  1.48, F-20. 26.2  $\pm$  2.38, F-21. 25  $\pm$  3.16 and F-22. 24.4  $\pm$  3.36, respectively. We selected 100 eggs randomly and measured the length  $65.7 \pm 0.74$  (mm), width  $41 \pm 0.87$  (mm) and weight  $165.56 \pm 4.09$  (g), respectively. Further distribution of this species was made to NJF in order to find out suitable habitat for its captive breeding. Since then, the performance of breeding was closely observed and found result in the total stock 133 individuals in NJF. Hatching rate of the species has been recorded low because only less numbers of eggs were hatched due to less availability of space in the ecological condition of three ponds in NJF

#### **Submitted Publications**

SYSTEMATIC STATUS OF CROCODILES IN THE AWASH RIVER CATCHMENT. Recent molecular studies investigating the systematics of the Nile crocodile indicated that there are actually two, highly divergent species in Africa (Hekkala et al. 2011). All populations of Nile crocodile in West Africa represent the species *Crocodylus suchus*, with *C*. niloticus in the rest of Africa [see Shirley et al. (2015) for an updated distribution map). The CSG is currently undertaking IUCN Red List assessments for crocodilian species, and the newly recognized C. suchus will be included. Because of this, there is a need to better understand the species' continental distribution. From eastern Africa, contemporary records exist for this species only in the Kidepo Valley of Uganda, while historic records from museum specimens collected in Sudan show it may have been, and still is, more widely distributed in the region (Hekkala et al. 2011).

During crocodile surveys carried out in Lake Chamo in Ethiopia (Shirley *et al.* 2014), the question of the status of the crocodiles of the Awash River basin was raised. Some animals from the Awash River drainage showed some morphological characteristics consistent with *C. suchus* (see Fig. 5). Size-capture records of hunters show that the largest crocodile ever shot in the Awash measured only 3.17 m total length (H. Pohlstrand, pers. comm.), which approaches the upper limit of the size of *C. suchus*, and is much too small for trophy *C. niloticus*.

In light of these recent taxonomic revisions, the study team recommended that an effort be made to confirm the species identification for this population, because if two species are present within Ethiopia it may impact on crocodile utilisation schemes introduced across the country.

#### Awash River Catchment

The Awash River originates west of Addis Ababa, and flows southeast and then east towards the rift valley. South of Mojo, the river is dammed to feed the Koka Dam, one of the oldest dams in the country. Farther east, the Awash forms the southern boundary of the Awash National Park (ANP). The Awash then turns north and flows through the Afar regional state to the Tendero-Semera area, where it turns east to end in four terminal lakes, the first of which is Lake Gemeri, and the largest and furthest south is Lake Abe. Lake Abe straddles the border with Djibouti.

In the rift valley close to the Awash there are a number of lakes that show volcanic activity. The first is Lake Beseka to the east of ANP, and the others are the Filoha Hot Springs, Bilen Hot Springs, and further north are Hertale and Gewane Lakes. All of these lakes contain crocodiles.

The Awash River was the first to be developed for irrigation agriculture. The earliest attempts were made under Emperor

Menelik's rule in the early 20th Century, and the first large agricultural schemes for cotton and sugar were established in the 1930s. At the traditional Afar capital of Aseita, close to the terminal lakes, the Afar have practised traditional irrigation agriculture for several hundred years. As a result of human impact, crocodiles have disappeared from some areas of the Awash, whilst in other areas they are still numerous (eg in the hot springs and the terminal lakes).

The Ethiopia Wildlife Conservation Authority (EWCA), as the regulating body for wildlife utilisation in Ethiopia, is receiving applications to establish crocodile ranches. EWCA also receives requests for advice on appropriate locations and how to establish ranches. The current ranches are located in Nechsar, which is around 1200 m asl. Lower-lying locations would provide a warmer climate, and thus a better environment for crocodiles to grow. The low-lying Awash basin could provide potentially prime locations for crocodile ranching. Knowledge of the status of the crocodiles in the Awash catchment is therefore crucial for EWCA.

#### Methods

Tissue samples from crocodiles in the Awash basin (Fig. 1) were collected and genetically screened. The original intention was to collect samples from the total length of the Awash, including the adjacent lakes and swamps, the latter being hot spring-fed. Samples were ultimately collected from Beseka and Gemeri Lakes, the swamps and hot springs of Filoha and Bilen, and the Awash River. Collection was carried out on 7-12 April 2016 by Dr. Ludwig Siege and Girma Ayalew (chief veterinarian at EWCA).

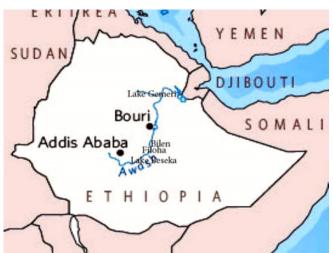


Figure 1. Sample locations on the Awash River.

Priority was given to collection of tissue samples from dead animals, parts of animals, and from skins in the Awash National Park Museum, etc. Where "dead" samples could not be found, attempts were made to catch small crocodiles, and to take small tissue samples. This was done in Filoha. One crocodile was shot in Lake Beseka, because this was the only means to obtain samples from there. On Lake Gemeri a sick half-dead crocodile was killed by the study group (sample 6). Technical advice was provided by Drs. Matthew Shirley and Dietrich Jelden.

The Sustainable Development of the Protected Area System of Ethiopia Project (SDPASE), headed by Dr. Ludwig Siege, facilitated and organised the collection of samples and their analysis. The EWCA issued the necessary permits (Ethiopian research permit and CITES Export Permits for the samples), and provided staff for the research (veterinary, supporting staff). Some samples were sent to the Museum Koenig in Bonn (Germany) for analysis.

#### Results

Samples analysed at Museum Koenig (Table 1) were used to determine the genetic status of the crocodiles, because they represent all locations from which samples were collected, and, except for Sample 1, they were fresh "wet" samples and thus more likely to produce results than the older "dry" samples that were collected.

Six samples were analysed, covering the whole length of the Awash River. No DNA could be extracted from one of these samples (Sample 8), leaving 5 samples (LS1, LS4, LS7, LS10, LS12) that were included in the analysis.

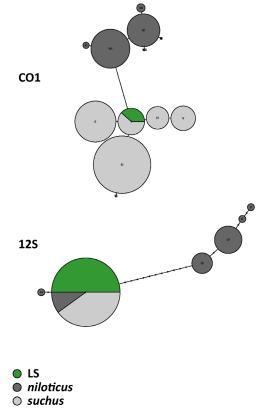


Figure 2. Network displays for CO1 and 12S gene sequences. The circles represent different haplotypes of each gene. The length (and number of dots) of each connecting line between two circles indicates the number of differences (substitutions) in the base pairs between the two haplotypes. The size of the circles depends on the number of sequences that share the same haplotype. The analysis was carried out using 3 gene sequences: 12S, COI and RAG1. RAG1 did not produce any results, so the following conclusions are based on two mitochondrial genes only. We added 124 sequences of CO1 (representing 44 specimens of *C. niloticus* and 80 specimens of *C. suchus*) and 15 sequences of 12S (representing 9 specimens of *C. niloticus* and 6 specimens of *C. suchus*) that are available on Genbank (https://www.ncbi.nlm.nih.gov/genbank/). The results are shown in network displays (Fig. 2) and neighbor-joining trees for each gene separately (Figs. 3 and 4).

C. niloticus

C. suchus

intragroup variation, whereas a large genetic difference exists between the two groups. Haplotypes I-VI belong to *C. suchus* and haplotypes VII-XII belong to *C. niloticus* (Fig. 2). All 5 Awash samples analysed ("LS" in Fig. 2) belong to haplotype I, signifying that they belong unambiguously to *C. suchus*.

For 12S, 6 haplotypes (I-VI) are present, of which haplotypes II-V and I+VI form two separated groups. Each group shows only minor differences in base pairs, whereas a large genetic difference exists between the two groups. Haplotypes I and VI belong to *C. suchus* and haplotypes II-V belong to *C. niloticus*. All sequences of haplotype VI and some sequences of haplotype I were erroneously filed as *C. niloticus* (Schmitz and Hekkala 2003), but actually represent *C. suchus*. All 5 Awash samples analysed ("LS" in Fig. 2) belong to *A. suchus*.

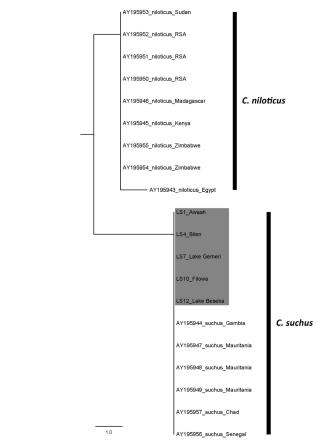
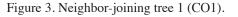


Figure 4. Neighbor-joining tree 2 (12S).

The neighbour-joining trees for each gene sequence (Figs. 3 and 4) show exactly the same pattern, with sample gene sequences clustering together with other reference sequences for *C. suchus*. Thus, the results show that the 5 samples from the Awash River belong unambiguously to *C. suchus*.

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1.0

In the network displays, for CO1 there were 12 haplotypes (I-XII) present, of which haplotypes I-VI and VII-XII form two separated groups. Each group shows very little

Croc. No.	Sample No.	Date Time, Location	GPS Coordinates	TL (cm), Sex	Type of Sample		Notes
1	LS1 *	7 April 2016	-	150, unknown	dry	Taxidermied	Museum Awash Park, old specimen; provenance and age unknown
2	LS2	7 April 2016	-	90, unknown	dry	Taxidermied	Museum Awash Park, old specimen; provenance and age unknown
3	LS3	7 April 2016	-	40, unknown	dry	Taxidermied	Museum Awash Park, old specimen; provenance and age unknown
4	LS4 *	9 April 2016 0900 h; Bilen	37 P 636251 1048451	180, unknown	wet	Dead	Shot by local people, about 10 d old
4	LS5	9 April 2016 0900 h; Bilen	37 P 636251 1048451	180, unknown	dry	Dead	Shot by local people, about 10 d old
5	LS6	10 April 2016 0700 h; Lake Gemeri	37 P 0788811 1274086	230, female	dry	Live	Sick, due to drought?
5	LS7 *	10 April 2016 0700 h; Lake Gemeri	37 P 0788811 1274086	230, female	wet	Live	Sick, due to drought?
6	LS8	10 April 2016 1900 h; Lake Gemeri	37 P 0788811 1274086	Unknown	dry	Dead	Dead, due to drought?; >1 mth old
7	LS9	10 April 2016 1900 h; Lake Gemeri	37 P 0788811 1274086	Unknown	dry	Dead	Dead, due to drought?; >1 mth old
8	LS10 *	11 April 2015 1930 h; Filowa, ANP	37 P 0611395 1007042	130, unknown	wet	Live	Caught and released
8	LS11	11 April 2015 1930 h; Filowa, ANP	37 P 0611395 1007042	130, unknown	dry	Live	Caught and released
9	LS12 *	12 April 2016 1700 h; Lake Beseka	37 P 0594619 0984643	190, female	wet	Live	Shot by study group
9	LS13	12 April 2016 1700 h; Lake Beseka	37 P 0594619 0984643	190, female	dry	Live	Shot by study group

Table 1. Crocodile tissue samples collected by EWCA/SDPASE in the Awash River Basin. Type of Sample: wet= in alcohol;ANP= Awash National Park; TL= total length; \*= samples analysed by Museum Koenig, Bonn, Germany.



Figure 5. Around 1000 crocodiles were estimated to be at this location in Lake Gemeri.

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