

**CROCODILE
SPECIALIST
GROUP
NEWSLETTER**

VOLUME 28 No. 4 • OCTOBER 2009 - DECEMBER 2009



IUCN • Species Survival Commission

CROCODILE

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COVER PHOTOGRAPH: 'Sacred' female Nile crocodile (*Crocodylus niloticus*) at the village of Bazoulé, Burkina Faso. Photograph: Christine Lippai.

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The CSG Newsletter provides information on the conservation, status, news and current events concerning crocodylians, and on the activities of the CSG. The Newsletter is distributed to CSG members and to other interested individuals and organizations. All Newsletter recipients are asked to contribute news and other materials.

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Editorial

A CSG Executive meeting with the Chair, Deputy Chairs and Executive Officer, was held somewhat opportunistically in Darwin, Australia (16-17 October 2009). It follows a similar successful meeting held before the 19th CSG meeting (Bolivia, June 2008). It was decided to move ahead with establishing an independent legal entity to manage the CSG's financial affairs, which was agreed at the last Steering Committee meeting. It is an important strategic step for sustaining the CSG over the long-term. It was also decided that there were benefits in broadening the CSG's membership, because there remain many people active with crocodile conservation and management around the world who are still not members: a draft CSG Membership Recruitment Strategy is being developed. It was decided that a small West Africa regional office is now warranted. A Crocodylian Capacity Building Manual, we believe, could help many people, especially within a CITES context. CSG representation at a series of up meetings was discussed (eg CoP15, Qatar, March 2010; West Africa Regional meeting, Burkina Faso, March 2010; International Workshop on Human-Crocodile Conflict, Sabah, June 2010; 20th CSG meeting, Manaus, September 2010).

The Philippine Department of Environment and Natural Resources, Protected Areas and Wildlife Bureau has offered to host the 21st CSG Working Meeting in Manila in 2012. The offer has been accepted, and we look forward to another meeting in Southeast Asia, the first since Singapore in 1998.

Concerns about oil palm development in Mesangat Lake, East Kalimantan, Indonesia, led to an exchange of letters with PT REA Kaltim Plantations regarding conservation and protection of the remnant populations of *Tomistoma schlegelii* and *Crocodylus siamensis*. The company has expressed a willingness to cooperate with the CSG on this issue. In Jakarta I met with senior officials from the Ministry of Forestry to discuss the possibility of Mesangat being declared as a RAMSAR site.

Following deliberations at the 58th meeting of the CITES Standing Committee (www.cites.org/eng/com/SC/58/sum/index.shtml), the CITES Secretariat, in collaboration with CSG, will deliver a Training Workshop for Government authorities in Madagascar, in February 2010.

CITES CoP15 (Qatar, 13-25 March 2010) will see the CSG well represented on delegations and among NGOs. The crocodylian issues being debated include: Ranching and trade in ranched specimens; Production systems for specimens of CITES-listed species; Review of the universal tagging system and trade in small crocodylian leather goods; and, Personal and household effects. Two amendment proposals (from Appendix I to Appendix II) are also on the agenda.

Mexico has developed its proposal on *C. moreletii* in close collaboration with the CSG [see Newsletter 28(3), page 3]. Mexico does not at this time intend to utilize wild populations of *C. moreletii*, and has sought an Appendix-II listing with a zero quota. If a use program involving the wild population is established in the future, a new proposal will be developed and submitted to a future CITES CoP. Egypt's proposal at CoP15 is based on ranching [Resolution Conf. 11.16 (Rev. CoP14)]. CSG members reviewed the proposal after its submission and provided comments back to Egypt. The CITES Secretariat has noted some technical anomalies with the submission date (ranching proposals need to be submitted 330 days prior to the CoP), and at this stage it is not clear where the proposal can be considered under Resolution Conf. 9.24 (Rev. CoP14).

The French fashion house *Lacoste* is proposing to contribute \$500,000 over the next three years to gharial (*Gavialis gangeticus*) conservation research in Nepal, under the "Save Your Logo" initiative (see page 5). This is a very significant contribution for which *Lacoste* are to be congratulated.

The CSG Action plan species accounts are now complete and a revised web-based document should be posted on the website in early 2010. I wish to thank all those CSG members who have contributed to this process over the past 12 months.

The CSG approved 21 Student Research Assistance Scheme projects in 2009 (see page 4), and with the continued support of the Bergen Aquarium (Norway) and the Krokodille Zoo (Denmark) the scheme will continue operating.

Let me take this opportunity to thank all CSG members and donors for their support during 2009, and to wish all a Happy New Year for 2010.

Professor Grahame Webb, CSG Chairman

CSG Lore

John Lever, one of our longest-serving CSG members, submitted the following poem that he wrote as he was flying in a DC3 to Maningrida in 1976, to attend the 3rd CSG Working Meeting. John had been working with crocodiles in Papua New Guinea since 1972, and tried to put his thoughts into words that reflected his involvement with “conservation through commerce” and its importance to the environment.

CROCODILE

Two burning embers
In the torchlight glow
We see the monster close and clear
Head above water - we come nearer, so near
We see his teeth and jaws like steel
That could crush away the life of man
And we shun such a beast as cruel and evil
Devoid of rights to water or land.
But yet in our efforts to kill him out
We find he has real worth
For he controls the riverine life
And designs a balance so fine
That allows all creatures within his domain
To live on natures vine.
To remove him from his place
Is to take a King from his throne
And his scores of subjects are leaderless
Controls and disciplines unknown
Thieves and beggars
Weeds and parasites
In numbers have grown and grown
So now, in reflection, we see the folly
Of removing natures controls with haste
Because of financial gain or distaste
For this animal that does not please our eye
Is an evolved creation like you and I
So why should he be hunted to death by man
If he is part of the creators plan.

Printed with permission of John Lever (Koorana Crocodile Farm, Rockhampton, Australia; koorana@westnet.com.au).

Student Research Assistance Scheme Update

In the last quarter of 2009, 5 projects were awarded Student Research Assistance Scheme (SRAS) funding of \$US1000.

- Vincent Shacks (Rhodes University, South Africa): Accumulation of persistent organic pollutants (POPs) in the Nile Crocodile (*Crocodylus niloticus*) as a consequence of aerial spraying to eradicate tsetse fly from the Okavango Delta, Botswana.
- Kristian Pahl (Alexander Koenig Research Museum and Cologne Zoo, Germany): Natural history of the Siamese crocodile (*Crocodylus siamensis*).
- Ashley Percy (Institute of Biology, Leiden University, The

Netherlands): Ecological and conservation implications of skull morphometrics in sympatric species of crocodylians.

- Alejandro Villegas (Instituto de Biología, Universidad Nacional Autónoma de México; Universidad Autónoma Metropolitana, Unidad Xochimilco, Mexico): Genetic structure and flow of genes in *Crocodylus moreletii* in Mexico.
- Dusty Savage (McNeese State University, Louisiana, USA): Nest defense strategies of the American alligator (*Alligator mississippiensis*)

A total of 21 projects were received and funded in 2009, involving 12 crocodylian species in 13 countries, including three of the most critically endangered species: *Alligator sinensis*, *Crocodylus siamensis* and *Crocodylus mindorensis*.

Tom Dacey, *CSG Executive Officer*, <csg@wmi.com.au>.

20th CSG Working Meeting

The 20th CSG Working Meeting will be hosted by the Amazonas State Government, and held in the city of Manaus, Brazil, from 13-16 September 2010. The CSG Steering Committee meeting will take place prior to the working meeting, on 12 September, and various “field trips” will be available to participants during and after the meeting.

Information is available on the meeting website (www.csgmeeting.com.br), which will be regularly updated with details on the agenda, accommodation, etc.

Tom Dacey, *CSG Executive Officer*, <csg@wmi.com.au>.

International Workshop on Human-Crocodile Conflict (HCC)

Organised by the CSG, Sabah Wildlife Department and University Malaysia Sabah, the “International Workshop on Human-Crocodile Conflict (HCC)” will be held on 9-11 June 2010, at the Shangri-La Rasa Ria Hotel, Tuaran, Sabah, Malaysia.

The theme of the workshop is “Crocodile Conservation Through Sustainable Use”, and a key objective is to develop an integrated and practical approach to crocodile management that incorporates better solutions to HCC problems in the Pan Borneo Region.

For further details contact Silvester Saimin (Silvester.Saimin@sabah.gov.my or slyluki@hotmail.com).

CITES Notification 2009/048: Revised List of Crocodylian Skin Tag Manufacturers

In November 2009, the CITES Secretariat sent Notification 2009/048 to the Parties, concerning the universal tagging system for the identification of crocodylian skins (Resolution

Conf. 11.12). The Notification communicated a revised list of manufacturers able to produce tags for marking crocodilian skins in accordance with Resolution Conf. 11.12.

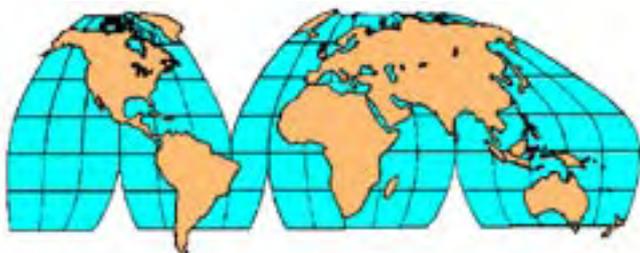
Full details of each manufacturer are available from the Notification, versions of which are available in English, Spanish and French at www.cites.org/eng/notif/2009/E048.pdf, www.cites.org/esp/notif/2009/S048.pdf and www.cites.org/fra/notif/2009/F048.pdf respectively.

Lacoste Supports Gharial Conservation

Lacoste will spend \$500,000 over the next three years to help conservation of endangered crocodiles, with the Gharial (*Gavialis gangeticus*) being the focus of their attention. Lacoste was encouraged into the scheme by people at the Global Environment Facility, the World Bank, and the IUCN's "Save Your Logo Initiative".

Lacoste were the first company to embroider a logo onto apparel 75 years ago, and have now become the first fashion company to support "Save Your Logo" initiative. According to "Save Your Logo", more than 300 large global brands use animals or plants threatened by dwindling biodiversity in their logos.

Regional Reports



East and Southeast Asia

Cambodia

DNA STUDY REVEALS PURE SIAMESE CROCODILES AT PHNOM TAMAO WILDLIFE RESCUE CENTRE, CAMBODIA. The critically endangered Siamese crocodile (*Crocodylus siamensis*) was once widespread throughout Southeast Asia, but its range and numbers have been severely reduced due to hunting for skins, habitat alteration for agriculture and, since the 1950s, the collection of live animals for the farming industry. The 1992 Crocodile Action Plan feared the Siamese crocodile to be "effectively extinct in the wild". It was not until biological surveys conducted by the Forestry Administration (FA) and Fauna & Flora International (FFI) in 2000 that wild *C. siamensis* were re-discovered in small numbers in the Cardamom Mountains of southwest Cambodia. Subsequent conservation efforts involving local communities have successfully stabilised the largest *C. siamensis* colonies in Cambodia, but this progress

is becoming undermined by emerging new challenges, notably major hydro-electric dam developments. Another problem is that many of the wild *C. siamensis* exist as isolated individuals or in very small, fragmented groups that are no longer capable of breeding. The entire reproductive output of the wild Cambodian population appears to be no more than 5 nests per year.

With a view to boosting Cambodia's wild stocks of *C. siamensis* through captive breeding, the FA and FFI have begun examining captive *C. siamensis* in Cambodia to identify pure stocks. More than 10,000 crocodiles are held in at least 1000 farms and other facilities in Cambodia, ranging from 'backyard' household operations with only a few individuals to large-scale production facilities with thousands of crocodiles (Jelden *et al.* 2005). Unfortunately, the captive *C. siamensis* gene pool has been compromised by hybridization with *C. porosus* and *C. rhombifer*, and very few facilities mark their animals or keep accurate breeding records. Pure-bred *C. siamensis* can be difficult to distinguish from hybrids on the basis of morphology alone.

The first facility to be screened in 2009 was the Phnom Tamao Wildlife Rescue Centre (PTWRC), which is managed by the FA for conservation and education purposes. The objective of this study was to determine whether PTWRC contained any pure-bred *C. siamensis* that could be used as founder stock for future re-introductions.

Background of Conservation Activities and Contemporary Threats

Upon the rediscovery of *C. siamensis* in the Cardamom Mountains in 2000 (Daltry and Chheang 2000), the Cambodian Crocodile Conservation Programme (CCCP) was founded by the FA and FFI to conserve the remaining populations in Cambodia. Surveys by CCCP staff since 2002 have confirmed 35 sites in Cambodia that contain *C. siamensis* (although most consist of only one or a few non-breeding individuals). Three sites in the Cardamom Mountains - Veal Veng, Areng and Chay Reap - contain critically important breeding colonies with an estimated 120 crocodiles, representing more than 50% of Cambodia's population of wild Siamese crocodiles. Another small group which may be reproductively active was observed in 2009 in Mondulkiri by the FA, FFI and (World Wildlife Fund (WWF)); more studies are needed at this site. Also in recent years, surveys throughout the region conducted by FFI, Wildlife Conservation Society (WCS) and other organizations have confirmed small remnant groups of *C. siamensis* in Thailand, Laos, Vietnam and Indonesia, though seemingly none as large as Cambodia's.

Conservation activities by the CCCP to date at the three breeding sites have included community engagement through alternative livelihoods development to reduce pressure on the wetlands (in partnership with CEDAC, a local NGO), participatory land use planning to establish permanent crocodile sanctuaries, recruitment and training of community wardens to conduct regular patrols, and providing school teachers, underpinned with regular monitoring and research

using camera trapping (eg Holden 2007) and radio-telemetry. In addition, other organizations such as Conservation International (CI) and WCS have also been actively involved in crocodile conservation. CI has been conducting its Community Stewardship Program in the Central Cardamom Mountains, to encourage local communities to help conserve protected species and habitats, and WCS, with the Fisheries Administration (FiA), is patrolling the Sre Ambel River (recently seizing two juvenile crocodiles from a fisherman) and has conducted radio-telemetry studies in the Tonle Sap.

As Cambodia emerges from decades of genocide, war, and poverty, other development activities threaten to have a negative impact on key *C. siamensis* population sites. In 2003, the Royal Government launched its Rectangular Strategy for National Development, which determined that Cambodia had the highest rates per kilowatt hour for electricity in the region and that energy development through hydro-power was a national priority to improve the economy and encourage foreign investment. Since this time, at least 14 hydro-power projects have been proposed, including 5 on rivers within the Cardamom Mountains of southwest Cambodia, of which two directly threaten the two largest *C. siamensis* colonies remaining in the wild (Veal Veng and Areng). As such, the Siamese crocodile conservation strategy has needed to be urgently revised in order to meet this challenge. With the impending loss of the species' main breeding sites in the Cardamom Mountains, it is imperative to identify alternative sites where the species can be re-established and fully guarded.

DNA Sampling and Analysis

Plans for DNA sampling and analysis of the PTWRC crocodile population began several years ago but were not actualized until 2009. The PTWRC is operated by the FA with technical support from Wildlife Alliance and other organisations, and houses native species rescued from illegal wildlife traders or confiscated from zoos. As of early 2009 there were 69 crocodiles that had been either donated or rescued; 15 adults and 54 young (mainly progeny of the adults at the centre).

Sampling of the PTWRC population was conducted in February 2009. Large crocodiles were captured by trained teams from the PTWRC and CCCP with support from Wildlife Alliance. Field veterinarians Dr. Verne Dove (WWF/FiA/Cambodian Mekong Dolphin Conservation Project) and Dr. Nhim Thy (FA/PTWRC) were on site to monitor the health of the animals and attend to any injuries. Once crocodiles were captured, they were measured (head length, snout-eye length, snout-vent length, total length), sexed and individually marked by scute-clipping. Adults were also injected with an "AVID" microchip under the epidermal scales of the mid-section of the tail.

DNA samples were obtained from adults and juveniles from: (i) 2 ml of blood drawn using 14G (50 mm) needles between the ventral scales near the base of the tail and placed in EDTA in vacuum-sealed tubes, and (ii) sections of the excised tail scutes placed in 70% ethanol/saline solution in vacuum-

sealed tubes. Samples were stored at -30°C until they could be transported to a laboratory. Samples from hatchlings were collected using similar methods, except needle size was smaller - 21G (40 mm). All 69 animals at the PTWRC were measured and sampled.

As there is no laboratory in Cambodia with the capacity to conduct DNA analysis, the project contacted CSG member Yosapong Temsiripong in Thailand for advice. On Mr. Temsiripong's recommendation, the Department of Genetics at Kasetsart University, under the direction of Dr. Somsak Apisitwanich, was approached to assist. This department had previous experience in screening 10 *C. siamensis* for a soft re-introduction to Pang Sida National Park, Thailand, in 2006 (Temsiripong 2007a, b). Once CITES export and import permits were prepared by the respective Government agencies, the samples were flown from Phnom Penh to Bangkok.

The analyses took approximately 3 months to complete. DNA was extracted from both blood and tissue samples and amplified by PCR using 10 specific primers (CscytB, CpcytB, CSA6, CSA7, CSA15, CSA38, CSA49, MSCro25, MSCro26, MSCro35). First, the cytochrome-b region in the mtDNA was analyzed to identify the crocodile's maternal line, with reference to two primer pairs for Cyt-b of *C. siamensis* (CscytB) and Cyt-b of *C. porosus* (CpcytB). The next, more important analysis, was conducted on the nuclear DNA using 8 dominant and co-dominant markers to reveal pure-bred or hybrid status. The control DNA was *C. siamensis* and *C. porosus*, but if an additional DNA band appeared (or was absent) that differed from the *C. siamensis* and *C. porosus* controls, it was considered to be an unknown species, most likely *C. rhombifer*.

The cytochrome-b gene of all samples analyzed was determined to be *C. siamensis*, confirming that all of the crocodiles sampled were the progeny of either a pure-bred female *C. siamensis* or a hybrid female. None of the sampled crocodiles were the progeny of a *C. porosus* mother. For the other 8 nuclear genes, 35 crocodiles were found to be pure-bred *C. siamensis*, 31 contained hybrid DNA of both *C. siamensis* and *C. porosus*, and three samples contained hybrid DNA of *C. siamensis* and an unknown species (*C. rhombifer?*).

Conclusions

Although there are likely other captive pure-bred *C. siamensis* in farms throughout Cambodia, DNA analysis is required to confirm the species. The significance of the present study is that this is the first time in Cambodia that DNA from a captive population has been successfully collected and analyzed.

The results from this DNA analysis of the PTWRC crocodile population indicates that 35 of the 69 animals housed here are indeed pure-bred *C. siamensis*. These comprise 3 mature males, 3 mature females, one sub-adult female, 20 juvenile males, two juvenile females (more than one year of age), and 6 hatchlings of unknown sex. These findings will allow CCCP to plan a conservation breeding program at the PTWRC.



Figure 1. Pure-bred *C. siamensis* at the Phnom Tamao Wildlife Rescue Centre, whose genetic identity was confirmed through DNA analysis.

The next steps in the captive breeding and reintroduction programme will be to:

1. separate purebred *C. siamensis* from hybrids to prevent cross-breeding;
2. separate male and female hybrids to prevent them from breeding (these animals may be transferred to a separate facility);
3. construct breeding pens for at least 3 *C. siamensis* pairs and rearing pens for offspring;
4. identify at least 3 sites with suitable habitat that can be adequately protected; and,
5. develop a re-introduction plan using the guidelines of the IUCN-SSC Re-introduction Specialist Group. The reintroduction will be presented to the CSG and Re-introduction Specialist Group for review. It is hoped that the first pure-bred young could be released in appropriate, secure sites in Cambodia as early as the end of 2012.

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Philippines

Crocodile meat has been on restaurant menus in many parts of Southeast Asia (eg Thailand, China, Singapore) for many years, but it is only now making an appearance in the Philippines. The Hai Kang Restaurant in Manila has been offering various crocodile dishes for a few months now. Patrons are enticed to try it, and if they don't like it they don't have to pay for it - so far everyone has paid!

According to Benedict Solco, Operations Manager for Coral Frams, other restaurants are slowly showing an interest in crocodile meat, which has never been a food source in the Philippines.

Source: Alastair McIndoe, "Filipinos snap up crocodile meat", *Vientiane Times*, 11 December 2009.

South Asia and Iran

Sri Lanka

OBSERVATIONS OF MUGGERS AT BLOCK 1, RUHUNA (YALA) NATIONAL PARK. Sri Lanka is the southernmost location for the distribution of the Mugger (*Crocodylus palustris*), and has the largest wild population of the species. The Mugger, a highly adaptable species that may live side by side with humans in anthropogenic habitats, is mainly distributed in the dry zone plains of Sri Lanka and commonly inhabits man-made fresh water pools known as 'tanks', most of which are 2-3 thousand years old. It is also found in rivers, streams, agricultural canals, and occasionally in agro-wells. Some may also be observed in hyper-saline lagoons adjoining the sea.

Ruhuna National Park, popularly known as Yala National Park (YNP) consists of five blocks covering an area of 1594 km² and is one of the most popular national parks in the country. Block 1 (140 km²) was gazetted as a National Park under the Department of Wildlife Conservation on 25 February 1938 (Department of Wildlife Conservation 2001). The present study on the status of *C. palustris* was conducted in Block 1 (Fig. 1), which contains 37 tanks of different sizes. Of these tanks, Paltupana, Buthawa, Heen wewa and Katagamuwa wewa (a few metres outside Ruhuna

NP boundary in the Kataragama Sanctuary), are well known to harbour appreciable mugger populations.



Figure 1. Map showing location of Block 1, Ruhuna (Yala) National Park.

We visited Block 1 in August 2009, spending 3 days and nights inside the park. We were informed by Wildlife Department staff that most of the tanks in the park were drying up due to drought, and that crocodiles had migrated to the few tanks with sufficient water. Counts were made during the day and at night using binoculars.

1. Day and night counts were made at Paltupana (Fig. 2), Buthawa (Fig. 3) and Heen wewa on 20-22 August. Maximum day counts were 78 (74 basking, 4 in water), 264 and 21 for Paltupana, Buthawa and Heen wewa respectively. Maximum night counts were 80, 300 and 83 eyeshines for Paltupana, Buthawa and Heen wewa respectively.



Figure 2. Paltupana wewa. Photograph: Anslem de Silva.



Figure 3. Buthawa wewa. Photograph: Anslem de Silva.

2. On 21 August we visited Katagamuwa tank and were informed by security personnel living a few metres away from the tank that there had been nearly 200 crocodiles the previous week. However, by the time of our visit the water had dried up to a mere 30 x 50 m area; 51 adults were counted in late afternoon (1700 h) and 42 eyeshines at night (1900 h). Due to drying up of Katagamuwa tank Muggers may have migrated to Menikganga (river), approximately 3-4 km overland through the forest, Heen wewa (approximately 5 km away) and Buthawa (approximately 10 km away). The Katagamuwa tank was completely dry by the first week of October 2009.

3. Day counts were also recorded at Mahaseela wewa (3), Siyambalagas wewa (0), Palugas wewa (11 adults), Uraniya wewa (0) and Buthawa lagoon (3 adults) on 21-22 August.

Based on the maximum counts at these 9 tanks, there is a minimum of 531 Mugger present. Taking into account individuals that were not sighted during the visit (ie underwater, obscured from vision, aestivating in tunnels and banks) and areas that were not surveyed, the number of *C. palustris* in Block 1 of Yala National Park is estimated to be in excess of 600 non-hatchling *C. palustris*.

On the evening of 21 August, during heavy rains, we observed several adults and sub-adults walking overland, perhaps to other tanks with water and the river. By the third week of October 2009, Katagamuwa tank (see above) and several other tanks were all completely dry and the Muggers had migrated to the saline lagoon and the Menikganga (river). Counts made by K.H. Susanthain in Platupana tank indicated a decrease in the Mugger population as the tank dried, from 101 on 26 August to 21 on 4 October 2009.

Comparison of these recent data with historical survey data suggest an increase in the Block 1 population over time (10-30 years):

1. Whitaker and Whitaker (1979) counted 109 Muggers in 14 water bodies (tanks, lagoons and Menik River) in October 1977.
2. Jayaratne (1993), a Wildlife officer working at Yala, counted 284 Muggers in 23 water bodies (tanks, lagoons and Menik River) during June, July and August 1993.

3. Whitaker (1999) reported 230 counts of Muggers in 16 water bodies (tanks, lagoons and Menik River) between 26 and 31 August 1999.
4. Mohamed (1999) counted 83 Muggers in 28 water bodies (tanks, lagoons and Menik River) during July 1999.

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Bangladesh

GHARIAL SURVEYS INITIATED. The Gharial (*Gavialis gangeticus*) is considered to be on the verge of extinction in Bangladesh, and it is possible that there are no breeding adults remaining in the wild. The Centre for Advanced Research in Natural Resources and Management (CARINAM) has started a survey to status of the breeding population of gharials in Bangladesh. The project is supported by the Mohamed bin Zayed Species Conservation Fund (Abu Dhabi, UAE).

With permission from the Forest Department, CARINAM began surveys in the Padma and Jamuna Rivers. CARINAM has requested people with any information on sightings of adult breeding gharials anywhere in Bangladesh to contact its Dr. S.M.A. Rashid (carinam95@yahoo.com), its Chief Executive.

The Mugger (*Crocodylus palustris*) is no longer found in the wild in Bangladesh, and the Saltwater crocodile (*C. porosus*) is only found in the Sunderbans, where it is facing various threats, including habitat destruction, human disturbance and incidental capture by fishermen.

Source: www.thedailystar.net/newDesign/news-details.php?nid=116619

Europe

PROTIVIN CROCODILE ZOO NEWS. Twenty (20) Cuban Crocodiles (*C. rhombifer*) were hatched successfully at the Protivin Crocodile Zoo (PCZ; Protivin, Czech Republic), the third time that the species has been bred there. Genetically tested and confirmed to be pure, the PCZ is offering the offspring to scientifically led zoos that are willing to participate in conservation programs for this Critically Endangered species. Mrs. Barbara Montalvo from the Embassy of the Republic of Cuba welcomed the hatchlings at a ceremony at PCZ.

The PCZ is also supporting the Danau Mesangat Crocodylian Conservation Project by Yayasan Ulin (Ironwood Foundation) in East Kalimantan, Indonesia. For this, the zoo is presenting a poster exhibition on Danau Mesangat and has edited a colored "Crocodile Atlas" in Czech language, which is available at the zoo. The income generated by this publication will go directly to the conservation project. The zoo is also working on further collaborative projects with Yayasan Ulin and its Chairman Rob Stuebing, and is supporting the CSG Tomistoma Task Force.

One male and three female sub-adult Philippine crocodiles (*C. mindorensis*) were imported to PCZ. Zoo personnel visited the Philippines in February 2009 and met with responsible authorities at Silliman University, Palawan Crocodile Farming Institute and others, to establish a breeding loan agreement, and discussed support for Philippine biodiversity conservation projects. Financial support was also provided for the construction of exhibits for critically endangered Hornbill species at the Kabankalan Rescue Center/DENR. Representatives of the PCZ, Avilon Zoo and DENR signed the Breeding Loan Agreement for the animals. Through this, PCZ has become an official participant in the Philippine Crocodile Conservation Program. Currently, 20 *C. mindorensis* are housed in European zoos, with 8 (3:5) of them at PCZ.

The PCZ is very grateful for substantial support of the European Union, which is not only supporting the zoo as a important tourist destination in South Bohemia, but also for

its contribution to conservation and education, following the WAZA strategy.

Miroslav Prochazka (*Director, Protivin Crocodile Zoo, porosus@atlas.cz*) and Ralf Sommerlad (*CSG Regional Vice Chair for Europe, crocodilians@web.de*).

Science



Recent Publications

Kundrát, M. (2009). Heterochronic shift between early organogenesis and migration of cephalic neural crest cells in two divergent evolutionary phenotypes of archosaurs: crocodile and ostrich. *Evol. Dev.* 11(5): 535-546.

Abstract: Living archosaurs (crocodiles and birds) represent an intriguing evo-devo model system. Although close in phylogenetic relationship, the two lineages show considerable divergence in trends of phenotypic evolution. The head anatomy of recent crocodylians has changed little in comparison with that of their crocodylomorph ancestors. The head phenotype of the avians (birds), as well as some non-avian theropods, shows numerous evolutionary innovations that differ considerably from the crocodylomorph pattern. Most of the novel head structures, such as features of the craniofacial skeleton, cranial nerves, head muscles, and integument are derived from the same cellular source common to all archosaurs, the cephalic neural crest (CNC). Therefore, other factors must be involved in the developmental disparity of homologous structures in the aforementioned lineages. The present study analyzes the earliest developmental events that are associated with the appearance of the neural crest cells in the two archosaur models: *Crocodylus niloticus* and *Struthio camelus*. I found that both models share unique developmental features, the presence of an unpaired, rostrally migrating population of CNC cells, showing that the two are closely related to each other. On the other hand, the crocodile and the ostrich differ substantially in (1) timing, (2) duration, and (3) expression patterns of the CNC. Compared with the crocodile, the CNC cells in the ostrich (1) migrate much later into the embryonic head, (2) but relocate to their terminal positions faster, and (3) take specifically directed migratory routes in the mandibular/oral region and head/trunk-interface regions. I suggest that accelerated relocation of CNC cells combined with delayed head organogenesis may represent important innovative conditions in the developmental evolution of a new archosaur head phenotype.

Rey, F., González, M., Zayas, M.A., Stoker, C., Durando, M., Luque, E.H. and Muñoz-de-Toro, M. (2009). Prenatal exposure to pesticides disrupts testicular histoarchitecture and alters testosterone levels in male *Caiman latirostris*. *Gen. Comp. Endocrinol.* 162(3): 286-292.

Abstract: The increased use of agrochemical pesticides, such as atrazine (ATZ) and endosulfan (END), may have a significant impact on ecosystem health and biodiversity. The aim of this study was to investigate the consequences of in ovum exposure to ATZ and END on *Caiman latirostris* gonadal histo-functional features. Caiman eggs were collected from environmentally pristine areas and incubated in controlled conditions at male producing temperature (33°C). At stage 20 of embryonic development, the sensitive stage for gonadal sex determination, eggs were exposed to one dose of either END or ATZ. Gonadal histo-morphology was examined in caiman hatchlings and serum levels of testosterone were measured. Regardless of treatment condition, all eggs incubated at 33°C resulted in male hatchlings. Tortuous seminiferous tubules with increased perimeter, disrupted distribution of peritubular myoid cells (desmin positive), and emptied tubular lumens characterized the testes of pesticide-exposed caiman. An imbalance between proliferative activity and cell death was observed in the testes of caiman exposed to the higher doses of END, mainly due to a high frequency of apoptosis in intratubular cells. This altered cell turnover was associated with decreased testosterone levels. Prenatal exposure to only one dose of END and ATZ disrupted neonatal male gonadal histo-functional features. Alterations described here could have detrimental effects on the sexual maturation of the caiman and, ultimately, on the success of male caiman reproduction.

Poletta, G.L., Larriera, A., Kleinsorge, E. and Mudry, M.D. (2009). Genotoxicity of the herbicide formulation Roundup (glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and the Micronucleus test. *Mutat. Res.* 672(2): 95-102.

Abstract: The genotoxicity of pesticides is an issue of worldwide concern. The present study was undertaken to evaluate the genotoxic potential of a widely used herbicide formulation, Roundup (glyphosate), in erythrocytes of broad-snouted caiman (*Caiman latirostris*) after *in ovo* exposure. Caiman embryos were exposed at early embryonic stage to different sub-lethal concentrations of Roundup (50, 100, 200, 300, 400, 500, 750, 1000, 1250 and 1750 microg/egg). At time of hatching, blood samples were obtained from each animal and two short-term tests, the Comet assay and the Micronucleus (MN) test, were performed on erythrocytes to assess DNA damage. A significant increase in DNA damage was observed at a concentration of 500 microg/egg or higher, compared to untreated control animals ($p < 0.05$). Results from both the Comet assay and the MN test revealed a concentration-dependent effect. This study demonstrated adverse effects of Roundup on DNA of *C. latirostris* and confirmed that the Comet assay and the MN test applied on caiman erythrocytes are useful tools in determining potential genotoxicity of pesticides. The identification of sentinel species as well as sensitive biomarkers among the natural biota is imperative to thoroughly evaluate genetic damage, which has significant consequences for short- and long-term survival of the natural species.

Moore, B.C., Milnes, M.R., Kohno, S., Katsu, Y., Iguchi, T. and Guillette, L.J. Jr. (2009). Influences of sex, incubation temperature, and environmental quality on gonadal estrogen and androgen receptor messenger RNA expression in juvenile American alligators (*Alligator mississippiensis*). Biol. Reprod. 82: 194-201.

Abstract: Gonadal steroid hormone receptors play a vital role transforming ligand signals into gene expression. We have shown that gonads from wild-caught, juvenile alligator express greater estrogen receptor 1 (ESR1) levels than estrogen receptor 2 (ESR2). Furthermore, sexually dimorphic ESR2 mRNA expression (female>male) observed in reference site animals, Lake Woodruff, was lost in alligators from the contaminated Lake Apopka. We postulated that environmental contaminant exposure could influence gonadal steroid hormone receptor expression. Here we address questions regarding gonadal estrogen and androgen receptor (AR) mRNA expression in one-year-old, laboratory-raised alligators. What are relative expression levels within gonads? Do these levels vary between sexes or incubation temperatures? Can contaminant exposure change these levels? We observed a similar pattern of expression (ESR1 >AR>ESR2) in ovary and testis. However, both incubation temperature and environment modulated expression. Males incubated at 33.5°C expressed greater AR levels than 30°C females; dimorphic expression was not observed in animals incubated at 32°C. Compared to Lake Woodruff alligators, Lake Apopka animals of both sexes showed lesser ESR2 mRNA expression levels. Employing cluster analyses, we integrated these receptor expression patterns with those of steroidogenic factors. Elevated ESR2 and CYP19A1 expressions were diagnostic of alligator ovary, whereas elevated HSD3B1, CYP11A1, and CYP17A1 expressions were indicative of testis. In contrast, AR, ESR1, and NR5A1 showed variable expressions that were not entirely associated with sex. These findings demonstrate that the mRNA expression of receptors required for steroid hormone signaling are modified by exposure to environmental factors, including temperature and contaminants.

Johnston, M.A., Porter, D.E., Scott, G.I., Rhodes, W.E. and Webster, L.F. (2009). Isolation of faecal coliform bacteria from the American alligator (*Alligator mississippiensis*). J. Appl. Microbiol.

Abstract: Aim: To determine whether American alligators (*Alligator mississippiensis*) are an unrecognized poikilothermic source of faecal coliform and/or potential pathogenic bacteria in South Carolina's coastal waters. Methods and Results: Bacteria from the cloaca of American alligators, as well as bacteria from surface water samples from their aquatic habitat, were isolated and identified. The predominant enteric bacteria identified from alligator samples using biochemical tests included *Aeromonas hydrophila*, *Citrobacter braakii*, *Edwardsiella tarda*, *Escherichia coli*, *Enterobacter cloacae*, *Plesiomonas shigelloides* and putative *Salmonella*, and these were similar to bacteria isolated from the surface waters in which the alligators inhabited. Based on most-probable-

number enumeration estimates from captive alligator faeces, faecal coliform bacteria numbered $8.0 \times 10^9 \text{ g}^{-1}$ (wet weight) of alligator faecal material, a much higher concentration than many other documented endothermic animal sources. Conclusions: A prevalence of enteric bacteria, both faecal coliforms and potential pathogens, was observed in American alligators. The high faecal coliform bacterial density of alligator faeces may suggest that alligators are a potential source of bacterial contamination in South Carolina coastal waters. Significance and Impact of the Study: These findings help to increase our understanding of faecal coliform and potential pathogenic bacteria from poikilothermic reptilian sources, as there is the potential for these sources to raise bacterial water quality levels above regulatory thresholds.

Merchant, M., Monroe, C. and Falconi, R. (2009). Dipeptidyl peptidase IV activity in the blood of the American alligator (*Alligator mississippiensis*). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 154(3): 341-345.

Abstract: Dipeptidyl peptidase IV (DPP4) enzyme activity, associated with cell surface and soluble CD26, was measured in alligator plasma and whole blood. DPP4 activity was higher in whole blood than in the plasma, presumably due to the inclusion of both membrane-bound CD26 on the surface of T-cells and sCD26 in the plasma. The plasma DPP4 activity was measured within 5 min after the addition of the substrate, and maximal accumulation of product was reached at 120 min. Alligator whole blood DPP4 activity peaked at 120 min. In addition, the DPP4 activity in both plasma and whole blood was temperature-dependent, with moderate activities at lower temperatures (5-20°C) and peak activity at 40°C. Alligator plasma DPP4 displayed classical Michaelis-Menten kinetics, with a V(max) value of $267 \pm 9 \text{ nmol/min}$, and a K(m) of $21.1 \pm 1.9 \text{ microM}$, while these values were $286 \pm 14 \text{ nmol/min}$ and $57.1 \pm 3.7 \text{ microM}$, respectively, for whole blood. The proteolytic activity was inhibited in a concentration-dependent manner by a specific DPP4 inhibitor, diprotin A, which indicates that the activity is probably due to the presence of DPP4. The significance of the presence of this activity in alligator blood is still unknown.

Merchant, M., Heard, R. and Monroe, C. (2009). Characterization of phospholipase A(2) activity in serum of the American alligator (*Alligator mississippiensis*). J. Exp. Zool. A Ecol. Genet. Physiol. 311A.

Abstract: PLA(2) is a diverse class of enzymes with a broad spectrum of physiological functions. Secretory PLA(2) isoforms have been reported to exhibit important innate immune function in higher vertebrates. This study was conducted to characterize PLA(2) activity in the serum of the American alligator (*Alligator mississippiensis*). We used a glycerophospholipid with a fatty acid in the sn-2 position labeled with a fluorescent probe (BODIPY) to detect and quantify alligator serum PLA(2) activity. Incubation of BODIPY-labeled bacteria with different concentrations of alligator serum resulted in a concentration-dependent

detection of PLA(2) activity. Kinetic studies showed that product formation was rapid, with substantial activity within 5 min, and maximal activity at approximately 20 min. The alligator PLA(2) activity was temperature-dependent, with activity at lower temperatures (5-10°C) approximately half of that observed at temperatures of 30-40°C. In addition, the generation of fluorescent product was reduced by a specific inhibitor (p-bromophenacyl bromide) of secretory PLA(2) in a concentration-dependent manner, enforcing the idea that the observed activities are due to a secretory PLA(2) enzyme in alligator serum.

Poole, D.F.G. (2009). Notes on tooth replacement in the Nile crocodile *Crocodylus niloticus*. J. Zool. 136(1): 131-140.

Abstract: The history and replacement of crocodile teeth are described. A new tooth erupts with only a short root which grows downwards into the socket as the pulp cavity in the crown begins to be occluded by the further deposition of dentine. After the root has become fully extended, it is gradually resorbed at its base to make room for an enlarging successional tooth which has been forming on the lingual side of the socket. The successional tooth eventually moves to a position within the partly resorbed functional root and directly under the functional crown. Progressive reduction of the functional root now occurs to accommodate the continuously enlarging successional tooth and, when resorption is largely complete, the functional tooth falls away, allowing the successional tooth, with a short, incomplete root, to take its place. As a result of this successional cycle it is found, within certain limits, that at any given time the form and condition of a functional tooth is related to the size and condition of its successor lying below. Consideration of various measurements made on a series of 24 skulls of different sizes indicates that a regular and continuous replacement of teeth occurs throughout a large part of a crocodile's life. It is estimated that in a crocodile which is 13 feet long, each tooth is likely to have been replaced 45 times.

Seebacher, F., Murray, S.A. and Else, P.L. (2009). Thermal acclimation and regulation of metabolism in a reptile (*Crocodylus porosus*): the importance of transcriptional mechanisms and membrane composition. Physiological and Biochemical Zoology 82(6): 766-775.

Abstract: Energy metabolism is fundamental for animal fitness because it fuels locomotion, growth, and reproduction. Mitochondrial capacities often acclimate to compensate for negative thermodynamic effects. Our aim was to determine the importance of transcriptional regulation and membrane fatty acid composition in modulating oxidative capacities at body temperatures selected in a cold and a warm environment by a reptile (*Crocodylus porosus*). In the cool environment (mean selected $T_b = 21^\circ\text{C}$), mRNA concentrations of the transcription factor peroxisome proliferator-activated receptor gamma (PPAR γ) and its coactivator PPAR γ coactivator 1 alpha (PGC-1 α), as well as of the cytochrome c oxidase (COX) subunits COX1 and COX5, were significantly

higher in the liver but not in skeletal muscle compared with animals in the warm environment (mean selected $T_b = 29^\circ\text{C}$). F(O)F(1)-ATPase subunit α mRNA concentrations were significantly higher in both muscle and the liver in the cool animals. A positive relationship between PGC-1 α and PPAR γ mRNA concentrations, with an indicator of mitochondrial density (16S rRNA) in muscle and COX and F(O)F(1)-ATPase subunit α mRNA concentrations in liver, suggest that these proteins regulate quantity increases of mitochondria during acclimation. The percent saturated fatty acids in liver membranes of cool animals was significantly lower, and the n3 fatty acid content was significantly higher, compared with in warm animals. The n3 fatty acid content was positively related to COX enzyme activity in the liver, and there was a negative relationship between n7 fatty acid content and COX activity in muscle. Hence, metabolic acclimation is mediated by both transcriptional regulation and membrane fatty acid composition. The importance of PGC-1 α and PPAR γ in a reptile indicate that the mechanisms that regulate metabolism are conserved among vertebrates.

Brochu, C.A., Wagner, J.R., Jouve Stephane, Sumrall, C.D. and Densmore, L.D. (2009). A correction corrected: Consensus over the meaning of Crocodylia and why it matters. Syst. Biol. 58(5): 537-543.

Wang, X., Wang, D., Zhang, S., Wang, C., Wang, R. and Wu, X. (2009). Why do Chinese alligators (*Alligator sinensis*) form bellowing choruses: a playback approach. J. Acoust. Soc. Am. 126(4): 2082-2087.

Abstract: Crocodylians are quite vocal relative to other reptile groups, and the alligators are among the most vocal of the crocodylians. The Chinese alligator, *Alligator sinensis*, is usually solitary but engages in bellowing choruses in certain waters during the mating season. This paper reports the organization of Chinese alligator's bellowing choruses based upon field observations and playback experiments. Alligators of both genders engaged in the choruses, remaining immobile throughout and inclining toward bellowing synchronously (i.e., starting and finishing at about the same time). The choruses lasted about 10 min with abrupt onset and offset. Moreover, playback experiments revealed that both male and female alligators responded equally to bellowing stimuli from the same and opposite sexes and that none of the tested alligators approached the loudspeaker in spite of playback of male or female stimuli. These suggest that Chinese alligators may not bellow to compete for or attract mates during the choruses. Instead, when their ecological behaviors, namely, dispersed inhabitation, multi-copulation, restricted mating season, etc., are considered, we hypothesize that they may synchronize bellows to enhance group detectability for assembling individuals into certain waters for subsequent copulations.

Miles, L.G., Isberg, S.R., Thomson, P.C., Glenn, T.C., Lance, S.L., Dalzell, P. and Moran, C. (2009). QTL mapping for two

commercial traits in farmed saltwater crocodiles (*Crocodylus porosus*). Anim. Genet.

Abstract: The recent generation of a genetic linkage map for the saltwater crocodile (*Crocodylus porosus*) has now made it possible to carry out the systematic searches necessary for the identification of quantitative trait loci (QTL) affecting traits of economic, as well as evolutionary, importance in crocodylians. In this study, we conducted genome-wide scans for two commercially important traits, inventory head length (which is highly correlated with growth rate) and number of scale rows (SR, a skin quality trait), for the existence of QTL in a commercial population of saltwater crocodiles at Darwin Crocodile Farm, Northern Territory, Australia. To account for the uncommonly large difference in sex-specific recombination rates apparent in the saltwater crocodile, a dual mapping strategy was employed. This strategy employed a sib-pair analysis to take advantage of our full-sib pedigree structure, together with a half-sib analysis to account for, and take advantage of, the large difference in sex-specific recombination frequencies. Using these approaches, two putative QTL regions were identified for SR on linkage group 1 (LG1) at 36 cM, and on LG12 at 0 cM. The QTL identified in this investigation represent the first for a crocodylian and indeed for any non-avian member of the Class Reptilia. Mapping of QTL is an important first step towards the identification of genes and causal mutations for commercially important traits and the development of selection tools for implementation in crocodile breeding programmes for the industry.

Lance, S.L., Tuberville, T.D., Dueck, L., Holz-Schietinger, C., Trosclair, P.L., Elsey, R.M. and Glenn, T.C. (2009). Multiyear multiple paternity and mate fidelity in the American alligator, *Alligator mississippiensis*. Mol. Ecol. 18(21): 4508-4520.

Abstract: We examined multiple paternity during eight breeding events within a 10-year period (1995-2005) for a total of 114 wild American alligator nests in Rockefeller Wildlife Refuge in south-west Louisiana. Our goals included examining (i) within population variation in multiple paternity among years, (ii) variation in multiple paternity in individual females and (iii) the potential for mate fidelity. To accomplish this, in the current study, eggs were sampled from 92 nests over 6 years and analysed along with 22 nests from a previous 2-year study. Genotypes at five microsatellite loci were generated for 1802 alligator hatchlings. Multiple paternity was found in 51% of clutches and paternal contributions to these clutches were highly skewed. Rates of multiple paternity varied widely among years and were consistently higher in the current study than previously reported for the same population. Larger females have larger clutches, but are not more likely to have multiply sired nests. However, small females are unlikely to have clutches with more than two sires. For 10 females, nests from multiple years were examined. Seven (70%) of these females exhibited long-term mate fidelity, with one female mating with the same male in 1997, 2002 and 2005. Five females exhibiting partial mate fidelity (71%) had at least one multiple paternity nest and thus

mated with the same male, but not exclusively. These patterns of mate fidelity suggest a potential role for mate choice in alligators.

Claessens, L.P. (2009). A cineradiographic study of lung ventilation in *Alligator mississippiensis*. J. Exp. Zool. A Ecol. Genet. Physiol. 311(8): 563-585.

Abstract: The skeletal and visceral kinematics of lung ventilation of the American alligator (*Alligator mississippiensis*) was examined using cineradiography, pneumotachometry, and intrapulmonary pressure recording. The respiratory pattern of *A. mississippiensis* is intermittent and diphasic. The inspiratory lung volume is retained during the non-ventilatory period through closure of the glottis. The aspiration pump of *A. mississippiensis* consists of multiple components: visceral movement, pubic rotation, gastralial movement, and costosternal movement, which vary independently in their contribution to lung ventilation. Vertebral flexion and extension is also observed, and may be a passive artifact of costal displacement. The amount of craniocaudal visceral movement during lung ventilation is variable, and can produce as much as 60% of the tidal volume. Pubic rotation is not directly coupled to visceral movement and contributes a relatively small percentage of the tidal volume, approximately 4% on average, as does vertebral flexion, which contributes less than 3%. Costosternal movement contributes the remaining majority of tidal volume, generally over 40%. The gastralial stiffen the abdominal wall and likely facilitate unified displacement of the abdominal wall. Tripartite ribs facilitate thoracic movement, allowing substantial excursion of the body wall. A relatively abrupt change in position of the vertebral parapophysis in the anterior thorax results in an increase in lateral rib movement in the posterior half of the thorax. The crocodylian aspiration pump appears to consist of a derived pelvic and diaphragmatic breathing pump combined with a basal costosternal and gastralial aspiration pump.

Moore, B.C., Hyndman, K.A., Cox, A., Lawler, A., Mathavan, K. and Guillette, L.J. (2009). Morphology and histochemistry of juvenile American alligator (*Alligator mississippiensis*) nephrons. Anat. Rec. 292: 1670-1676.

Abstract: Here we present a detailed morphological description of the alligator (*Alligator mississippiensis*) kidney and nephron. We present a series of histological, histochemical, and immunohistochemical markers that clearly define the seven regions of the alligator nephron. The alligator kidney is composed of many paired (mirrored) lobules on each kidney (lobe). Single nephrons span the width of lobules three times. The fine structure of glomeruli, lying in rows spanning the height of the lobule, is resolved by periodic acid methionine silver (PAMS) and periodic acid Schiff's (PAS) histochemistry. Glomeruli are connected to the proximal tubule (PT) via a neck segment. The PT is alcian blue-negative, making it distinct from the distal tubule (DT), connecting segment (CS), and collecting duct (CD). The PT is clearly identifiable by a PAS-positive brush border

membrane. The PT is connected to the DT via an intermediate segment (IS) that makes a 180 degrees turn to connect these tubules. PAMS-positive material is found in the lumens of the PT, IS, and DT. Also, PAMS-positive granules are found in the DT, CS, and CD. Immunolocalization of the Na(+), K(+)-ATPase to the basolateral membrane of the DT, CS, and CD suggests a role of this enzyme in driving primary and secondary transport processes in these segments, including bicarbonate transport into the lumen of the DT (leading to an alkaline urine). Through the techniques described here, we have identified a series of distinct markers to be used by pathologists, veterinarians, and researchers to easily identify alligator nephron segments.

Kohno, S., Katsu, Y., Urushitani, H., Ohta, Y., Iguchi, T. and Guillet, L.J. (2009). Potential contributions of heat shock proteins to temperature-dependent sex determination in the American alligator. *Sex Dev.* DOI: 10.1159/000260374.

Abstract: Sex determination in the American alligator depends on the incubation temperature experienced during a thermo-sensitive period (TSP), although sex determination can be 'reversed' by embryonic exposure to an estrogenic compound. Thus, temperature and estrogenic signals play essential roles during temperature-dependent sex determination (TSD). The genetic basis for TSD is poorly understood, although previous studies observed that many of the genes associated with genetic sex determination (GSD) are expressed in species with TSD. Heat shock proteins (HSPs), good candidates because of their temperature-sensitive expression, have not been examined in regard to TSD but HSPs have the ability to modify steroid receptor function. A number of HSP cDNAs (HSP27, DNAJ, HSP40, HSP47, HSP60, HSP70A, HSP70B, HSP70C, HSP75, HSP90alpha, HSP90beta, and HSP108) as well as cold-inducible RNA binding protein (CIRBP) and HSP-binding protein (HSPBP) were cloned, and expression of their mRNA in the gonadal-adrenal-mesonephros complex (GAM) was investigated. Embryonic and neonatal GAMs exhibited mRNA for all of the HSPs examined during and after the TSP. One-month-old GAMs were separated into 3 portions (gonad, adrenal gland, and mesonephros), and sexual dimorphism in the mRNA expression of gonadal HSP27 (male>female), gonadal HSP70A (male<female), and adrenal HSP90alpha (male>female) was observed. These findings provide new insights on TSD and suggest that further studies examining the role of HSPs during gonadal development are needed.

Eme, J., Gwalthney, J., Blank, J.M., Owerkowicz, T., Barron, G. and Hicks, J.W. (2009). Surgical removal of right-to-left cardiac shunt in the American alligator (*Alligator mississippiensis*) causes ventricular enlargement but does not alter apnoea or metabolism during diving. *J. Exp. Biol.* 212(21): 3553-3563.

Abstract: Crocodylians have complete anatomical separation between the ventricles, similar to birds and mammals, but

retain the dual aortic arch system found in all non-avian reptiles. This cardiac anatomy allows surgical modification that prevents right-to-left (R-L) cardiac shunt. A R-L shunt is a bypass of the pulmonary circulation and recirculation of oxygen-poor blood back to the systemic circulation and has often been observed during the frequent apnoeic periods of non-avian reptiles, particularly during diving in aquatic species. We eliminated R-L shunt in American alligators (*Alligator mississippiensis*) by surgically occluding the left aorta (LAo; arising from right ventricle) upstream and downstream of the foramen of Panizza (FoP), and we tested the hypotheses that this removal of R-L shunt would cause afterload-induced cardiac remodelling and adversely affect diving performance. Occlusion of the LAo both upstream and downstream of the FoP for approximately 21 months caused a doubling of RV pressure and significant ventricular enlargement (average approximately 65%) compared with age-matched, sham-operated animals. In a separate group of recovered, surgically altered alligators allowed to dive freely in a dive chamber at 23 degrees C, occlusion of the LAo did not alter oxygen consumption or voluntary apnoeic periods relative to sham animals. While surgical removal of R-L shunt causes considerable changes in cardiac morphology similar to aortic banding in mammals, its removal does not affect the respiratory pattern or metabolism of alligators. It appears probable that the low metabolic rate of reptiles, rather than pulmonary circulatory bypass, allows for normal aerobic dives.

Vergne, A.L., Pritz, M.B. and Mathevon, N. (2009). Acoustic communication in crocodylians: from behaviour to brain. *Biol. Rev. Camb. Philos. Soc.* 84(3): 391-411.

Abstract: Crocodylians and birds are the modern representatives of Phylum Archosauria. Although there have been recent advances in our understanding of the phylogeny and ecology of ancient archosaurs like dinosaurs, it still remains a challenge to obtain reliable information about their behaviour. The comparative study of birds and crocodiles represents one approach to this interesting problem. One of their shared behavioural features is the use of acoustic communication, especially in the context of parental care. Although considerable data are available for birds, information concerning crocodylians is limited. The aim of this review is to summarize current knowledge about acoustic communication in crocodylians, from sound production to hearing processes, and to stimulate research in this field. Juvenile crocodylians utter a variety of communication sounds that can be classified into various functional categories: (1) "hatching calls", solicit the parents at hatching and fine-tune hatching synchrony among siblings; (2) "contact calls", thought to maintain cohesion among juveniles; (3) "distress calls", induce parental protection; and (4) "threat and disturbance calls", which perhaps function in defence. Adult calls can likewise be classified as follows: (1) "bellows", emitted by both sexes and believed to function during courtship and territorial defence; (2) "maternal growls", might maintain cohesion among offspring; and (3) "hisses", may function in

defence. However, further experiments are needed to identify the role of each call more accurately as well as systematic studies concerning the acoustic structure of vocalizations. The mechanism of sound production and its control are also poorly understood. No specialized vocal apparatus has been described in detail and the motor neural circuitry remains to be elucidated. The hearing capabilities of crocodylians appear to be adapted to sound detection in both air and water. The ear functional anatomy and the auditory sensitivity of these reptiles are similar in many respects to those of birds. The crocodylian nervous system likewise shares many features with that of birds, especially regarding the neuroanatomy of the auditory pathways. However, the functional anatomy of the telencephalic auditory areas is less well understood in crocodylians compared to birds.

Submitted Publications

MAXIMUM WIDTH OF ADULT CRANIUM. Recently, Whitaker and Whitaker (2008) reported Maximum Head Width (MHW) data for large crocodylian skulls (see Table 1 of that publication). However, the diagram of the dorsal view of a skull presented by Whitaker and Whitaker (2008) (Fig. 1 below) indicated a width measurement (MCW; Maximum Cranium Width) that is not used elsewhere in the article. Thus, to untrained people the MHW data in their Table 1 could possibly be misinterpreted as being measured as shown by MCW in their figure (see Fig. 1).

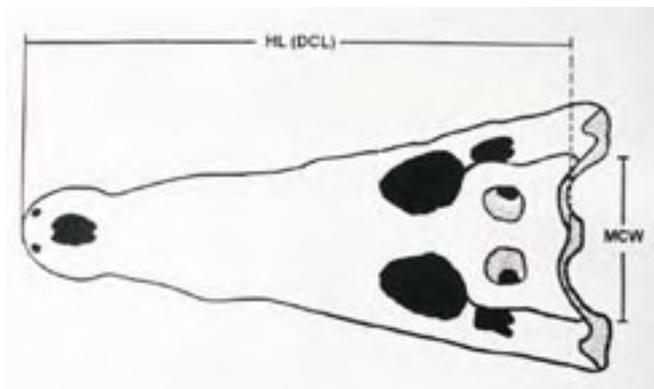


Figure 1. Dorsal view of skull from Whitaker and Whitaker (2008), showing MCW (Maximum Cranium Width), which could be mistaken for Maximum Head Width which is referred to elsewhere in the article.

As Whitaker and Whitaker (2008) used data from Webb and Messel (1978), it is clear that MHW in the former's Table 1 is indeed Maximum Head Width.

It should be noted that Webb and Messel (1978) used HMW as the acronym for the measurement of maximum head width (see #3 in Fig. 2), and Hall (1989) called it "Posterior Cranial Width" (see #2 in Fig. 3).

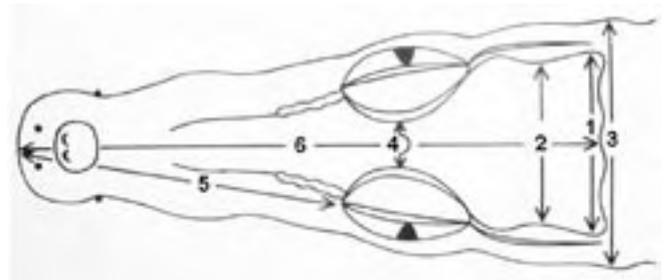


Figure 2. Dorsal view of skull from Webb and Messel (1978) showing maximum head width (3 = "HMP") and maximum width of the cranial platform (1 = "HPP").

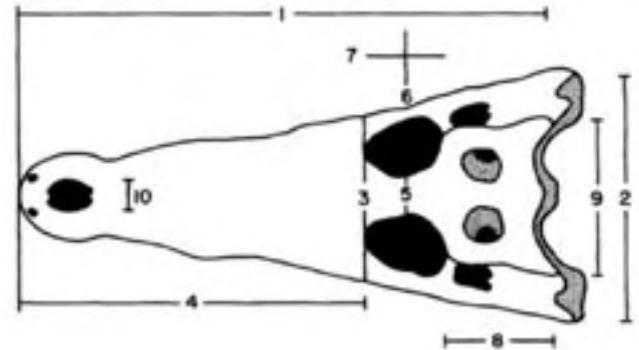


Figure 3. Dorsal view of skull from Hall (1989), showing maximum width of the head (2 = "posterior cranial width") and maximum width of the cranial platform (9 = "posterior cranial table width").

Much of the MHW data in Whitaker and Whitaker's (2008) Table 1 is new and extremely valuable, and the table itself is correctly defined with head length (HL; midline to the back edge of the cranial table) and maximum head width (MHW; across the quadrato-jugal bones) - only the diagram is potentially misleading.

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