Parasites and People: Crocodile Parasite Interactions in Human Impacted Areas of Belize – Final Report

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Introduction

This study, which was partly funded by the CSG SRAS grant scheme, examines the effect of parasite presence on the innate immune response and overall health of the American crocodile (*Crocodylus acutus*) and Morelet's crocodile (*Crocodylus moreletii*) in response to human presence and disturbance across Belize. More spec, it focuses on the relationship between the crocodiles and their parasites from various habitats within developed and rural landscapes in Belize. Investigations into the host's health response to haemoparasite presence and load, and to the presence of the parasitic nematode *Paratrichosoma* sp. were also conducted.

Examining the interaction between the host and its parasite may provide supplemental knowledge on the biodiversity and overall health of an ecosystem (Combes 2001, Tellez 2014, Tellez and Merchant 2015). Results from this study may in turn be used to detect changes in crocodile health during periods of anthropogenic disturbances, such as during pollution events and additional deterioration of the crocodiles preferred ecosystems.

Parasites and pathogens are experienced by most living organism, and are considered an important evolutionary driving force as they have the potential of altering fitness, fecundity and behavior of their host (Anderson and May 1978, May and Anderson 1978, Combes 2001, Tellez 2014). With an evolutionary lineage reaching back more than 200million years, crocodilians are a prime study candidate to investigate the evolutionary relationship between parasites and their hosts (Tellez 2014).

Contradictory to the general definition of a parasite, research conducted on American alligators (*Alligator mississippiensis*) indicate that some host-specific parasites of the digestive system of American alligators may have a beneficial effect on the hosts health rather than being deleterious (Tellez 2014, Tellez and Merchant 2015). Due to some parasites having a free-living life stage researchers are investigating the possibility for utilizing the presence of parasites such as the benign ectoparasite species *Paratrichosoma* sp. as biomarkers for changes in habitat quality. Changes in ecosystems would likely affect the nematodes free living stage, which in turn would impact the presence of the parasite in crocodilians. Utilizing this knowledge researchers are looking into using these parasites as early bioindicators for increased pollution and toxicity in the environment (Tellez and Paquet-Durand 2011, Tellez and Merchant 2015, Charruau et al. 2017).

The increase in anthropogenic interaction with crocodilians in Central America, and the lack of knowledge about immune system response and host-parasite interactions within the Order Crocodilia, as well as the interplay between these have been the driving force behind this study.

Methods

Study site

For this study the sampling locations were spread across Belize and divided into two

categories: urban/developed/developing areas (*developed* for short), and rural/isolated areas (*rural* for short) (**Fig. 1**).

Developed areas are areas that have been or are being developed for human habitation, industry or other commercial use.

Rural areas are areas with limited or no human impact. These areas include national parks, national reserves, field stations, and inhospitable and remote areas.



Figure 1: Developed and rural areas sampled in Belize marked with red and green pins, respectively. Inset shows Belize's placement in Central America (red pin). Picture retrieved from Google Earth, Jan 2019.

Captures, data collection and blood sample analysis

The collection of samples was performed throughout the range of *C. acutus* and *C. moreletii* in Belize from June 2018 and ended in the beginning of August 2018 (**Fig. 1**). All sample collections were done in partnership with the Crocodile Research Coalition (CRC), the Belize Forest Department, and several other national organizations. All data collection was performed with permits issued by the Belize Forest Department as well as the Belize Fisheries Department.

Before any captures commenced a nocturnal eyeshine survey (NES) was conducted. This data is used by the CRC to estimate the population size and density (Thorbjarnarson and Hernandez 1992, Tellez 2011, Fukuda et al. 2013, Tellez et al. 2016, Tellez et al. 2017).

During captures, crocodiles shorter than 120cm were caught by hand, whilst larger individuals were captured using a pole noose. The same method for capture and release of animals were used throughout the sample collection period (Tellez 2011, Mazzotti et al. 2012, Grigg and Kirshner 2015, Brandt et al. 2016).

Whilst the animal was under restraint morphometric measurements were registered to the nearest 0.1cm using the guidelines provided by the CRC Crocodile Population Survey Methods Manual, and all crocodiles were marked with a specific numbered code using scute clipping *(Tellez 2011)*. Local water pH, water temperature, air temperature and time of capture were also registered.

The presence of the parasitic nematode *Paratrichosoma* sp. was recorded by searching for the tell-tale *zig-zag* pattern female parasites creates in the skin of crocodilians. (Ashford and Muller 1978, Buenviaje et al. 1998, Moravec and Vargas-Vazquez 1998, Moravec 2001, Seijas 2007, Tellez and Paquet-Durand 2011, Padilla and Weber 2016, Charruau et al. 2017).

Blood samples were collected from 56 individuals, where 49 were registered as *C. acutus*, 6 registered as *C. moreletii*, and 1 registered as a hybrid with traits associated to both Morelets and American crocodiles. The blood was drawn from the caudal artery from the lateral side of the tail. Blood samples were added to 6mL vials containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) for hematological analysis (Stacy and Whitaker 2000, Sykes and Klaphake 2008). The samples were then fixated and stained for analysis. Eosinophil analysis, identification of blood cells, parasite identification, and manual parasite counts were performed at the CRC's headquarters in Placencia, Belize, using a binocular stereoscopic microscope at 100X magnification.

Body condition was used as a proxy for an animals health and was calculated with the assumption of an isometric growth as the investigation aimed at comparing body condition amongst localities. (Zweig 2003, Mazzotti et al. 2012, Brandt et al. 2016, Shirley et al. 2017)

Blood sample analysis

Blood smears were produced via a common pull-push method and fixated by bathing them in methanol (99,8%). They were then stained using Sigma-Aldrich modified Giemsa stain for cellular blood *components and blood parasites* and left to incubate according to Sigma-Aldrich protocol.

After the blood was prepared and stained a Differential White Blood Cell Count (DWBC), parasite counts, and total cell counts was conducted using a modified differentiation count method (Godfrey Jr et al. 1987).

Total blood cell counts were registered, as well as erythrocytes, eosinophils, signs of blood parasites and other leukocytes (**Figure 2**). Cells and parasites bordering the field of view were counted as ½. The erythrocytes were recognized as oval shaped cells with a clear border and a deep purple stained nucleus.

Leukocytes were counted as a way to measure the crocodile's immune response. Especially eosinophils (**Figure 2D**) were of interest as these are specifically linked to combatting parasite infections in other vertebrates, and the lack of evidence of the same function in crocodilians (Montali 1988, Jain and Jain 1993, Veiga et al. 1998, Sykes and Klaphake 2008, Zimmerman et al. 2010, Weiss and Wardrop 2011).

The percentage of infected erythrocytes were calculated from the total count to create a parasite: erythrocyte, haemoparasite: eosinophil ratio and haemoparasite: white blood cell ratio.

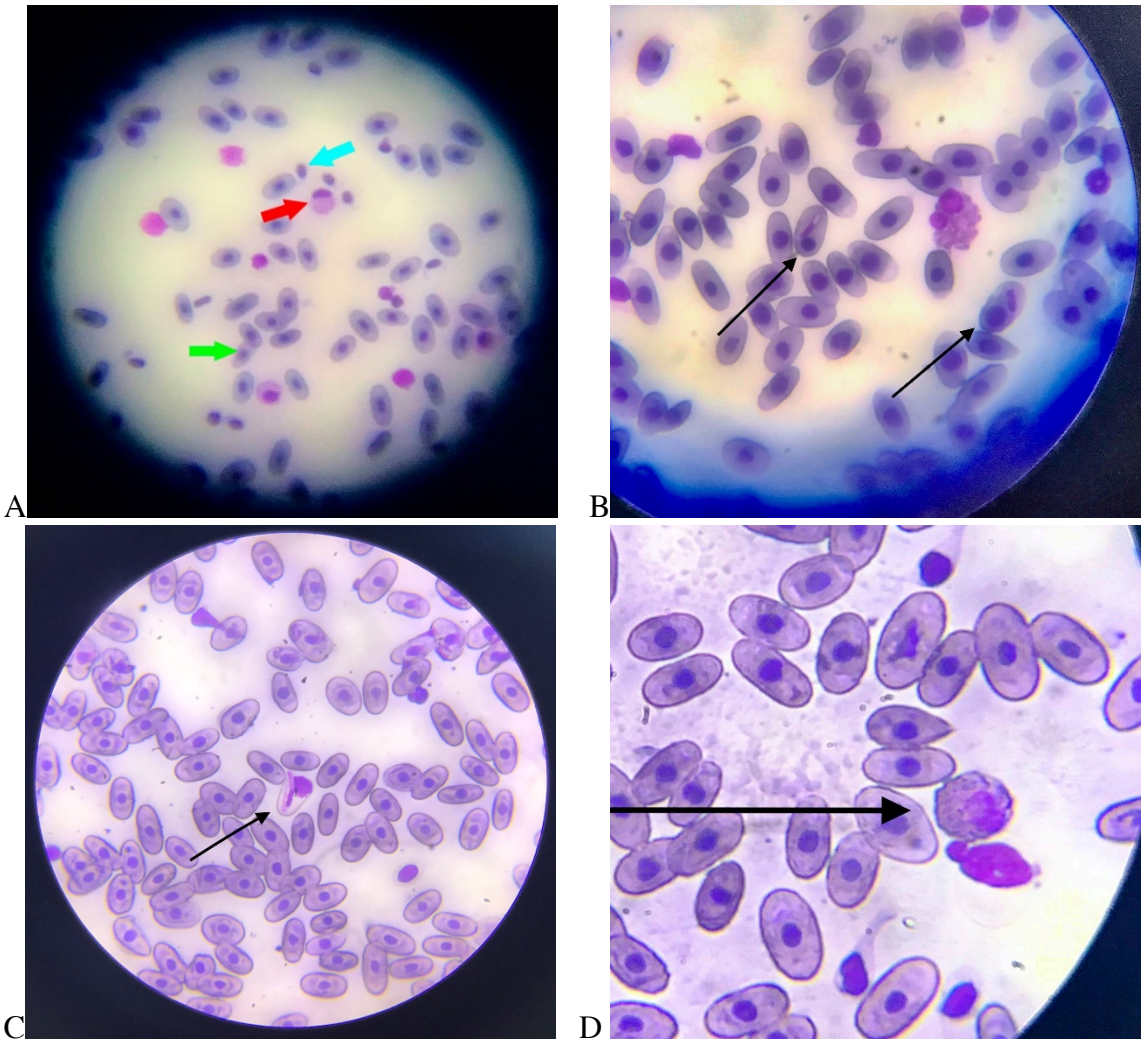


Figure 2: A: Blue arrow pointing towards thrombocyte, red arrow pointing towards a heterophil, green arrow pointing towards erythrocyte. B: Arrows pointing towards developed blood parasite inside erythrocyte. C: Arrow pointing towards blood parasite inside granulocyte. D: Arrow pointing to eosinophil with round granules

Interesting findings

The findings suggesting a 100% infection rate amongst both species of crocodiles in Belize, an infection rate previously not registered in crocodilians. The high parasite prevalence did not correlate with changes in the crocodilian immune response or their body condition, and neither did the ratio of infected blood cells. These finding could suggest either that these parasites have little or no adverse effect on the health of the crocodiles, or that we do not yet fully comprehend how the immune system of these animals handle parasite infections and that a potential effect will need to be measured using novel techniques specifically adapted to crocodiles.

One factor that did have an effect on the crocodiles' body condition was the animals proximity to humans. Crocodiles living close to humans had a significantly lower body condition compared with crocs in rural habitats. Additionally, crocodiles in developed areas also displayed a higher white blood cell count, suggesting that animals living in close to

humans are exposed to an unknown stressor more often than those in rural habitats, which may explain the lowered body condition of these animals.

Potential follow-up work

After conferring with Dr.Lutz Bachmann at the Norwegian Natural History Museum I am currently planning to perform genetic studies on the samples collected in Belize. The goal is to investigate whether the species of *Hepatozoon* infecting the blood of crocodiles in Belize are the same or separate species of haemoparasites. With the knowledge that most parasites are host specific the results from a study like this may contribute to further understanding of crocodilian evolution in the region, as well as increase our understanding of the host – parasite relationship (Combes 2001).

Another potential project would be to launch an investigation into the haemoparasites life cycle aiming at determining the parasites vector and its infection pathway into crocodilians. There are two invertebrate groups that have been put forward as the potential vectors of the *Hepatozoon* and *Haemogregarines* infecting crocodilians in the Americas, leeches and mosquitos (Khan et al. 1980, Viana et al. 2014). Studies have accomplished experimental transmission of the parasites on *Caiman yacare* from mosquitos, but similar studies have yet to be preformed on *Crocodylus acutus* or *C. moreletii*.

Reference list

Anderson, R. M., and R. M. May. 1978. Regulation and stability of host-parasite population interactions. I. Regulatory processes. Journal of animal ecology **47**:219-247

Ashford, R. W., and R. Muller. 1978. Paratrichosoma-Crocodilus N-Gen N-Sp (*Nematoda Trichosomoididae*) from Skin of New-Guinea Crocodile. Journal of Helminthology **52**:215-220.Doi 10.1017/S0022149x0000540x

Brandt, L. A., J. S. Beauchamp, B. M. Jeffery, M. S. Cherkiss, and F. J. Mazzotti. 2016. Fluctuating water depths affect American alligator (*Alligator mississippiensis*) body condition in the Everglades, Florida, USA. Ecological Indicators **67**:441-450.10.1016/j.ecolind.2016.03.003

Buenviaje, G. N., P. W. Ladds, and Y. Martin. 1998. Pathology of skin diseases in crocodiles. Australian Veterinary Journal **76**:357-363.DOI 10.1111/j.1751-0813.1998.tb12368.x

Charruau, P., J. S. Perez-Flores, and D. Labarre. 2017. Skin parasitism by *Paratrichosoma recurvum* in wild American crocodiles and its relation to environmental and biological factors. Diseases of Aquatic Organisms **122**:205-211.10.3354/dao03071

Combes, C. 2001. Parasitism: The Ecology and Evolution of Intimate Interactions. The University of Chicago Press.0-226-11446-5

Fukuda, Y., K. Saalfeld, G. Webb, C. Manolis, and R. Risk. 2013. Standardised method of spotlight surveys for crocodiles in the tidal rivers of the Northern Territory, Australia. Northern Territory Naturalist **24**:14

Godfrey Jr, R. D., A. M. Fedynich, and D. B. Pence. 1987. Quantification of hematozoa in blood smears. Journal of wildlife diseases **23**:558-565

Grigg, G., and D. Kirshner. 2015. Biology and Evolution of Crocodylians

Cornell University Press, CSIRO, USA.978-0-8014-5410-3

Jain, N. C., and A. Jain. 1993. Essentials of veterinary hematology. Lea & Febiger Philadelphia.081211437X

Khan, R. A., D. J. Forrester, T. M. Goodwin, and C. A. Ross. 1980. A haemogregarine from the American alligator (Alligator mississippiensis). J Parasitol **66**:324-332

May, R. M., and R. M. Anderson. 1978. Regulation and stability of host-parasite population interactions: II. Destabilizing processes. The Journal of Animal Ecology:249-267

Mazzotti, F. J., M. S. Cherkiss, L. A. Brandt, I. Fujisaki, K. Hart, B. Jeffery, S. T. McMurry, S. G. Platt, T. R. Rainwater, and J. Vinci. 2012. Body Condition of Morelet's Crocodiles (*Crocodylus moreletii*) from Northern Belize. Journal of Herpetology **46**:356-362.10.1670/11-188

Montali, R. 1988. Comparative pathology of inflammation in the higher vertebrates (reptiles, birds and mammals). Journal of Comparative Pathology **99**:1-26

Moravec, F. 2001. Some helminth parasites from Morelet's crocodile, *Crocodylus moreletii*, from Yucatan, Mexico. Folia Parasitologica **48**:47-62.DOI 10.14411/fp.2001.008

Moravec, F., and J. Vargas-Vazquez. 1998. First description of the male and redescription of the female of *Paratrichosoma recurvum* (Nematoda: *Capillariidae*), a skin-invading parasite of crocodiles in Mexico. Parasitology Research **84**:499-504

Padilla, S. E., and M. Weber. 2016. External injuries of Morelet's crocodile *Crocodylus moreletii* in Campeche, Mexico. Diseases of Aquatic Organisms **120**:151-158.10.3354/dao03015

Seijas, A. E. 2007. Injuries and parasites in orinoco crocodiles (*Crocodylus intermedius*) in a river with high impact by anthropic activities. Interciencia **32**:56-60

Shirley, M. H., B. Burtner, R. Oslisly, D. Sebag, and O. Testa. 2017. Diet and body condition of cave-dwelling dwarf crocodiles (*Osteolaemus tetraspis*, Cope 1861) in Gabon. African Journal of Ecology **55**:411-422.10.1111/aje.12365

Stacy, B. A., and N. Whitaker. 2000. Hematology and blood biochemistry of captive mugger crocodiles (*Crocodylus palustris*). Journal of Zoo and Wildlife Medicine **31**:339-347

Sykes, J. M., and E. Klaphake. 2008. Reptile hematology. Veterinary Clinics: Exotic Animal Practice **11**:481-500

Tellez, C. M. 2011. Crocodile Populations Survey Methods Manual.*in* T. C. R. Coalition, editor.

Tellez, C. M. 2014. Alligator Parasitism- the Mysterious Frontier Unfolded: Exploration of the Ecological Interaction between an Archaic Predator (*Alligator mississippiensis*) and its Parasites. University of California.

Tellez, M., B. Arevalo, I. Paquet-Durand, and S. Heflick. 2017. Population status of Morelet's Crocodile (*Crocodylus moreletii*) in Chirquibul Forest, Belize. Mesoamerican Herpetology **4**:8-21

Tellez, M., M. Boucher, and K. Kohlman. 2016. Population status of the American crocodile (*Crocodylus acutus*) in Caye Caulker, Belize. Mesoamerican Herpetology **3**:450-460

Tellez, M., and M. Merchant. 2015. Biomonitoring Heavy Metal Pollution Using an Aquatic Apex Predator, the American Alligator, and Its Parasites. Plos One **10**.ARTN e0142522
10.1371/journal.pone.0142522

Tellez, M., and I. Paquet-Durand. 2011. Nematode Infection of the Ventral Scales of the American Crocodile (*Crocodylus acutus*) and Morelet's Crocodile (*Crocodylus moreletii)* in Southern Belize. Comparative Parasitology **78**:378-381.10.1654/4469.1

Thorbjarnarson, J. B., and G. Hernandez. 1992. Recent Investigations of the Status and Distribution of the Orinoco Crocodile *Crocodylus intermedius* in Venezuela. Biological Conservation **62**:179-188.Doi 10.1016/0006-3207(92)91046-U

Veiga, J. P., A. Salvador, S. Merino, and M. Puerta. 1998. Reproductive effort affects immune response and parasite infection in a lizard: a phenotypic manipulation using testosterone. Oikos **82**:313-318.Doi 10.2307/3546971

Viana, L. A., P. Soares, F. Paiva, and R. Lourenço-De-Oliveira. 2014. Caiman-biting mosquitoes and the natural vectors of Hepatozoon caimani in Brazil. Journal of medical entomology **47**:670-676

Weiss, D. J., and K. J. Wardrop. 2011. Schalm's veterinary hematology. John Wiley & Sons.047096183X

Zimmerman, L., L. Vogel, and R. Bowden. 2010. Understanding the vertebrate immune system: insights from the reptilian perspective. Journal of Experimental Biology **213**:661-671

Zweig, C. L. 2003. Body condition index analysis for the American alligator (*Alligator mississippiensis*). University of Florida Gainesville.