ABSTRACT

Transition Metal Accumulation in Caudal Scutes of American Crocodiles (*Crocodylus acutus*) from Belize

Derek R. Newberger

Director: George P. Cobb, Ph.D.

Contamination of aquatic environments is a global concern that poses risks to wildlife and human health. Due to their high trophic status, broad diet, long life span, and occurrence in a variety of aquatic habitats, crocodilians are susceptible to exposure and accumulation of numerous persistent environmental contaminants, including metals. Exposure to these metals may have potential health hazards and have a more pronounced effect on populations already subject to other stressors (e.g., habitat loss, deliberate killing). Previous studies have documented transition metals in caudal (tail) scutes of crocodiles from remote areas of mainland Belize; however, no such data are available for crocodiles living on the country's offshore islands (cays). In this study, we examined transition metal concentrations in caudal scutes from American crocodiles (*Crocodylus acutus*) sampled from various localities on Ambergris Cay, Belize. In addition, a smaller number of *C. acutus* scutes from Costa Rica were also examined for comparative purposes. Sixteen metals were detected in scutes: Pb, As, Cu, Ag, Be, Cd, Al, Cr, Ni, Co, Mo, Sb, Se, Tl, Sn, and Zn, with Al, Zn, Cu, and Sn exhibiting the highest concentrations. Metal concentrations differed by sex, body size, site, and proximity to putative contaminant sources. Juvenile crocodiles generally contained the highest metal concentrations, and for many metals concentrations decreased with increasing body size.

APROVED BY DIRECTOR OF HONORS THESIS:

____

Dr. George P. Cobb, Department of Environmental Science

APPROVED BY THE HONORS PROGRAM:

____

Dr. Andrew Wisely, Director

Date: ________________________

TRANSITION METAL ACCUMULATION IN CAUDAL SCUTES OF AMERICAN CROCODILES (*CROCODYLUS ACUTUS*) FROM BELIZE

A Thesis Submitted to the Faculty of

Baylor University
In Partial Fulfillment of the Requirements for the
Honors Program

By
Derek R. Newberger

Waco, Texas
May 2014

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

For field collection I thank Cherie Chenot-Rose and Vince Rose of the American Crocodile Education Sanctuary (ACES), Punta Gorda, Toledo District, Belize; Thomas R. Rainwater from the U.S. Fish and Wildlife Service, Ecological Services Field Office, Charleston, SC 29407; and Frank J. Mazzottie from the department of Wildlife Ecology and Conservation, University of Florida, Fort Lauderdale Research and Education Center, Davie, FL 33314.

For the laboratory methods I thank Abhilasha Acharya from the department of Environmental Science, Baylor University, Waco, TX for teaching me the necessary procedures and analyzing the samples through the ICP-MS.

I thank George P. Cobb from the department of Environmental Science, Baylor University, Waco, TX for being my mentor and friend.

It should be noted that the findings and conclusions in this poster are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service. I am grateful that the Conservation Division, Forest Department, Belmopan, Belize and the Ministry of Atmosphere and Energy, San José, Costa Rica issued the research permits. The ACES is fully funded by The Rufford Small Grant Foundation.

CHAPTER ONE

Introduction

The Environment under Study

One of the most polluted rivers in the Neotropics is the Tarcoles River in west-central Costa Rica, (Fuller et al., 1990; Whelan, 1989). Fauna residing in the Tarcoles River and utilizing its resources could be exposed to industrial, agricultural, and municipal contaminants and be vulnerable to various pollutant-related health hazards.

The largest caye in Belize, Ambergris Caye, is located about 35 km northwest of Turneffe Atoll. The coral sand island is flat, and is protected by the Mesoamerican Barrier Reef System on the east side. The red mangrove (Rhizophora) has a formidable population within the fresh, brackish, and saltwater lagoons along the west coast. Unregulated garbage has been dumped in lagoon areas. In previous studies, nesting sites were located on Blackbird (17019'N, 87047'W) and Northern (17029'N, 87047'W) Cays in the Turneffe Atoll where coarse sands appear to be the preferred nesting habitat in Belize (Platt, 2000).

Bioaccumulation in Alligators and Crocodiles

Bioaccumulation is the process in which a substance such as a contaminant is accumulated by the organism whose rate of intake exceeds the organism’s ability to remove the substance from their body (International Union of Pure and Applied

Chemistry, 1993). Toxic metals are excreted through urine, feces, skin, and eggs. American alligators have high toxic metals concentrations in the liver and the kidneys but low in bile.

Reptiles, and more specifically crocodiles, are appropriate indicators to demonstrate bioaccumulation due to their biological processes. A bioaccumulation indicator must exhibit correlation between their tissue contaminant concentrations and the contaminant concentrations in the surrounding environment, typically water, sediment, or both. In comparison to birds or mammals, reptiles have lower metabolic rates, lower energy demands, and lower dietary exposure. Reptiles are different from their endothermic counterparts since their enzymatic detoxification system is thought to be less developed which could minimize detoxification processes that eliminate heavy metals from the body. As a consequence, when reptiles utilize stored energy reserves like metabolizing lipids in the liver, the contaminants that were previously stored there will be remobilized during those periods changing the accumulation of contaminants in other locations. Contaminant concentrations correspond to the trophic level of reptiles. It should be noted that a wide variation exists in toxicant bioaccumulation among reptiles due to the diversity of feeding strategies and hence diets concerning mobility, foraging, location, migratory traits, as well as differences in contaminant assimilation of each food type (Schneider et al. 2013) A long lifespan of 60 years (in the wild) gives the crocodiles an opportunity for long term exposure to contaminants (Schardt, 2008). More specifically, an American crocodile is chosen as a bioaccumulation indicator since it meets these requirements well. More literature addresses bioaccumulation in alligators than crocodiles, but a correlation between crocodiles and alligators can be made due to similarities in morphology, habitat, and diet. In regards to this study, the contrast that alligators prefer freshwater and crocodiles prefer saltwater is most relevant, even though only Ambergris Cay has a salt water environment and the Tarcoles River consists of freshwater. American alligators and American crocodiles are similar and the results of a study from either organism can be applied to the other in general terms. Alligators typically consume large meals (>30% TBW) every few weeks or couple of months. This interval creates a pulse that is ideal for bioaccumulation. Age, size, and non-migratory patterns make for an effective indicator. American crocodile portray these characteristics. A long-term exposure to natural sources of contaminants increase the amount of contaminant accumulated (Schneider et al. 2013).

A few factors should be considered while viewing these data to understand fluctuations that are expected in the bioaccumulation process. As the crocodile grows older it can take down larger prey. In consequence, the consumption of different prey items exposes them to different contaminants. Furthermore, contaminant assimilation is different among male and female individuals because of reproduction affects and mating behavior. It is possible for the female to excrete some contaminant within her eggs (Hopkins, 2006). During this time, the movement of female crocodiles is limited, and there is a change in diet. Larger crocodiles can potentially accumulate more due to slowed elimination of contaminant and larger prey items. It has been found that there is the lowest dietary overlap between the largest crocodiles and the smallest crocodiles than any other size class (Platt et al.

2013). This change in diet creates a difference in the contaminant concentrations taken up by the crocodiles in relation to their size. Ideally an increase in age corresponds to an increase in size so that an accurate measure of age can be taken. Other measures of age could be snout-to-vent length (SVL) which is the length between the snout and to cloaca of the crocodile, curved carapace length (CCL), and body weight, but the true measurement of age continues to be elusive (Schneider et al. 2013). After the crocodile has reached maturity, the slowed rates of somatic growth will cause errors in the estimated age of the individuals whether determined by back-calculation, skeletochronology, or growth-curve analysis (Tucker, 1997). However, as found in C. *johnstoni* from the Lynd River, it is reported that coefficients of variation in age estimates are about ±1 year across all ages in a regression of SVL (cm) versus age (years) (Tucker, 1997).

Caudual scutes were used to determine the amount of bioaccumulation that took place by studying the concentrations s of toxicants. In most cases within this study, the crocodilian caudal scutes were triangular in nature, vertically extended, and laterally flattened dermal scales found on the dorsal surface of the tail (Richardson et al., 2002). Liver and muscle samples are more efficient to determine bioaccumulation; however, the removal of caudual scutes is a non-lethal sampling procedure that does not appear to undermine the fitness of the animal (Schneider et al., 2013). American crocodiles are currently considered to be vulnerable by the International Union for the Conservation of Nature and Natural Resources (IUCN) (IUCN, 2014). In Florida, the American crocodile was reclassified from "endangered" to "threatened" by the U.S. Endangered Species List in 2007 (Schardt, 2008).

Additionally, American crocodiles are listed under Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Groombridge, 1987; Platt and Thorbjarnarson, 2000a, b). Observations of low population densities in Belize's coastal zone coupled with the loss of nesting and nursery habitats further advocate the protection for these crocodiles that are classified as critically threatened in Belize (Platt et al. 2000c). As a Charismatic species that can promote coastal conservation efforts, a sampling procedure that is non-lethal should be pursued and verified for American crocodiles (Thorbjarnarson et al. 2006). After all, those are the only procedures that are allowed (Burger et al., 2000). Removing scutes in a unique pattern permanently marks the crocodiles for future identification (Rainwater et al. 2006). In most injuries to crocodiles, the wound shows epidermal scarring after the granulation of soft tissue covers the wound (Brazaitis, 1981). A dominant characteristic in contaminant assimilation is an increase in muscle-contaminant with increasing trophic levels. Fortunately, it has been shown that alligator skin can be used to predict contaminant concentrations in muscle after taking the origin and the time the alligator was exposed to Hg into account but variation is still expected (Schneider et al. 2013). This can be applied to the expectation of how other toxicants will accumulate. The morphology of the skin consists of non-living keratin layers in a pattern where contaminants can be stored and metabolically unavailable in these tissues. Once stored in these keratin layers, contaminants availability to tissues are significantly limited, there by inhibiting negative effects on the physiological systems. In these scutes, keratin is the dominant epidermal structural protein and consists of sulfur rich metal-binding proteins, concentrate thiol reactive metal ions, and metalloid elements. These components may be immobilize to prevent toxicity by sequestering them into these metabolically inactive areas of keratin and form a relatively immobile inorganic complex. Vertical tail scutes do not contain bone; however, they are hardened and provide protection. These vertical tail scutes increase the surface area of the tail substantially which has the potential to provide a role in swimming efficiency. Furthermore, they have a good blood supply and are sites of heat exchange between the crocodile and its environment (Richardson, 2000).

Previous Studies

Previous studies have been performed in the Tarcoles River. In 2003, Adult American crocodiles (*Crocodylus acutus*) found in the lower reaches of the Tarcoles River contained various organochlorine pesticides (OCPs) in addition with heavy metals in their caudal (tail) scutes. However, a reduced fitness was not observed among these crocodiles (Rainwater et al., 2007). Samples from Costa Rica were obtained during 12-15 September and 12-13 December 2007, when crocodiles were captured and examined. The focus of that study was to determine if an association between an ocular disease of unknown etiology and the accumulation of chemical pollutants within the environment was present. Of 11 crocodiles, 3 (27.3%) male crocodiles were diagnosed with the unilateral ocular disease if symptoms such as corneal opacity, corneal scarring, anterior synechia, and phthisis bulbi, were present. Although various pollutants such organochorine pesticides (OCPs) and metals were detected in caudal scutes, crocodilian blood, and sediments, no associations were determined between contaminant accumulation and the incidence of the eye disease. Nonetheless, due to the small sample size, neither infection nor chemical toxicants can be eliminated as primary causes of the eye disease. It was also speculated that the ocular disease could have been due to injury-induced trauma either primarily or indirectly through secondary infection. Such injuries are the probable result of aggressive encounters caused by territorial disagreements among large adult crocodiles. Ocular disease is not traumatic, but a blunt force directed at the eye through a closed eyelid could leave the eye lid with minimal visible damage while permanent eye damage occurs.

Arsenic, copper, vanadium, and zinc were detected from the scute samples. All the samples contained copper and zinc, six (86%) contained vanadium, and three (43%) contained arsenic. Non-diseased crocodiles had a significantly higher mean of copper (X^2=4.50, df=1, P=0.0339) and was the only significant difference between contaminant concentrations in the scutes of diseased and non-diseased crocodiles. Through a linear regression analyses, significant negative relationships were found between crocodile body size (SVL) and copper (R^2=0.75; P=0.0122; n=7) and vanadium (R^2=0.62; P=0.0349; n=7) concentrations in scutes. Contaminant concentrations in caudal scutes (ng/g wet weight) of the diseased and non-diseased were noted separately. In the 3 diseased crocodiles, arithmetic means were; arsenic 801.3±347.2, copper 655.7±77.4, vanadium 202.7±99.4, and zinc with 3,886.7±613.3. In the 11 non-diseased crocodiles, arithmetic means were; arsenic 405.5±291.5, copper 1,212.3±340.2, vanadium 530.5±254.5, and zinc with 3,627.8±951.9 (Rainwater et al., 2011).

American Crocodiles In Belize

Crocodiles are seldom found more than 10 km into the coast of Belize and the majority of the population resides in offshore islands and atolls. In a study on three coral atolls, Turneffe, Lighthouse, and Glovers atoll was performed to determine the diet of American crocodiles. Crocodiles were classified as hatchlings (SVL < 15.0 cm), small juveniles (SVL = 15.0–40.0 cm), large juveniles (SVL =40.1–65.0 cm), subadults (SVL = 65.1–90.0 cm), or adults (SVL >90.0 cm). Some hatchlings had not begun to feed, and contained only fat globules from metabolized egg yolk while the others contain insects and crustaceans. Insects were consumed by all age classes except for adults. Although dietary diversity was uniformly low for all age classes, small and large juveniles had the most diverse diet since they consumed primarily insects and crustaceans but also included mollusks, fish, and non-fish vertebrates. Subadults primarily consumed crustaceans and consumed fewer insects and non-fish vertebrates and no fish or mollusks. Adults consumed mostly crustaceans, but their diet also included fish, non-fish vertebrates, and mollusks. Crustaceans were the top prey item and were found in 73 (75.2%) of the crocodiles. Crabs contain large amounts of calcium, phosphorus, and particular long-chain polyunsaturated fatty acids that crocodiles need. Fish was the most frequent vertebrate prey item but overall was not a common part of diet. Fresh prey was the greatest in the smaller size classes and decreased with increasing SVL. However, fresh prey was uniformly high in all classes (Platt et al. 2013). Bias may have been induced by the chitinous exoskeleton of insects and crustaceans that can stay in the stomach of crocodiles for several months (Villegas, 2008). A high dietary overlap was found with the largest age class differing from the smallest age class. Most of the crocodiles were found to have prey items in their stomachs. Smaller individuals feed more frequently than larger individuals. Twenty-three crocodiles (23.7%) contained nonfood items such as stones, coral, wood, seeds, pumice, parasites, and vegetation. Small stones and other hard objects are deliberately consumed which function as gastroliths that help to facilitate the breakdown of ingested prey especially for smaller crocodiles that consume chitin-rich diets. However, the study did not find that the smaller crocodiles did not show that behavior (Platt et al. 2013).

Subjective population surveys have observed that smaller size classes inhabit mangrove swamps and landlocked hypersaline lagoons meanwhile the subadults and adults forage in areas that are more open, such as tidal flats, turtle-grass beds and along the barrier reef. These crocodiles are experiencing a high intake of Na^+ from their environment and their diet of crustaceans. Dredging and water pollution has led to the loss of a few sea-turtle beds and mangroves are being cleared locally but increasingly (Platt et al. 2013). Among the cays surrounding Ambergris Caye, it has been postulated that crocodiles migrate from cay to cay (Chenot-Rose, 2012).

American crocodiles in Costa Rica

There is little literature concerning the diets and habitats of American crocodiles in Costa Rica. As a freshwater system, there may be common characteristics with other freshwater river systems with crocodiles. In these areas diets of crocodiles are more diverse with including mammals, birds, anurans, and most primarily, fish. All size classes of crocodiles in freshwater river systems consume mollusks. Crocodile juveniles inhabit shallow and dense vegetated microhabitats, meanwhile the larger size classes inhabit large canals and open shorelines (Platt et al. 2013)

Initial Concern

The ACES is a non-profit organization that was developed to conserve Belize's critical habitats and protected species, specifically the American crocodiles. From the photographs received from Cherie Chenot-Rose of the ACES, trash is over abundant within these lagoons where the crocodiles were sampled. The illegal dumping of the unregulated trash could potentially release harmful heavy metals within this area. Water samples tested by Cherie Chenot-Rose will be further analyzed to confirm this. In the past six years, the crocodile population has declined, which is caused by the destruction of habitat and nesting grounds, low hatching survival rates, and unnecessary human predation in locations where protection laws are inadequately enforced. Only two nesting sites were found in 2008 in comparison to 15-16 in previous years. Due to habitat destruction, crocodiles wander into areas inhabited by humans, creating a local concern when the crocodilian prey items consisted of the pets of residents. Upon capture of a crocodile that had wandered into the areas inhabited by humans, a health concern stemmed from the whitish pigmentation around the tip of the snout which represented an immunosuppression response and that the lack of teeth. Transportation for this obese crocodile was organized in vain. Shortly after the arrival of the boat captain the crocodile named bubbles began to quickly exacerbate and die. This is the third large American crocodile (*C. acutus*) to have been found within this particular region in an unhealthy condition. Upon further review of the crocodile, the whitish pigmentation was found to be caused by a degenerative skin disease and is also a symptom of Chronic Septicemia. The distended stomach was bloated and jelly in nature prior to death and through a biopsy of the stomach the diet of this crocodile was determined to be composed of largely chicken and dog. The accumulation of toxicants may have contributed to the untimely death. The absent or not fully-grown diaphanous teeth are speculated to be caused by poor mineralization from improper diets like nutritional osteomalacia or Botulism from the toxins produced by bacteria such as *Clostridium botulinum*. The reproduction rate of these bacteria is enhanced by anaerobic environments caused by the contained nature and added by-wastes from humans therefore enriching the nutrients that algae consume which only enhance the anaerobic environment where the bacteria thrive under (Chenot-Rose, 2012). This reoccurrence of these observations has caused an interest to further investigate the origins of this issue and lead to the sampling of American crocodiles (n=39) for the contaminants within their scutes to be analyzed. Habitat destruction forces crocodiles to wander into areas inhabited by humans where concerns arise about pets becoming crocodilian prey items.

CHAPTER TWO

Methods

Field Methods

Crocodilian caudal scutes from Costa Rica were obtained 12-15 September and 12-13 December 2007 along a 5-km stretch of the lower Tarcoles River (9 °48952.740N, 84 °34955.770W to 9 °46957.750N, 84 °37907.270W) (Rainwater, 2011). Scutes from Belize were obtained in Ambergris Caye (18°00'N, 88°00'W) from the 20 October 2010 to 5 September 2011. Scutes were removed from the dorsal surface of the tail, placed into plastic bags, and frozen for storage. Snout-to-vent length was used as the primary measure of age in this study and is the distance between the snout and the cloaca of the crocodile. From SVL juvenile (27.6cm-62cm), sub-adult (77.5cm-85.2cm), and adult (94.2cm-241.3cm) size classes were determined.

Laboratory Methods

A Mainstays glass cutting board, razor blades, hand saw, and a glass scrapper by Allway Tool Inc GS 07007 were purchased from a local supermarket. The saw clogged, which was contrary what was stated in its description on the packaged and so was not used. A razor blade did not clog and was precise. A glass cutting board was used as a surface for dicing scutes. After dicing, glass cutting board had a cloudy residue. However, since the razor blade with the handle was made to scrape paint from a surface such as a window, the razor blade was then used to dislodge the residue which was added to the vial with the rest of the sample. Furthermore, to see if there were any scute fragments left, the glass cutting board was lifted over the individual who was dicing the scute. Ideally, this individual is wearing a white laboratory coat, and provides an excellent contrast to the dark scute fragments that can be added into the vial.

A total of 60 samples of finely diced organic material were prepared from American crocodiles from Belize (n=51) and Costa Rica (n=9). Fist, the glass cutting board, the razor blade, and the handle for the razor blade were cleaned as to avoid cross contamination. Samples were then diced to less than an eighth of an inch, placed into vials, weighed, and then stored in the freezer.

After a single use, each item underwent a cleaning process that included three rinses with soap and water, three rinses with reverse osmosis water, three washes of ethyl alcohol, and three washes of Nanopure ™ water. A rinse bottle was filled with 3/4ths tap water and 1/4th liquinox. Paper towels from a nearby dispenser were placed into two different stacks, one of which consisted in whole sheets and the other into sheets torn into thirds. One third of a sheet was placed aside to place the razor blade and handle after they were coated with soap on both sides and ran under tap water. This was performed three times for each item. The razor blade and the handle were then dried with a 1/3rd sheet of paper towel and placed on the 1/3rd sheet that was placed aside. The cutting board was then covered in soap using a zigzag motion on both sides. The backside of the cutting board was only covered in soap once, while the front smooth side was covered with soap and thoroughly washed three times. The cutting board was then dried with paper towels. Often heavy suds accumulated in the cutting board in the attempt of drying. The cutting board was then run through reverse osmosis water three times on both sides. If suds were still visible in either of the washes, then an extra rinse was performed. The cutting board was then dried with paper towels. The razor blade and the handle were run under reverse osmosis water three times on both sides and if suds were still visible than an extra rinse was provided. The razor blade and the handle were then dried and then blotted by 1/3rd of a paper towel that was wetted with a solution of 70% ethyl alcohol three times. The cutting board was then wiped down with 1/3rd of a paper towel sheet that was wetted with a solution of 70% ethyl alcohol three times. The razorblade and the handle were then wiped down with a 1/3rd of a paper towel sheet that was wetted with a solution of Nanopure ™ water and then dried. A slight amount of Nanopure ™ water was then poured onto the cutting board, which was then dried with 1/3rd of a paper towel three times. If at any time during this process an item was dropped and contaminated, the procedure was done anew for that specific object. If at any time there was a doubt in the number of washes or blots, and additional wash or blot was performed. Gloves worn during the washing period were then discarded before touching the next scute sample.

The dicing of scute samples then commenced, with the batch from Costa Rica performed first and then followed by the Batch from Belize. Scutes from Costa Rica, although more difficult to dice due to their size, were consistent in packaging and their ability to provide 2 g of finely diced organic material. The cutting process incorporated a strategy of "through any means possible." Belize scutes varied in size, packaging, and other discrepancies (Table 1). However, due to their smaller size and general shape, they were less difficult to dice. The shape of most scutes from Belize were similar to a scalene triangle, with the longest end being from the scute excision site and the shortest side was the back of the scute in reference to the American Crocodile facing forward. The scute would be placed on the glass cutting board and vertical sections would be cut parallel with the shortest end. These vertical sections were grouped into plies that contained three to four of them lined adjacent with each other so that one horizontal cut could cut three vertical sections at a time. Scutes were diced; piled together and then the razor blade was used to scrape any remaining scute residue from the glass cutting board in the direction of the finely diced scute material. During this time, the razor blade could catch the glove and cut a fragment off of it. The fragment of glove was promptly removed from the glass cutting board and thrown away along with the glove that was cut and a new glove was put on.

Once the dicing was completed, the lab identification number was written on the vial and cap. They were then weighed together (Mettler MS205DU, Switzerland). The cap was then removed and the vial centered before weighing again. The balance was then zeroed and the scute sample was then transferred into the vial with the aid of a disposable polyproplyene spatula. After most of the scute was within the vial, the cutting board was placed slightly hanging over the edge of the table so that the rest of the scute sample could then be scrapped into the vial that was placed under the edge of the glass cutting board. The scute sample was weighed, recorded, and placed into the freezer. For every three scutes, the samples were then taped together with the labels on the vial kept visible. The gloves worn were then discarded, and new gloves were used during the cleaning process.

Scutes were taken from 7 American crocodiles in Costa Rica from which 9 samples of finely diced scute were made. Samples from Costa Rica (n=9) were packaged in one or at time two zip lock bags. Scutes from Costa Rica (n=1-9) weighed 2.004±0.0016g (Rainwater et al., 2007. Sci. Tot. Environ). The center of the scute was taken for analysis. Some samples from Costa Rica had multiple scutes from one American crocodile, and when one of these additional scutes could provide a little over 2 g of finely diced organic material, a duplicate was made. The two duplicates 6102A07 and 6107A02 came from the same American crocodile as 61102B03 and 6107B05 respectively.

Scutes from Belize varied in weight and the packaging method was not consistent. Scutes from the same crocodile were combined into one sample if either scute weighed less than 0.3 g. Belize scutes were acquired from 37 American crocodiles from which 51 samples were made. Scutes from Belize fell into two groups, 45 weighed 0.7112 ±0.5793 g and 6 weighed 0.0148± 0.0114 g. Some scutes were: duplicates, contained sediments, and/or were significantly small. One scute was: packaged in a glass vial suspended in ice, contained a significant amount of blood residue, reported as missing, or had an unpleasant odor. Neither sediment nor blood was a main focus of this study. Thus the scutes that contained such items were recorded, and the sediment or blood was removed until no great amount was visible.

EPA method 3050B was modified and used for this study. Although this digestion is primarily developed for soils and sediments, it was proposed that the method could also be applied to American crocodile scute samples (as observed by results from the practice digestion with samples P154251 and P154252). Flat bottomed vials that contained the finely diced American crocodile scutes predigested in 10 ml q 1:1 of HNO_3 for 21 hr (Rainwater et al., 2006. Sci. Tot. Environ). For the predigestion, 10 ml q 1:1 of HNO_3 was added. The heating block was set for 95° C, and a vial with water and thermometer were placed into the heating block to assure the temperature was correct. Samples were placed into the center of the block to ensure consistent heating, refluxed for 10-15 minutes while avoiding boiling, and removed from the heating block to cool for 2 minutes. Five ml of HNO_3 was then added and the samples were then again refluxed for 30 minutes. If brown fumes were generated, another 5 ml of HNO_3 was added and the sample was then refluxed again for 30 minutes. This process was repeated until no brown fumes emanated or no solid sample was visible. Solutions were refluxed until the solution evaporated to approximately 5 ml or refluxed for 120 minutes. For either option, boiling was avoided. Solutions were then removed from the heating block and cooled for 2 minutes. Two ml of H_2O and 3 ml of 30% H_2O_2 was then added to the solutions slowly as to prevent the solution to bubble over. Solutions were then returned to the heating block and heated until the effervescence had subsided. Afterwards, 30% H_2O_2 in 1 ml-aliquot was added, and solutions were then heated until the effervescence was minimal. No more than 10 ml of H_2O_2 was added in this step. Solutions were then heated until either the volume was reduced to approximately 5 ml or heated at 95° C without boiling for 30 minutes. Solutions were then cooled and filtered. Filter paper of 110 mm was then folded into a cone shape and placed into a funnel that rested on a 50 ml flask. The solution was then gently poured into the funnels. The vial with that carried the solution during the digestion was then rinsed three times with 2% HNO_3 water to avoid any solution residue remaining at the bottom of the vial. Once all the solution had eluted, the filter paper was discarded, and the solution was diluted to 50 ml using 2% HNO_3 with a Pasteur pipet. New flat-bottomed vials and caps were then labeled. Respected solutions were then transferred into these vials. Along with the solutions and their duplicates, a 5 ml solution of Nanopure ™ water was used as a blank and the 0.5g of reference material used was 8704 Buffalo River sediment by the U.S. department of commerce national institute of standards and technology of Gaithersburg, MD 20899. With the acid digestion complete, solutions were prepared to go through the ICP.

Digests were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) to quantify Pb, Zn, As, Cu, Ag, Be, Cd, Al, Cr, Ni, Co, Mo, Sb, Se, Tl, and Sn. The solution was further diluted by taking 5 ml of the solution and adding it to an ICP vial that contained 5 ml of 2% HNO_3. In light of high concentrations of Zn, a 1:10 solution was made using the previous technique. A Fisher Mini Vortexer of 120V model 945404 which was made in the U.S.A vortexed 120 dilutions which were then given to a co-worker to use the ICP-MS to analyze. A spike of mixed ICP standards(Ricca Chemical Co., Arlington, TX) , with 16 regents (1 ppm each Ag, Be, Cd, Zn; 3 ppm each Al, Cr, Ni; 5 ppm each As, Co, Cu, Fe, Mo, Pb, Sb, Se, Tl; 10 ppm Sn in 2%

HNO_3) was used to make the calibration points of 1 ppb, 5 ppb, 25 ppb, 50 ppb, and 100 ppb for all analytes except for Zn. Our calibration points were 1 ppb, 5 ppb, 25 ppb, and 50 ppb for Zn. An ICP-MS Elan 9000 by Perkin Elmer was used daily to acquire this data.

Statistical Methods

Calibrations were primarily linear; however, Ag and Zn fit non-linear regressions. Sigma plot was then used for all statistics involving metal concentrations in scutes. Two-way ANOVA used the log transformed concentrations for proximity versus sex, sex versus size, and proximity versus size. A regression line was formed between metal concentration and SVL by using (semi-log regression). Pearson correlation analysis was performed for all metals within groups, Costa Rica, Belize adults, and Belize juveniles.

CHAPTER THREE

Results

Toxicant Concentrations

For Costa Rica, metals with the highest concentration were Al (62373.5<x<699842.0 ng/g), Zn (2349.6<x<4775.3 ng/g), and Cu (2074.9<x<5000.3 ng/g). For adults from Belize, metals with the highest concentration were Al (24945.9<x<48194.8 ng/g), Cu (4698.9<x<10764.7 ng/g), and Zn (6526.8<x<8115.2 ng/g). For juveniles from Belize, metals with the highest concentration were Al (43451.0<x<169044.1 ng/g), Sn (3475.4<x<121618.6 ng/g), and Zn (3396.3<x<19186.7 ng/g). Although not among the highest concentrations of contaminants from Costa Rica, it should be noted that concentrations of Pb, As, and Se in the scutes were (37.9<x<238.2 ng/g), (135.8<x<1462.2 ng/g), and (458.1<x<803.5 ng/g) respectively. For the adults from Belize, the concentration of Pb, As, and Se in scutes were (236<x<360.2 ng/g), (287.5<x<454.4 ng/g) and (562.7<x<786.5 ng/g) respectively. For juveniles from Belize, concentrations of Pb, As, and Se in scutes were (348.3<x< 8472.3 ng/g), (258.2<x<2766.9 ng/g), and (381.1<x<2786.1 ng/g), respectively.

Five juveniles from Belize generally contained the highest metal concentrations within the data set (Table 1). These scutes obtained from juveniles and their sample masses were quite low. No comparative statistics were performed on Be concentrations because 20 of the 43 signal intensities were either detectable but non-quantifiable or non-detectable. Concentrations of Mo and Tl were either detectable but non-quantifiable or non-detectable in 7 scutes. Remaining analytes were quantified in all scutes.

Two Way ANOVA for Proximity, Sex, and Size

The two way ANOVA (proximity, sex) showed that crocodile proximity to a local sewage plant contributed to differences for Al; whereas, sex contributed to significant concentration differences for Pb, As, Ag, Cd, Al, Cr, Ni, Co, Mo, Sb, Se, Tl, and Sn. Two way ANOVA (proximity, size) showed that crocodile proximity to a local sewage plant contributed to differences for Al, Cr, and Ni; whereas, size contributed to significant concentration differences for Pb, Ag, Cd, Cr, Co, Mo, Sb, Tl, and Sn. The two way ANOVA (sex, size) showed that crocodile sex contributed to differences for Pb, As, Ag, Cd, Cr, Ni, Co, Mo, Sb, Se, Sn, and Zn; whereas, size contributed to no significant concentration differences for any metals.

As and Se were the only metals that exhibited a dependence on sex but were not explained by size or proximity. No analyte concentrations differences were uniquely explained by crocodile size. Al was the only analyte with proximity dependent concentrations in both ANOVAs for, but sex was found to be significant in sex and proximity also.

Regressions of Metal Concentration Versus Snout-to-Vent Length

Regression slopes of log transformed data ranged from $-4.89X10^{-3}$ to $-5.27X10^{-3}$ for each metal in the adult and -0.0242 to -0.101 for each metal in the juvenile crocodiles. Eight metals (Pb, Ag, Cd, Cr, Co, Mo, Sb, and Sn) indicated significant concentration differences based on crocodile size.   For these metals, concentrations declined as a function of SVL for juvenile crocodiles from Belize. For juvenile crocodiles, the three most significant regressions were Mo (R^2=0.83), Pb (R^2=0.71), and Cd (R^2=0.61). For adult crocodiles, the three most significant regressions were Ag (R^2=0.36), Sn (R^2=0.30), and Co (R^2=0.27). It should be noted that slopes are for log-transformed concentrations, and small differences may be more pronounced than one would expect. In the regression for the juveniles from Belize, a female collected far from the sewage treatment plant (lab ID#12) contained low metal concentrations in all regressions (Figures 1, 2, and 3) Care should be used in interpreting the influence of proximity. The designations close, intermediate, and far were generalized and as such can only be used in general terms. Tl was not reported because the number of zeros skewed these data.

Toxicant Correlations

Metals in scutes were highly correlated to one another. For scutes from juvenile crocodiles, 13 metals were correlated to at least 12 other metals. However, [Cu] and [Tl] correlated with 6 and 5 metals, respectively. For Belize adults [Ag], [Al], and [Sn] correlated with six metals. [Cd], [Sb], and [Cr] correlated with five. One correlation was determined for each of [As], [Cu], and [Se]. For scutes from Costa Rica, [Pb] had significant correlation with nine metals. [Co], and [Tl] correlated with 8. Most other metals had varying degrees of correlations. [Sb], [Se], [Sb], and [Cr] had no significant correlations.

Method Validation

Filter papers were then dried (Jeio Tech, model OF-21E, Korea) for 24 hr in the Lab and cooled at ambient temperature for 30 min before weighing. Filter papers were reheated for 30 min and cooled for 30 min before reweighing. To maintain consistency, filter papers were weighed in the same order. Filter paper weights revealed a mean sample loss of 0.818% with a range of $0<x<15.78$. It should be noted that in the process of filtering a scute from Costa Rica (lab #01), approximately 20% of the sample was spilled on the table. Metal concentrations from that scute were not found to be outliers.

Mean contaminant concentrations found in the scutes were compared to the reference concentration values for 8704 Buffalo River sediment. Mean Ni and Co concentrations were within the 95% confidence interval of the reference concentrations while mean Pb, Zn, Cd, Cr, and Sb were not. All other metals were not within the reference material. Aluminum was over the calibration range. Nanopure ™ water had 5 contaminant concentrations that were either detectable but non-quantifiable or non-detectable. The blank had 9 contaminant concentrations that were either detectable but non-quantifiable or non-detectable.

CHAPTER FOUR

Discussion

Toxicant Concentrations

Environmental factors such as diet, sediments, and anthropogenic sources play a role in the accumulation of toxicants. Juvenile crocodiles are utilizing their nutrient intake to grow, and their physiological system does not have the appropriate systems to excrete substances since their system is trying to absorb as much as possible. Smaller crocodiles have a higher surface area to volume ratio, which can lead to higher contaminant concentrations than larger crocodile. Juvenile's intake is much higher than an adult's intake in relation to size. To make a distinction between similar elements is a challenge for the body, therefore, young organisms readily uptake contaminants than the older organisms which utilize their nutrients for maintenance. Juveniles might have had higher contaminant concentrations in their scutes and have less developed physiological systems than adults.

Ca is essential for ossification. Pb and Ca have similar covalent radii and can be considered as analogues (1.74A, 1.47A). Pb concentrations from the scutes of the Juveniles from Belize were 5.9 times the Pb concentrations for adults from Belize and 18.1 times more than crocodiles from Costa Rica. Juveniles could be absorbing Pb as an analogue of Ca for bone growth which the adults are not. In humans, approximately 95% of the total body Pb burden is stored in the skeleton (Pemmer et al. 2013). A lack of Ca in Belize could explain the health concerns of the crocodiles such as loosing teeth.

In breeding females of C. *niloticus*, Ca mobilization accounts for elevated concentrations of plasma Ca. (Tucker, 1997). Female crocodiles had a mean Pb concentration of 285.9 ng/g (LCL: 163.0 ng/g; UCL: 505.1 ng/g) and males had a mean Pb concentration of 211.3 ng/g (LCL: 89.6 ng/g; UCL: 503.1 ng/g). A higher Pb concentration in potentially breeding females was found, which supports the notion that Pb behaves similarly to Ca.

In deer mice exposed to 100 μg/ml Pb in drinking water, dietary intake of Cu and Zn reduced the dose-dependent accumulation of Pb concentrations in the tissues with Zn serving as the most significant protector. Additionally, elevated dietary Zn and Cu can decrease Pb accumulation in blood (McFarland, 2005). Crocodile scutes were found to have Cu and Zn as one of the highest concentrations of measured analytes, which could decrease Pb concentrations in scutes. Lead concentrations found in scutes have the potential to not be able to accurately represent the amount of Pb in the environment that the crocodiles inhabit. In deer mice, increased Cu and Zn exposures did not increase Cu and Zn concentrations in the blood, rather maintained a consistent concentration with Zn being higher (McFarland, 2005). In consequence, Cu and Zn concentrations found in crocodiles may not accurately represent the metal concentrations in the environment.

Phosphorus is predominately found in bone and energy containing nucleoside triphosphates like ATP. Arsenic is a phosphate analogue, which has the potential to enter cells via phosphate transporters (Dani, 2011). P and As are in group VB. However, As concentrations are not the highest, and they do not greatly differ between crocodiles from Belize or Costa Rica. Although it would be expected to see higher As concentrations in the scutes from juveniles of Belize who are promoting bone growth, phosphate transporters are used throughout the crocodilian lifetime causing a consistent uptake.

Sulfur is present in two amino acids that give proteins their shape. This is important throughout the crocodilian life regardless of life stage. It is known that Se is an essential trace element and S is an essential element (Collins et al. 2012) (Amich et al. 2009). Since Se is in group VIB, Se is likely to behave like S. Selenium and sulfur analogues have comparable activity (Zhao et al. 2012) Se concentrations in crocodiles scutes are moderate and do not vary greatly. Se itself is used for certain enzymes like anti-oxidants, and the concentrations in scutes do not show a difference in the crocodiles. .

In both prokaryotes and eukaryotes, cobalamin species are required as cofactors for numerous enzymes. Vitamin B_{12}, the best known corrinoid species is a naturally occurring coordination complex with a Co as its central ion (Park et al. 2013). Vitamin B_{12} is required as a cofactor in numerous enzymatic reactions and serves a necessary role in organic metabolism (Domaç et al. 2014). Vitamin B_{12} is also important in red blood formation, nerve function, and bone health (Mayo Clinic, 2013). Juveniles from Belize had 9.9 times higher Co concentrations in their scutes than the adults from Belize. In addition, juveniles from Belize had 12.2 times higher Co concentrations in their scutes than the adults from Costa Rica. This could be due the nutrient demands asserted by growth in the juveniles. Older crocodiles recycle the components used for blood, but the juveniles need to make more blood as their size is rapidly increasing. In addition, juveniles consume more and their metabolism demands are higher as a consequence.

High concentrations of Cu and Zn are in part natural as they are micronutrients that are utilized in the natural physiologic processes of homeostasis and modulated in the body. Zn is an essential trace element in multiple biological processes (Pemmer et al. 2013). Cu is used as an electron donor. As micronutrients they have higher concentrations but do not greatly vary between the crocodiles from Belize and Costa Rica.

Elevated concentrations of Al are found in sediments of high clay content. However, sediments from Ambergris Cay were mostly sand, minimizing the contribution from clays. The lack of clay in Belize gives reason to believe that the contaminant concentrations originate from an anthropogenic source. Cocos is the tectonic plate off the west coast of Costa Rica where the mouth of the Tarcoles River is lies. Cocos Plate's upper limit consists of clays and muds (dominantly silty clay) (Kopf, 2013). Although offshore, this clay-rich stratum could be one source of Al for the crocodiles in Costa Rica. Crocodiles deliberately consume stones and other hard objects within their environment to serve as gastroliths that help to facilitate the breakdown of ingested prey (Platt et al. 2013). If there is a high concentration of Al in the sediments of Costa Rica, then the direct dietary exposure could increase Al concentrations in the crocodiles. Batteries are one of the only major known sources for Sb in that region and detectable traces of this contaminant are possibly due to anthropogenesis by the unregulated dumping of waste.

Our data indicates the need for further evaluation of crocodile exposures to metal contaminants for adverse effects of these toxicants include: neurotoxicity, anemia, carcinogenesis, and teratogenesis.

Two Way ANOVA for Proximity with Sex or Size

Two Way ANOVA for Sex

Sex was found to be the best indicator to explain differences in metal concentrations found in scutes within this study. Sex had the most significant p-values, which was 25. Both Two way ANOVAs were shown to have significant p-values in 11 analytes (Pb, As, Ag, Cd, Cr, Ni, Co, Mo, Sb, Se, and Sn). As and Se are shown to be specific to sex in that they only had two significant p-values each which were both for sex. Physiological systems between the two sexes can explain some of the differences in contaminant concentration in scutes. Female crocodiles are known to excrete contaminants in the eggs they lay. In eastern narrow-mouth toads, significant quantities of Se and Sr are transferred to their eggs. However, for egg masses, >50% of As concentrations were BDL which precluded reliable statistical analysis being performed (Hopkins, 2006). In the coastal zone of Belize, clutch sizes were 22.3 ± 6.0 and egg dimensions being 70.5mm ± 4.3mm in length and 44.1mm ± 1.6mm in width (Platt, 2000).

In my study the mean Se concentrations was 651.4 ng/g (LCL: 369.2 ng/g and UCL: 1152.9 ng/g) in females and 654.3 ng/g (LCL: 501.4 ng/g; UCL: 856.3 ng/g) in males. In this study the mean As concentrations was 314.0 ng/g (LCL: 167.5 and UCL: 592.4) in females and 434.7 ng/g (LCL: 195.4 ng/g and UCL: 971.7 ng/g) in males. All juveniles were removed for the purpose was to see if a decrease in Se was observed for female crocodiles laying eggs. Contrary to what would be expected due to the data received in the study on the eastern narrow-mouth toads, our study showed similar Se concentrations in the male crocodiles. Male crocodiles had a much higher As concentrations. In studies concerning human adult urinary As concentrations, men were reported to having higher urinary As concentrations than women. In regards to adult women, the only factor that was found to be significantly associated with As urinary concentration was creatinine. Soils samples were taken and it was found that As in those samples were high; however, they were variable and no link between As concentrations in the soil and urinary As concentrations could be found for either sex (Fillol et al. 2010). Proximity was not deemed to be significant in either two way ANOVA for As. Uptake of As is heavily influenced by gender in humans, and it appears that this study reveals similar findings.

Sex had a significant p-value in both the two way ANOVA for proximity and sex and the two way ANOVA for sex and size. This response could relate to the breeding female crocodiles which have elevated concentrations of plasma Ca.

Two Way ANOVA for Size

Size had 10 significant p-values in the two way ANOVA for proximity and size and had no significant p-values in the two way ANOVA for sex and size. Size was the second best indicator to explain the differences in the metal concentrations found in the scutes. However, no analyte was specific for size since any significant p-value found for size, that analyte was found to have significant p-values both of the two way ANOVAs for sex except for Al and Tl. However, proximity is a better indicator to explain the Al concentrations found in the scutes and Tl was reported to have either detectable but non-quantifiable or non-detectable in 7 scutes.

Two Way ANOVA for Proximity

Proximity was found to have a total of 4 significant p-values in the two way ANOVAs. Designations for proximity were generalized; in consequence, care must be taken when interpreting these data. Aluminum was the only analyte in both two way ANOVAs for proximity to have a significant p-value. Therefore proximity has an impact on Al concentrations in the scutes. Crocodiles, which were close to the sewage plant, had a mean Al concentration of 30251.8 ng/g (LCL: 14951.9 ng/g; UCL: 61211.7 ng/g), which was the second highest concentration. Crocodiles which were intermediate to the sewage plant had a mean Al concentration of 61960.2 ng/g (LCL: 25341.0 ng/g; UCL: 151501.4 ng/g), which was the lowest concentration. Crocodiles which were far to the sewage plant had a mean Al concentration of 64481.8 ng/g (LCL: 18378.4 ng/g; UCL: 226245.1 ng/g), which was the third highest concentration. Crocodiles, which were from Costa Rica, had a mean Al concentration of 208941.0 ng/g (LCL: 56532.98 ng/g; UCL: 772234.7 ng/g), which was the highest concentration. Clay rich sediment from the tectonic plate Cocos could explain those results. Even including the juveniles which had much higher metal concentrations than the other crocodiles from Belize, it was still shown that Costa Rican crocodiles (with no juveniles) had the highest Al concentration. Proximity to the sewage plant was found to have a large impact on the mean Al concentrations found in scutes from the crocodiles from that area was almost 4 times more. Although scutes collected from crocodiles far from the sewage plant had higher Al concentrations in their scutes than did crocodiles that were in the intermediate category for proximity. The difference is not much and can be contributed by other factors other than the sewage plant. It is important to note that there was only one subadult that was found at an intermediate distance from the sewage plant, while there was two juveniles and a subadult that were far away from the sewage plant. Although one of those juveniles contained much lower contaminant concentrations than the other crocodiles in their size class, these small crocodiles generally had higher contaminant concentrations. The intermediate distance appears to be far enough away from the sewage plant to not be impacted by it. Data from the water samples will determine the differences in contaminant concentrations in Belize to give light as to why the two way ANOVAs for proximity behaved in that fashion.

Cu and Zn Two Way ANOVAs

Both essential trace elements, Cu and Zn, were found to have the least number of significant p-values with Cu having none and Zn one for sex in the two way ANOVA of sex and size (Zelikoff et al. 2005). This might support how Cu and Zn are essential for a crocodile regardless of size (age), location, and sex. This coincides with the study of the deer mice in that proximity would not affect Cu and Zn concentrations for an increasing exposure to these essential trace elements does not elevate the concentrations in the organism for they are able to properly regulate them.

Regressions of Metal Concentration Versus Snout-to-Vent Length

Regressions for the juveniles were able to explain a large portion of the variance. All crocodiles in this study (n=44) were plotted on the same regression for each analyte. Five data points strayed from the rest. These crocodiles with high concentrations of contaminants were all juveniles and so were then graphed separately along with the sub-adult population. These five juveniles can still be observed as having a much higher contaminant concentration (Figures 1, 2, and 3). Scutes from Costa Rica did not deviate from the adults in Belize and kept together. This could mean that the abiotic environment where these crocodiles reside does not differ greatly or that the differences are small enough that the crocodiles are able to utilize similar physiological or behavioral mechanisms to acclimate. This diminishing metal concentration as the crocodile increases in size could indicate a difficulty in previous studies. Total lengths were similar to the crocodiles from Costa Rica since most of the samples were above 300cm (Rainwater, 2007). For Belize, four crocodiles had a total length greater than 300cm but the SVL regressions showed how at these longer lengths the metal concentrations in scutes decreased, making the differences between the length of the crocodile and the metal concentrations difficult to compare (Rainwater, 2007).

In regressions for the juveniles and subadults, there are two distinct data clusters (Figure 1, 2, and 3). Five are juveniles from Belize that have high concentrations of metal contaminants in their scutes where most were near the sewage plant and one juvenile being far removed from that possible source of metals. All three crocodiles with much lower metal concentrations in their scutes were female, with the smallest crocodile being in the juvenile size class and the other two being subadults. Only the largest subadult was at an intermediate distance from the sewage plant and the other two were listed as being far. This split can be caused by multiple factors, such as the change in diet and foraging location. Subadults consume fewer insects and more crustaceans and spend less time in mangrove swamps and landlocked hypersaline lagoons and instead forage with the adult crocodiles. In a study in western Maryland assessed the concentrations of As, Se, and Cd in atmospheric deposition, stream water, and biota in two streams. This study found that As, Cd, and Se concentrations in the organisms tended to decrease with increasing trophic levels. In crayfish and fish, concentrations of As, Se, and Cd are lower than in the herbivorous insects. Higher As and Cd concentrations were found in crayfish than in trout. It was reported that As is taken up in the carapace of the crayfish (Mason et al. 2000). Juvenile crocodiles consume insect prey items that may have a higher metal concentration than the crustaceous prey items consumed by the adults. In comparison with the other analytes, the difference of As and Se concentrations in juveniles to adults were not well pronounced. Although As concentrations were found to be higher in insects which would cause juveniles to have higher As concentrations, As is found to be stored in the carapace of the crayfish which the adults consume. Cadmium concentrations in the scutes from the juveniles were 10.1 times more than the Cd concentrations found in the scutes of adult crocodiles from Belize and 37.7 times more than the scutes of the adults from Costa Rica. There were no scutes collected from juveniles from Costa Rica, and it is only proposed that the adults consume as high amounts of fish as do other crocodiles in similar habitats; however, further investigation could show a more pronounced difference in As and Se concentrations between the insect consuming juveniles and the fish consuming adults. As reported by the ACES, there has been illegal dumping in mangroves and lagoons, which could explain that higher concentrations in juveniles crocodiles than the larger crocodiles that reside outside of those areas. Further investigation of the amount of unregulated dumping into the ocean needs to be investigated before elaborating more on this aspect.

In the regressions for the juveniles and subadults, there is a female juvenile crocodile from Belize that shows much lower metal concentrations from the scute (Figure 1, 2, and 3). First, the juvenile is the largest juvenile and it may be more accurate to classify this crocodile as a subadult which behaves differently from a juvenile. In previous studies, a SVL of 62cm is classified as a juvenile, but at 65cm the crocodile is classified as a subadult (Platt et al. 2013).This crocodile is 3 cm shy from being considered a subadult. Perhaps this crocodile should be reclassified as a subadult and the different metal concentrations between the juveniles and subadults from Belize would become more prominent. Additionally, SVL is not an impeccable measurement to determine age. The female juvenile could be much older than expected and had experienced a stunt in growth due to a lack in nutrition. A crocodile has the potential to increase their body size 500-fold from hatching to adulthood (Platt et al. 2013). Any stunt of growth could give rise to a smaller crocodile which is actually older than the other crocodiles in its size class. Although this juvenile is far away from the sewage plant which could contain a multitude of contaminants in prey items, water sheds, or sediments, the two way ANOVAs performed with proximity fails to support this notion.

Toxicant Correlations

High correlations have been found among metal constituents in the environment. In these scute tissues we found that these metals show high metal concentrations. There could be similar waste disposal in these areas, or that the crocodiles uptake the same toxicants but struggle to excrete them. More specifically the juveniles fail to excrete the contaminants they acquire. Unlike adult crocodiles, juveniles do not have fully developed physiological systems in place which may contribute to higher contaminant concentrations. Although potentially harmful, juveniles may absorb contaminants that have similar chemical properties of essential elements due to their high physiological demands. High metal correlation limits the ability to designate one specific toxicant as the cause of an adverse effect observed in the crocodile. With the exception of Be, Mo, and Tl, a signal was detected by every single crocodilian scute for each of the other 13 metals. High concentrations alone would not be sufficient evidence to pin point which toxicant inflicted an adverse effect for each organism has a distinct toleration range for each toxicant. For example, copper is necessary in the diet for iron utilization and a cofactor for an enzyme associated with metabolism; however at elevated copper concentrations are toxic to cells (Zelikoff et al. 2005). In this study, high Cu concentrations were detected. Even though the crocodiles from Costa Rica that had no observable health defects had Cu concentrations that we less than half of the Cu concentrations in the adults from Belize, crocodiles from Costa also had lower Pb, Ag, Cd, Al, Cr, Co, Mo, Sb, Se, Sn, and Zn concentrations. Adverse effects could be caused by any of these 11 analytes.

Analogues of essential trace elements overlap significant correlations. As different analytes, there is a wide range of trace-metal adsorption characteristics. However, such distinct characteristics can be simplified if the surfaces of these particulates are coated in a limited array of materials which then occludes the underlying bulk material matrix which decreases the degree of surface chemical heterogeneity. It has been speculated that hydrated metal oxides of Fe and Mn and organic coatings, along with the underlying bulk material matrix has the potential to make a model particulate phase with a given metal adsorption behavior. The adsorption of Cd, Cu, Pb, and Zn onto amorphous $Fe_2O_3{\bullet}H_2O$ and other hydrous oxides has been observed in a wide range of adsorbate-to-metal concentrations as a function of variable pH. Such studies support the suggestion that general characteristics are likely to be observed in multicomponent natural solid systems (Lion et al. 1982). This would explain such high metal correlations between metals that are chemically distinct, but we are unable to explore this any further.

Overall Findings

In the regressions for contaminant concentrations and snout-to-vent length, the crocodiles from Costa Rica, which had no health issues, contained similar contaminant concentrations in their scutes as did the unhealthy crocodiles from Belize. Nonetheless, we must impede the unregulated dumping of waste for more reasons than just preserving the lives of the crocodiles.

High contaminant concentrations in juveniles may be in part be natural, in their regulation of their uptake, their diet, and foraging location. Although it is not known the specific concentrations that compromise the tolerance range of the juveniles, however, there is a tolerance range that can be reached that compromises the health of a juvenile crocodile. This study shows how their contaminant concentrations are already high as shown in their scutes. Introducing more contaminants into this environment could initiate the tipping point for these crocodiles and other organisms. An important emphasis lies on crocodiles, being an apex keystone species that helps to regulate the food chain and maintain species diversity. Even if the other organisms fair fine contrary to increasing release of contaminants, once crocodiles are affected it will be difficult to reverse the process. In areas such as Belize, crocodiles are at risk simply due to their small population size, and any loss of a crocodilian's life can have an impact on the ecosystem.

As observed by the high metal concentrations these crocodiles are accumulating, crocodiles do not show major discrimination in the accumulation of these contaminants. Since these crocodiles cannot regulate the uptake of these contaminants that well, we need to regulate our output of contaminants.

Laws are already in place that prohibit this dumping, but it seems like there is not enough resistance. Our Sb concentrations found in the scutes most likely indicates that there are batteries being disposed in these areas. These individuals who seem careless may just need the appropriate services to manage their waste products. Implementation of these services might help to also spread awareness; in that the locals may not that they are creating a negative impact on the environment but not to a great extent. In addition to enforcing the laws through higher penalties, more individuals watching the sites with the most frequent dumping, and signs that discourage improper disposal of waste can help. In Florida, a strategy that was implemented was to eliminate the presence of any humans in the surrounding areas that obtained crocodile nests. It is believed that this is the reason as to why the population of crocodiles in Florida has increased as much as it has. However Ambergris Caye is much smaller and is a populated area by in many areas, both crocodiles and people. The strategy utilized in Florida may not be possible to apply to Ambergris Caye, but certainly another approach needs to be implemented to ease the burden of the crocodiles due to their contaminant concentrations.

Table 1. Summary statistics for metal concentrations* in American crocodile scutes from Central America.

Location	Costa Rica (n=7)			Belize Adult (n=28)			Belize Juvenile (n=8)		
Analyte	Mean	LCL§	UCL†	Mean	LCL§	UCL†	Mean	LCL§	UCL†
Pb	95.1	37.9	238.2	291.7	236.3	360.2	1717.9	348.3	8472.3
As	445.7	135.8	1462.2	361.4	287.5	454.4	845.3	258.2	2766.9
Cu	3221.1	2074.9	5000.3	7112.1	4698.9	10764.7	5248.1	2023.0	13614.5
Ag	131.8	78.0	222.8	337.3	272.6	417.3	2837.9	501.2	16069.4
Be	2.8	1.0	7.5	2.3	1.6	3.2	22.8	2.4	212.3
Cd	5.2	4.0	6.8	19.4	14.9	25.1	195.9	31.5	1219.0
Al	208929.6	62373.5	699842	34673.7	24946.0	48194.8	85703.8	43451.0	169044.1
Cr	1629.3	1361.1	1950.3	1875.0	1585.6	2217.2	5407.5	1442.1	20276.8
Ni	437.5	193.6	988.6	437.5	372.6	513.7	618.0	233.9	1633.1
Co	168.7	74.3	382.8	207.5	163.7	263.0	2065.4	325.1	13122.0
Mo	30.1	14.0	64.6	88.7	37.2	211.3	445.7	6.3	31477.5
Sb	122.5	107.3	139.8	375.0	316.9	443.7	3475.4	579.4	20844.9
Se	606.7	458.1	803.5	665.3	562.7	786.5	1030.4	381.1	2786.1
Tl	22.7	21.9	23.5	18.4	9.0	37.8	5.4	0.7	44.7
Sn	629.5	582	680.9	2162.7	1739.4	2689.1	20558.9	3475.4	121618.6
Zn	3349.7	2349.6	4775.3	7277.8	6526.8	8115.2	8072.4	3396.3	19186.7

* : ng/g
§: LCL= lower 95% confidence interval
† : UCL= upper 95% confidence interval

Table 2. P values[a] of proximity, sex, and size based on metal concentrations (ng/g) in American crocodile (*Crocodylus acutus*) scutes from Central America.

Analyte	Proximity[b]	Sex	Proximity[b]	Size	Sex	Size
Pb	0.315	<0.001	0.057	<0.001	0.003	0.436
As	0.294	0.01	0.156	0.161	0.01	0.386
Cu	0.853	0.841	0.602	0.353	0.325	0.229
Ag	0.386	<0.001	0.128	<0.001	0.002	0.188
Cd	0.541	<0.001	0.124	<0.001	0.004	0.257
Al	0.048	0.046	0.043	0.081	0.344	0.698
Cr	0.108	<0.001	0.028	0.023	0.003	0.812
Ni	0.061	0.016	0.015	0.352	0.002	0.272
Co	0.151	<0.001	0.075	<0.001	0.002	0.163
Mo	0.092	0.003	0.073	0.004	0.049	0.102
Sb	0.269	<0.001	0.08	<0.001	<0.001	0.153
Se	0.259	0.011	0.105	0.431	0.003	0.409
Tl	0.132	0.046	0.172	0.008	0.695	0.082
Sn	0.292	<0.001	0.118	<0.001	0.003	0.167
Zn	0.765	0.111	0.62	0.923	0.007	0.09

[a]: Statistical analysis was performed with log transformed data

[b]: Proximity of the crocodile from a sewage plant in Ambergris Caye Belize in general terms of near, far, and intermediate.

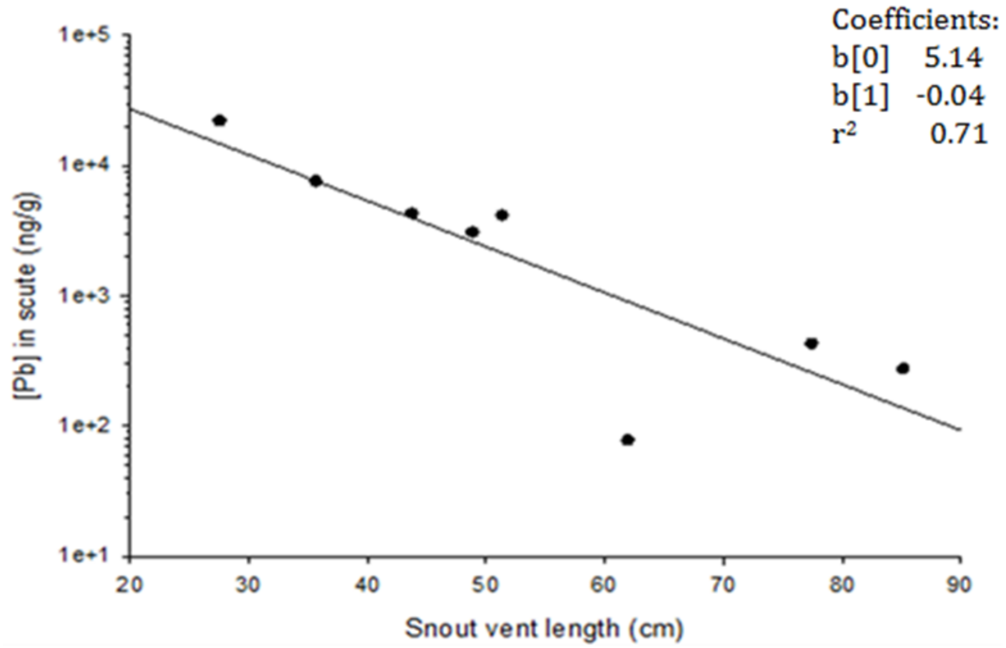

Figure 1. Lead concentrations in juvenile and subadult crocodiles from Belize versus snout to vent length. Note: Metal concentrations on this graph are slightly higher than measured since the data was graphed as log[1+M].

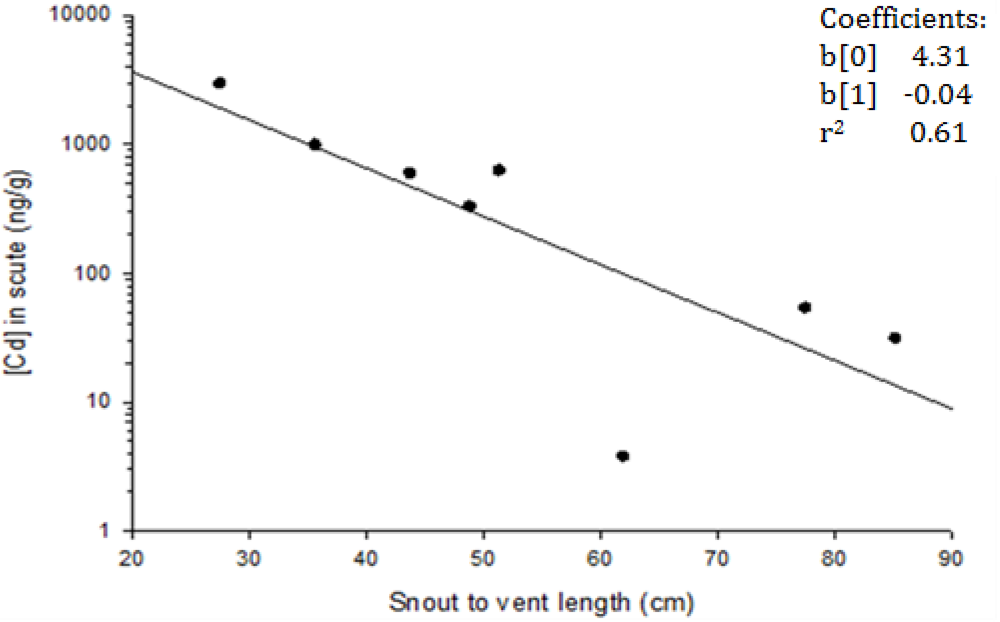

Figure 2. Cadmium concentrations in juvenile and subadult crocodiles from Belize versus snout to vent length. Note: Metal concentrations on this graph are slightly higher than measured since the data was graphed as log[1+M].

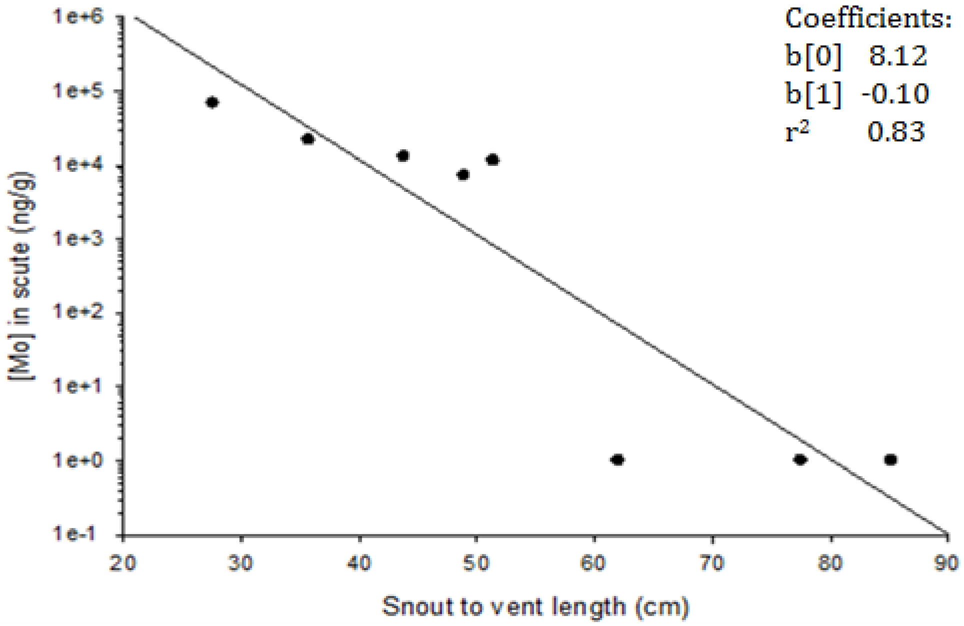

Figure 3. Molybdenum concentrations in juvenile and subadult crocodiles from Belize versus snout to vent length. Note: Metal concentrations on this graph are slightly higher than measured since the data was graphed as log[1+M].

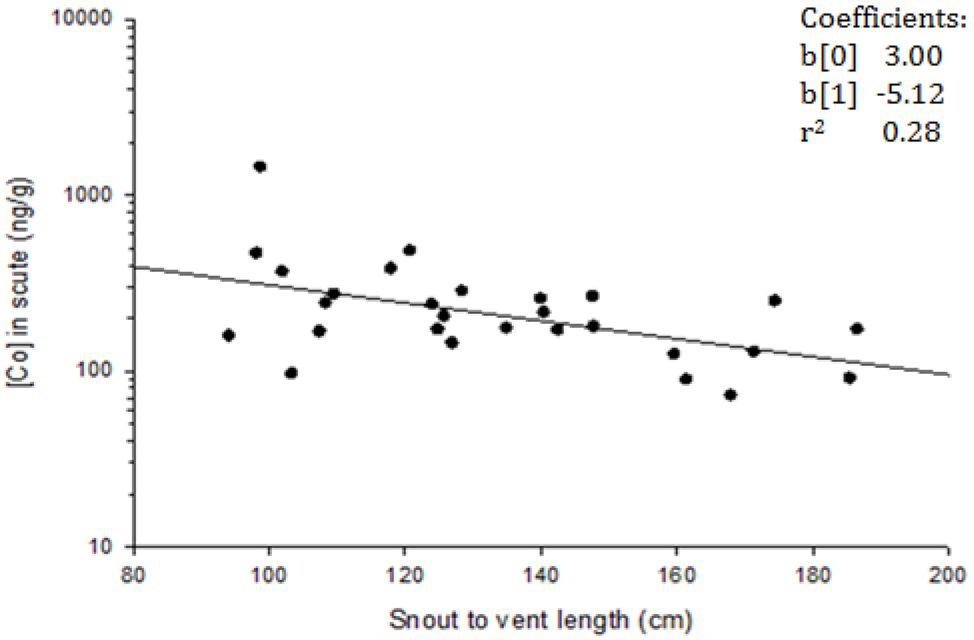

Figure 4. Cobalt concentrations in adult crocodiles from Belize and Costa Rica versus snout to vent length. Note: Metal concentrations on this graph are slightly higher than measured since the data was graphed as log[1+M].

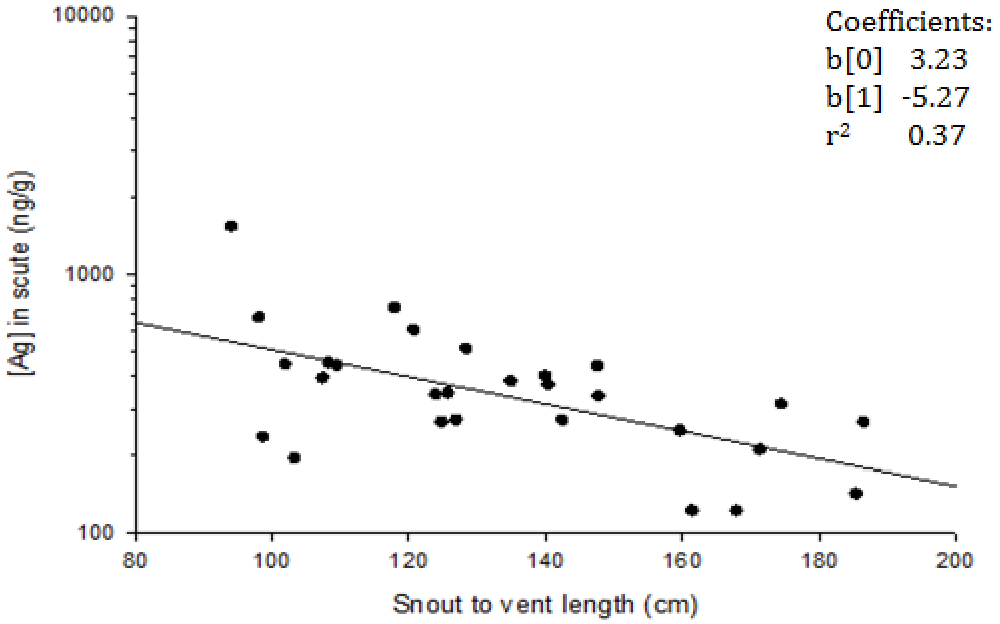

Figure 5. Silver concentrations in adult crocodiles from Belize and Costa Rica versus snout to vent length. Note: Metal concentrations on this graph are slightly higher than measured since the data was graphed as log[1+M].

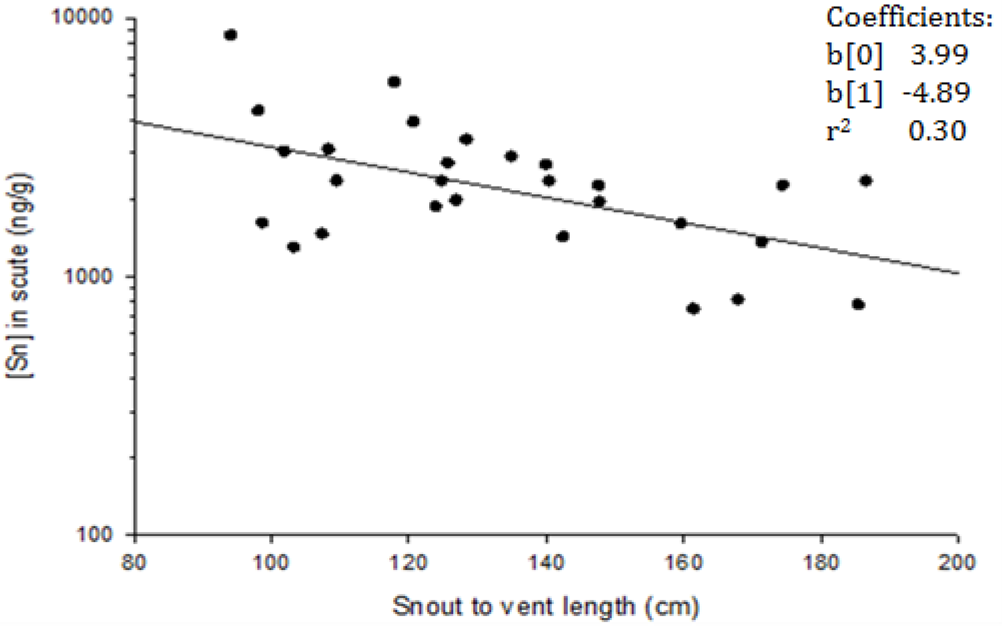

Figure 6. Tin concentrations in adult crocodiles from Belize and Costa Rica versus snout to vent length. Note: Metal concentrations on this graph are slightly higher than measured since the data was graphed as log[1+M].

APPENDIX

American crocodile scute sample weights

Adults from Costa Rica

Batch #	Field #	Lab #	Label #	Sample Weight (g)	Observations
1	1	O4	610104	2.0033	
1	2A	O7	6102A07	2.0021	
1	2B	O3	6102B03	2.0052	
1	3	O1	610301	2.0046	
1	4	O9	610409	2.0043	
1	5	O8	610508	2.0067	
1	6	O6	610606	2.0017	
1	7A	O2	6107A02	2.0033	
1	7B	O5	6107B05	2.0045	

Juveniles from Belize

Batch #	Field #	Lab #	Label #	Sample Weight (g)	Observations
3	1	55	830155	0.00345	
3	6	59	830659	0.0207	
3	7	56	830756	0.01817	
1	16	12	811612	3.417	
3	20	60	832060	0.01078	
3	24	58	832458	0.03253	Two Scutes

Subadults from Belize

Batch #	Field #	Lab #	Label #	Sample Weight (g)	Observations
2	11	33	821133	0.1011	Two Scutes
1	27	11	812711	0.301	

Adults from Belize

Batch #	Field #	Lab #	Label #	Sample Weight (g)	Observations
2	2	34	820234	0.2013	
2	4	32	820432	0.3211	
3	5	57	830557	0.00296	
2	8	36	820836	1.5178	
3	9	54	830954	0.105	
2	10	35	821035	0.2504	
2	12	38	821238	0.2489	Two scutes and sand
2	13	37	821337	0.3209	Two scutes and sand
2	14	31	821431	0.4055	Two Scutes and bad smell
2	15	26	821526	0.347	
2	15	27	821527	0.4405	
2	17	30	821730	0.6931	Two Scutes
3	18	44	831844	0.5619	Two Scutes
2	19	29	821929	0.4577	Two Scutes
1	21	17	812117	0.7962	
2	22	39	822239	0.6025	
2	22	40	822240	0.421	
3	23	52	832352	0.9461	Blood
3	23	53	832353	0.7033	
2	25	22	822522	0.5611	
2	25	23	822523	0.9849	
1	26	20	812620	0.4482	
2	26	21	822621	0.6199	
1	28	13	812813	0.4419	
3	28	47	832847	0.5345	
3	28	48	832848	0.8832	
2	29	24	822924	0.4324	Two Scutes
2	29	25	822925	0.3901	
2	30A	28	8230A28	0.7052	
1	30B	16	8130B16	0.6181	Two Scutes
3	32	50	833250	0.4927	
3	32	51	833251	0.4252	
1	33	18	813318	0.3745	Sand
1	33	19	813319	0.7779	
3	34	41	833441	0.4186	Two scutes and sand
1	35A	10	8135A10	0.4925	
1	35B	14	8135B14	1.0228	Sand
1	35B	15	8135B15	0.9544	Sand
3	42	42	834242	1.7333	

3	42	43	834243	1.6242
3	42	49	834249	0.9513
3	50/60	45	8350/6045	1.8375
3	50/60	46	8350/6046	1.1185

Data Collected in the Field

Crocodiles from Belize

Lab #	Field #	Proximity to Pollution	Sex	Size Class	SVLp (cm)
55	1	UNK	UNK	Juvenile	27.6
34	2	Intermediate	M	Adult	118
32	4	Far	M	Adult	128.5
57	5	Intermediate	UNK	UNK	101
59	6	Close	UNK	Juvenile	51.4
56	7	Close	M	Juvenile	43.8
36	8	Close	M	Adult	168
54	9	Close	F	Adult	94.2
35	10	Far	M	Adult	98.2
33	11	Far	F	Subadult	77.5
38	12	Far	F	Adult	120.8
37	13	Intermediate	F	Adult	102
31	14	Intermediate	M	Adult	140
26 and 27	15	Intermediate	F	Adult	108.4
12	16	Far	F	Juvenile	62
30	17	Close	F	Adult	142.5
44	18	Close	F	Adult	124
29	19	Close	M	Adult	147.7
60	20	Far	UNK	Juvenile	35.7
17	21	Intermediate	M	Adult	98.8
39 and 40	22	Intermediate	M	Adult	140.5
52 and 53	23	Close	M	Adult	171.4
58	24	Close	UNK	Juvenile	48.9
22 and 23	25	Close	F	Adult	159.7
20 and 21	26	Close	M	Adult	186.6
11	27	Intermediate	F	Subadult	85.2
13, 47, and 48	28	Close	F	Adult	147.8
24 and 25	29	Far	F	Adult	125.8
28	30A	Close	M	Adult	107.5
16	30B	Close	M	Adult	127
50 and 51	32	Intermediate	M	Adult	174.5

18 and 19	33	Intermediate	M	Adult	124.9
41	34	Close	M	Adult	109.6
10	35A	Close	F	Adult	135
14 and 15	35B	Close	F	Adult	103.4
42, 43 and 49	42	Close	M	Adult	185.5
45 and 46	50/60	Close	M	Adult	161.5

Crocodiles from Costa Rica

Lab #	Field #	Proximity to Pollution	Sex	Size Class	SVLp (cm)
4	CR 1	Costa Rica	M	Adult	218.44
3 and 7	CR 2	Costa Rica	M	Adult	241.3
1	CR 3	Costa Rica	M	Adult	223.52
9	CR 4	Costa Rica	M	Adult	231.14
8	CR 5	Costa Rica	M	Adult	220.98
6	CR 6	Costa Rica	F	Adult	154.94
2 and 5	CR 7	Costa Rica	M	Adult	187.96

Table 3. Summary statistics for metal concentrations in Buffalo River sediment, Nanopure ™ water, and blank standards for the inductively coupled plasma mass spectrometry.

Anaylte	Buffalo R.[b]	LCL[c]	UCL[d]	Nanopure ™	LCL[c]	UCL[d]	Blank	LCL[c]	UCL[d]
Pb	125091.2	111581.0	140239.0	37.7	21.6	69.2	31.5	10.5	101
Zn	93697.0	81131.0	108212.0	73.5	25.3	219.6	24.0	0.0	0.0
As	12768.4	11115.0	14671.0	0.0	0.0	0.0	0.0	0.0	0.0
Cu	93204	73293.0	118527.0	0.0	0.0	0.0	0.0	0.0	0.0
Ag	3935.6	3436.4	4509.7	25.7	6.6	108.4	1.6	1.9	3.6
Be	714.3	631.6	810.0	0.0	0.0	0.0	0.0	0.0	0.0
Cd	2387.7	2138.2	2668.6	4.6	2.8	10.9	0.0	0.0	0.0
Al	0[e]	0[e]	0[e]	2766.9	1972.0	3886	0.0	0.0	0.0
Cr	101779.4	78144.0	132567.0	257.4	223.5	298.8	0.0	0.0	0.0
Ni	45763.5	35672.0	58713.0	11.1	8.4	17.3	0.0	0.0	0.0
Co	13975.1	11590.0	16854.0	96.4	76.8	123.5	12.9	1.4	140
Mo	3075.6	2773.3	3413.1	0.0	0.0	0.0	0.0	0.0	0.0
Sb	372.1	324.4	429.0	145.1	119.1	179.1	98.4	45.3	218
Se	592.9	505.2	698.2	0.0	0.0	0.0	0.0	0.0	0.0
Tl	602.6	505.4	720.8	10.3	1.1	111.6	13.6	2.9	74.5
Sn	1422.2	1281.6	1580.5	1144.9	1045.0	1256.0	44.9	16.4	129

[a]: ng/g

[b]: Values were compared to reference concentration values for 8704 Buffalo River sediment that have a 95% confidence interval. Mean Ni and Co concentrations were within the 95% confidence interval of the reference concentrations while mean Pb, Zn, Cd, Cr, and Sb were not. All other metals were not within the reference material.

[c]: LCL= lower 95% confidence interval

[d]: UCL= upper 95% confidence interval

[e]: Over range

Figure A1. Practice scute residue left around the center of the filter paper. This visualization demanded for a modification in the digestion method EPA 3050B.

Figure A2. Filter paper used for a scute that was predigested. This modification showed improved results in that with the exception of the very tip, no scute residue was visual.

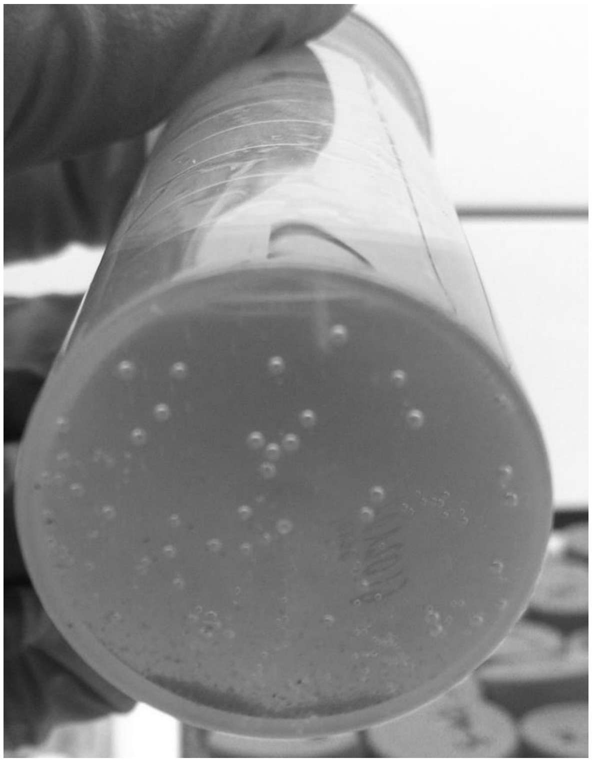

Figure A3. This solution is in mid-digest. Due to the visible suspended and the particles that settled, the digestion was deemed to not be complete.

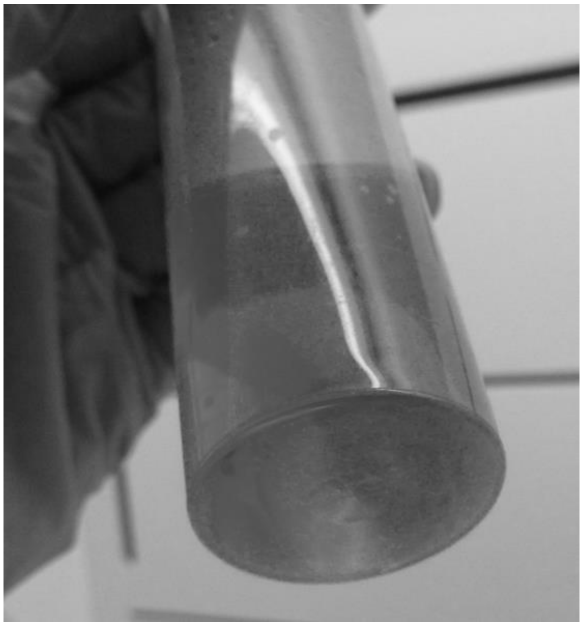

Figure A4. There are no suspended or settled particles and the digestion was deemed to have been complete.

BIBLIOGRAPHY

Abdallah, H. H.; Ramasami, P. Rotational barrier, energy difference and thermodynamic parameters of 2-furoylfluoride, its sulfur and selenium analogues in the gas and solution phases: A theoretical study. *Journal of Molecular Structure: THEOCHEM.* **2009**, 913, 157-161.

Brazaitis, P. Maxillary regeneration in a march crocodile Crocodylus palustris. *Journal of Herpetology*. **1981**, 15, 360-362.

Burger, J.; Gochfeld, M.; Rooney, A.A.; Orlando, E.F.; Woodward, A.R.; Guillette, Jr L.J. Metals and metalloids in tissues of American alligators in three Florida lakes. *Arch Environ Contam Toxicol.* **2000**, 38, 501–8.

Chenot-Rose. C. RSF concerns: American crocodile, Crocodylus acutus, population and habitat viability assessment and conservation in Ambergris Caye, Belize. **2012**, 1-4.

Collins, R.; Johansson, A.; Karlberg, T.; Markova, N.; van den Berg, S.; Olesen, K.; Hammarström, M.; Flores, A.; Schüler, H.; Holmberg Schiavone, L.; Brzezinski, P.; Arnér, E. S. J.; Högbom, M. Biochemical discrimination between selenium and sulfur 1: a single residue provides selenium specificity to human selenocysteine lyase. *PLoS ONE*. **2012**, 7, 1-10.

Dani, S. U. The arsenic for phosphorus swap is accidental, rather than a facultative one, and the question whether arsenic is nonessential or toxic is quantitative, not a qualitative one. *Science of the Total Environment*. **2011**, 409, 4889-4890.

Domaç, F.M.; Karlikaya, G.; Çetinkaya, Y.; Gencer, M.; Tanridağ, T.; Us, Ö. Electrodiagnostic studies in neurologically asymptomatic patients with vitamin B12 deficiency. *Journal of Neurological Sciences*. **2014**, 31, 1-10.

Fillol, C.; Dor, F.; Clozel, B.; Goria, S.; Seta, N. Does arsenic in soil contribute to arsenic urinary concentrations in a French population living in a naturally arsenic contaminated area? *Sci Total Environ*. **2010**, 408, 6011-6.

Fuller, C. C.; Davis, J.A.; Cain, D.J.; Lamothe, P. J.; Fries, T. L.; Fernandez, G.; Vargas, J. A.; Murillo, M. M. Distribution and transport of sediment-bound metal contaminants in the Rio Grande de Tarcoles, Costa Rica (Central America). *Water Research*. **1990**, 24, 805-812.

Groombridge, B. The distribution and status of world crocodilians. In: Webb GJW, Manolis SC, Whitehead PJ, editors. Wildlife management: crocodiles and alligators. *Chipping Norton: Surrey Beatty and Sons Pty Limited*. **1987**, 9–21.

Hopkins, W. A.; DuRant, S.E.; Staub, B. P; Rowe, C.L.; Jackson, B.P. Reproduction, Embryonic Development, and Maternal Transfer of Contaminants in the Amphibian Gastrophryne carolinensis. *Environ Health Perspect*. **2006**, 114, 661–666.

International Union of Pure and Applied Chemistry. Glossary for chemists of terms used in toxicology: Pure and Applied Chemistry. **1993**, 65, 2003-2122.

IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on **2014**.

Kopf, A. Effective strength of incoming sediments and its implications for plate boundary propagation: Nankai and Costa Rica as type examples of accreting vs. erosive convergent margins. *Tectonophysics*. **2013**, 608, 958-969.

Mason, R.P.; Laporte, J.M.; Andres, S. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. *Archives of Environmental Contamination and Toxicology*. **2000**, 38, 283-297.

McFarland, C.A., Delta-aminolevulinic acid dehydratase (ALAD) characterization and use in metal exposure assessments. Ph.D. Thesis, Texas Tech University, Lubbock, TX, **2005**.

Lion, L. W.; Altmann, R. S.; Leckie, J. O. Trace-metal adsorption characteristics of estuarine particulate matter: evaluation of contributions of iron/manganese oxide and organic surface coatings. *Environ. Sci. Technol.* **1982**, 16, 660–666.

Pemmer, B.; Roschger, A.; Wastl, A.; Hofstaetter, J. G.; Wobrauschek, P.; Simon, R.; Thaler, H. W.; Roschger, P.; Klaushofer, K.; Streli, C. Spatial distribution of the trace elements zinc, strontium and lead in human bone tissue. *BONE*. **2013**, 57, 184-193.

Platt, S.G.; Thorbjarnarson, J. B. Nesting Ecology of the American Crocodile in the Coastal Zone of Belize. *Copeia*. **2000a**, 2000 (3), 869-873.Platt, S.G.;

Thorbjarnarson, J. B. Status and conservation of the American crocodile, *Crocodylus acutus*, in Belize. *Biological Conservation.* **2000c**, 96, 13-20.

Platt, S.G.; Thorbjarnarson, J. B.; Rainwater, T.R.; Martin, D. R. Diet of the American Crocodile (Crocodylus acutus) in Marine Environments. *Journal of Herpetology.* **2013**, 47, 1–10.

Platt, S.G.; Thorbjarnarson, J.T. Population status and conservation of Morelet's crocodile, Crocodylus moreletii, in northern Belize. *Biol Conserv*. **2000**a, 96, 21–9.

Platt, S.G.; Thorbjarnarson, J.T. Status and conservation of the American crocodile, Crocodylus acutus, in Belize. *Biol Conserv*. **2000**b, 96, 13–20.

Rainwater, T.R.; Millichamp, N.J.; Barrantes, L. D. B.; Barr, B. R.; Montero, J. R.B.; Platt, S.G.; Abel, M.T.; Cobb, G.P.; Anderson, T.A. Ocular disease in American crocodiles (Crocodylus acutus) in Costa Rica. *Wildlife Disease Association*. **2011**, 47(2), 415-426.

Rainwater, T. R.; Wu, T. H.; Finger, A. G.; Cañas, J.E.;Yu, L.; Reynolds, K. D.; Coimbatore, G.; Barr, B.; Platt, S. G.; Cobb, G. P.; Anderson, T.A.; McMurry, S.T. Metals and organochlorine pesticides in caudal scutes of crocodiles from Belize and Costa Rica. *Science of the Total Environment*. **2007**, 373, 146-156.

Richardson, K.; Webb, G.; Manolis, C. Crocodiles: Inside and Out. *Surrey Beatty and Sons*. **2000**.

Richardson, K.C.; Webb, G.J.W.; Manolis, S.C. Crocodiles: inside out: a guide to the crocodilians and their functional morphology. Sydney: *Surrey Beatty and Sons, Ltd*. **2002**, 172.

Schneider, L.; Maher, W.; Green, A.; Vogt, R.C. Mercury contamination in reptiles: An emerging problem with consequences for wildlife and human health. *Nova Science*. **2013**, 173-215.

Thorbjarnarson, J.;Mazzotti, F.; Sanderson, E.; Buitrago, F; Lazcano, M; Minkowski, K.; Muñiz, M.; Ponce, P.; Sigler, L.; Soberon, R.; Trelancia, A.; Velasco, A. Regional habitat conservation priorities for the American crocodile. *Biological Conservation*. **2006**, 128, 25-36.

Tucker, A. D. Validation of skeletochronology to determine age of freshwater crocodiles (*Crocodylus johnstoni*). **1997**, 48, 343-51.

Schardt, H. Why is this crocodile smiling?. *National Wildlife*. **2008**, 46, 31-35.

Whelan, T. Environmental contamination in the Gulf of Nicoya, Costa Rica. *Ambio*. **1989**, 18, 302-304.

Villegas, A.; Schmitter-Soto, J. J. Feeding habits of the American crocodile, *Crocodylus acutus* (cuvier, 1807) (reptilia: crocodylidae) in the southern coast of Quintana Roo, Mexico*. Acta Zoológica Mexicana*. **2008**, 24, 117-124.

Vitamin B-12 deficiency. *Mayo Clinic Health Letter*. **2013**, 31, 7-7.

Zelikoff, J.T.; Vohr, H.; Trace metals and the immune system. *Encyclopedic Reference of Immunotoxicology*. **2005**, 654-657.

Zhao, L.; Li, J.; Li, Y.; Liu, J.; Wirth, T.; Li, Z. Selenium-containing naphthalimides as anticancer agents: Design, synthesis and bioactivity. *Bioorganic & Medicinal Chemistry*. **2012**, 20, 2558-2563.