ASSESSMENT OF CROCODILE ABUNDANCE AND SEASONAL EFFECTS OF SALINITY ON DISTRIBUTION USING BOTH BOAT BASED AND AERIAL DRONE SURVEYS

by

Jenell Black

A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Biology

Fort Wayne, Indiana

August 2019

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Frank V. Paladino, Chair
Department of Biology
Dr. Robert Gillespie
Department of Biology
Dr. Bruce A. Kingsbury
Department of Biology

Approved by:
Dr. Jordan M. Marshall
Head of the Graduate Program

To all the Giants whose shoulders I have been fortunate enough to stand upon

ACKNOWLEDGMENTS

My deepest gratitude goes to such a large number of individuals. First and foremost I thank Dr. Frank V. Paladino, my primary advisor. He took a chance on me as a graduate student, and gave me the opportunity to begin to pursue a life-long dream. Under him I have learned how to design a research project, and make the best of never-ending changes incurred from the nature of studying wildlife biology. I would also like to extend thanks to my committee members, Dr. Bruce Kingsbury and Dr. Robert Gillespie for helping advise me along the way.

I would not have met Dr. Paladino without the help of Dr. James “Jim” Spotila, who saw something in me. Jim gave me the opportunity to really wet my feet in fieldwork, and while that first season of sea turtle work was the most challenging of my life, it was one of the most fulfilling aspects as well and led to further pursuit in conservation biology. Of course, I never would have been in such close contact with Dr. Spotila, had it not been for Dr. Walter Bien, who gave me my first real taste of field ecology.

Dr. Nathan J Robinson and Dr. Pilar “Bibi” Santidrián Tomillo can never be thanked enough. The amount of support, knowledge, and opportunity afforded to me by them was limitless. Not to mention I could never have gotten through permits without Bibi’s endless help and patience. Beyond my thesis, they have helped me become a competent field manager and researcher. I will always remember our nights in Cabuyal playing dominoes with Margarita Flores.

Dr. Chelsea Clyde-Brockway has been my best friend, and often roommate, throughout this entire journey and Dr. Aliki Panagopoulou has been my own personal Philadelphian cheer squad.

I extend a special thank you to all of the people who have gone out canoeing with me, because I would never have been able to do it without you. Nathan Robinson, Shannon Kuznar, Chris Gatto, Brett Butler, Lauren “Lo” Hackney, Lexi Baker, Quintin Bergman, Rhys Pegrume, and Dana Neel. Thank you to the staff at Las Tortugas Hotel for letting me rent the canoes needed to conduct this research. Further thanks to all of the people who assisted in drone surveys as my diurnal spotters: Lexi Baker, Christian Díaz “Chuqui” Chuquisengo, Laura St. Andrews, Quintin Bergman, Alysa Hopkins, Kelcey Tolliver, and Chelsea Clyde-Brockway. And of course no drone survey would have been possible without the help of Enrique Chavarrier Rodriguez, who went above and beyond in taking the time to take us surveying on his tour boat.

From MINAE I would like to thank first and foremost Elizabeth “Ely” Velez, Rotney Piedra, Bernal Cortés, and Alvaro. We made the best crocodile team, I learned so much about crocodile survey and capture, and I will cherish those memories for the rest of my life.

From The Leatherback Trust I would like to thank the field teams from 2014-2017. If they were not supporting me in my surveys, they were picking up nights I would have been walking the beach to allow me to conduct research. I would also like to thank so much more of the staff for their support. Ely Solano, who helped me culture a good relationship with MINAE, and helped me practice my garbled Spanish along the way. Julianne Koval might not have helped me collect data, but she’s been with me throughout the entire journey. And of course, Giuselle, Jesús, and the entire Kike’s crew (Yanira, Kike, and Carlos most especially), who comprised my little Costa Rican family.

From my Drexel cohort, I would like to also thank Dr. Mike O’Connor, who patiently sat with me and taught me statistical basics and then some. And to thank Dr. Kevin Smith and Dr. Dane Ward for letting me assist in their study of the northern pine snake, which solidified my decision to change my career. I would like to thank Dr. Shaya Honarvar for encouraging me further in this career switch, and encouraging me along the way. I extend my gratitude to Dr. Abby Dominy and Dr. Hal Avery for all of the opportunity afforded by me to gain tremendous experience acting as a field manager for a red-bellied turtle project, and for all of their support and career advice along the way. Although she is in no way related to conservation biology, I extend a special thank you to Dr. Elizabeth Spudich, I would not be half the successful student I am without you.

Last, but in no way the least, I would like to thank my friends, and particularly my family (the core) who were supportive of me, despite not necessarily having any idea what I was talking about.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Author: Black, Jenell. MS
Institution: Purdue University
Degree Received: August 2019
Title: Assessment of Crocodile Abundance and Seasonal Effects of Salinity on Distribution Using Both Boat Based and Aerial Drone Surveys
Committee Chair: Frank Paladino

The American crocodile (*Crocodylus acutus*) is a common resident of Central American waterways, with one of the largest ranges of all crocodilians. It is very salt-tolerant, with only Nile crocodiles and Saltwater crocodiles superseding it in terms of being the most saline tolerant. Globally it has lost 9% of its range, with habitat destruction and fragmentation as the leading threat to this species. Overall there is a great need to understand the habitat uses by this species in order to mitigate consequences of further anthropogenic incursions along its range. Thus, this study aims to investigate seasonal effects of salinity on habitat selection of American crocodiles in a natural mangrove habitat, and to validate use of a commercial drone as a surveying tool for crocodilians in such habitats.

This study found that as the seasons shift from wet to dry there was an associated increase in salinity of the estuarine waterway that resulted in an increase in the halocline of the estuary waters the greater the distance from the ocean. When broken down into the following groups: Hatchlings and juveniles (HJ) vs subadults and adults (SbA) it was found that salinity as well as season both had a correlation with the presence of crocodiles from the HJ group ($p<.05$) and there was no correlation in regard to SbA crocodiles. Further, HJ size classes predominantly were found in the further reaches of the estuary, regardless of season.

Surveys taken via drone were as efficacious as surveys taken diurnally via boat, with a survey encounter rate of 0.40 km-1, but still less efficacious than nocturnal eyeshine surveys,

which had an encounter rate between 1.2 – 2.9 km-1. The drone was able to identify animals that were submerged under water, but was unable to ID animals resting on banks in thick mangroves. However, the drone was able to identify crocodiles as small as 0.7m. Further, the drone showed encounter rates for sharks and rays that were much higher than crocodiles, 0.84km-1 and 0.64km-1 respectively.

INTRODUCTION

Background Information & Premises

The American crocodile (*Crocodyllus acutus*) is a common resident of the waterways of Central America (Thorbjarnarson, 2010). It has one of the largest ranges of all crocodilians, spanning across 18 countries as far north as the southern tip of Florida, USA, and as far south as northern Peru (Thorbjarnarson, 2010; IUCN, 2014). It is a euryhaline species, which requires a diverse habitat, and most often can be found in mangroves of coastal estuaries and lagoons. Although they prefer a brackish environment, they can be found in freshwater rivers or hypersaline landlocked lakes and estuaries (Thorbjarnarson *et al*., 2006). Beyond the large fraction of time these animals spend in the mangroves, they also require access to sandy riverbanks or beaches for nesting purposes (Charruau, 2012; Mazzotti, 1989). Some mangroves lacking sandy riverbanks or beaches are often associated with a smaller population of crocodiles (Thorbjarnarson *et al.,* 2006).

Although previously listed as endangered, it was re-listed in 1994 as vulnerable due in large part to protection which allowed populations to recover somewhat (IUCN, 2014). Historically hunting decimated the crocodile populations throughout the North and Central American regions, but the implementation of hunting bans has likely led to resurgence in populations (Ross, 1998). Despite this, some areas have seen limited to no increase in local populations. Currently, habitat loss is the highest-ranking threat faced by American crocodiles (Thorbjarnarson *et al*., 2006). Globally, American crocodiles have lost almost nine percent of their historic range, largely reflecting problems of habitat loss as well as other human interactions (Thorbjarnarson *et al.,* 2006)

It's important to note that not only is habitat loss the leading cause of decrease in crocodilian numbers, but smaller and fragmented habitat can lead to other negative impacts on population. Initially of note, habitat fragmentation has been shown to interfere with genetic variation in populations, especially if habitat fragmentation is severe (Cotroneo, 2010). In addition, decreased habitat is often due to human development along coastlines and subsequent destruction of mangrove forests (Alfaro, 2010; Lai *et al.*, 2015; Airoldi *et al.*, 2008). Mangrove forests play a vital role in the coastal ecosystem, and as much as 40% of the mangroves found in Pacific Costa Rica are considered threatened (IUCN, 2010). Mangroves have historically been considered nothing better than a useless swamp full of foul odor, and they have often been destroyed to make way for agriculture, mariculture, and urbanization (Valiela *et al.*, 2001).

Since *C. acutus* requires a diverse habitat both terrestrially and aquatically, these animals can act as an indicator of general habitat quality and overall condition of the area they occupy. Further, conservation of this species will have an overlap effect where the protection of their required habitat will encompass not only terrestrial and aquatic areas the crocodiles occupy, but also will protect these same areas that are important to many other species that use these habitats. The large range of this species will also require habitat conservation on an international level. Some areas, such as U.S. and Costa Rica host healthy populations of American crocodiles which have increased in numbers since the ban on hunting (Cotroneo, 2010), but it remains to be seen whether or not this is the case for countries such as Panama and Nicaragua where protection is minimal (Thorbjarnarson *et al.,* 2006). Conservation priorities for this species have noted Nicaragua and lacustrine areas of Panama as very high priority areas (Thorbjarnarson *et al.,* 2006), but overall there is limited data on habitat uses by this species for management practices and conservation purposes in these countries.

As habitat for these crocodilians dwindles, coupled with increased human encroachment, there is an associated increase in human crocodile interactions. Crocodiles are often perceived negatively as a dangerous species, which is not helped by an increase in media coverage surrounding crocodile human interactions (Murray *et al.*, 2015). Recently there has been a conflict at our study site between crocodiles and humans that garnered quite a bit of negative media attention. A crocodile bite recorded in the Tamarindo estuary led to the amputation of a U.S. surfer visiting the region (Fendt, 2015; Krumholtz, 2016; Christian Díaz Chuquisengo, unpublished). This interaction resulted in the relocation of a large (3+m) crocodile at the mouth of the Tamarindo estuary by SINAC (*Sistema Nacional de áreas de conservación costa rica*). The Guanacaste providence of Costa Rica has a skew toward male crocodiles in its populations, which may lead to more competition between male crocodiles and thus more aggressive males (Murray *et al.*, 2015). With a yet largely unknown spatial ecology of this species, it is difficult to manage and mitigate these types of occurrences.

One of the most prominent issues in terms of global climate change and conservation is land use changes, both natural and anthropogenic (Getzin, 2011). As discussed previously, access to sandy river shorelines and beaches is one of the nesting requirements for the American crocodile, and also happens to be one of the most sought after areas for human development. Mangrove forests are also negatively influenced by human development, and overall there is a great need to mitigate the loss of mangroves and work toward their conservation (Gilman *et al.*, 2008; Benfield *et al.*, 2005; Alongi, 2002). Human development along coastlines often comes at the expense of resident flora and fauna, and can often impact humans as well. Particularly in the case of crocodiles, reductions in crocodile habitats in close proximity to areas now utilized by humans, leads to an increase in negative interactions between humans and crocodilians (Fendt, 2015; Krumholtz, 2016; Christian Díaz Chuquisengo, unpublished). One way to mitigate these interactions is to understand population size and spatial use of resident crocodilians. In order to assist with this, this study aims to look at seasonal affect of salinity on habitat selection of American crocodiles in a natural mangrove habitat, and to validate use of a commercial drone as a surveying tool for crocodilians in such habitats.

Methods Used Across Both Projects

Study Site

I conducted this study in Parque Nacional Las Baulas (PNLB), located in the Guanacaste province of Costa Rica and established in 1991 (Figure 1). Specifically this study was conducted along the Tamarindo estuary (10 degrees 19 minutes North, 85 degrees 50 minutes West), which was designated as site #610 on 9-June-1993 as a wetland of international importance (RAMSAR, 2014). This 500-hectare wetland constitutes a portion of the largest mangrove swamp complex in dry Central America (Cotroneo, 2010). Eighty percent of this area is made up of mangroves, with the red mangrove representing 75% of vegetation (RAMSAR, 1990). Tamarindo estuary separates PNLB from the rapidly growing town of Tamarindo, and conservation of this area in particular may rapidly become critical as the town expands

The Tamarindo estuary consists of two branches, with a centralized island of mangroves approximately 300 m in diameter occurring at the intersection of these two branches (Figure 2). The average distance from the mouth of the estuary to the furthest accessible point of the right branch is 3.14 km, and the average distance from the mouth of the estuary to the furthest accessible point of the left branch is 4.16 km.

Previous assessment of this site confirmed the presence of numerous hatchlings and juveniles, which together comprise over 60% of the size-class distribution (Mauger, *et al.,* 2012).

Current adult population at time of study was estimated at 40 individuals (Elizabeth Velez, unpublished).

Crocodile Capture & Morphometrics & Identification

A 15-24 ft flatbottom aluminum boat with 25 hp outboard motor was used to slowly approach crocodiles. Crocodiles were then captured by either by hand, snake tongues or noosing. For noosing, a stiff wire loop was used to create a noose that attached to a long rope and was mounted to a long pole with duct tape. This allowed the crocodile sufficient room to thrash away from the boat without injury before being noosed and subsequently reeled in to a shore point (Chabrek, 1963). After restraint of the mouth, rubber bands were wrapped around the snout, and a wet black rag was used to cover the animal's eyes. Crocodiles were then individually marked by surgically removing caudal scutes with an alcohol-sterilized knife. Using a flexible tape measure the following animal dimensions were taken: total length from the most rostral point of the snout to the most caudal point of the tail (TL), tail circumference at the widest point, just caudal to the pelvis (TC), distance between the most rostral point of the snout and the most cranial edge of the vent (SVL), length of the entire head (HL), and width of the entire head (HW) (Cotroneo, 2010). The crocodile was then weighed using a mesh holding bag and a spring scale; we were unable to weigh crocodiles larger than 50kg.

Survey Methods – night

Nocturnal spotlight surveys were taken from an aluminum boat, using an outboard engine in a standard manner (Bayliss, 1987) A 10,000 lumen handheld light was used for a spotlight, with a 1,000 lumen LED headlamp used only while capturing crocodiles.

Surveys began either at the mouth of the estuary, or as far away from the mouth as would be capable to navigate by boat. Surveys would then occur in a unidirectional fashion, to avoid double counting, and to the furthest accessible reaches of the estuary using 10,000 lumen handheld light to look for red eyeshines. All eyeshines were counted as individual crocodiles, and the GPS location was recorded for each. Surveys were conducted beginning an hour around low-tide, to allow for more crocodile visibility.

Survey Methods – diurnal

Diurnal surveys were conducted primarily to assess crocodile location in terms of salinity/temperature. Diurnal surveys were conducted via canoe, and opportunistically from a flatbottomed tour boat conducted in the local estuary.

In a similar fashion to nocturnal spotlight surveys, the transects began at the mouth of the estuary or as far away from the mouth as the canoe was safely navigated from this point and survey was conducted in a unidirectional fashion to avoid double counting. When a crocodile was spotted, size was estimated to the nearest half meter, GPS location was recorded, and salinity/temperature was taken.

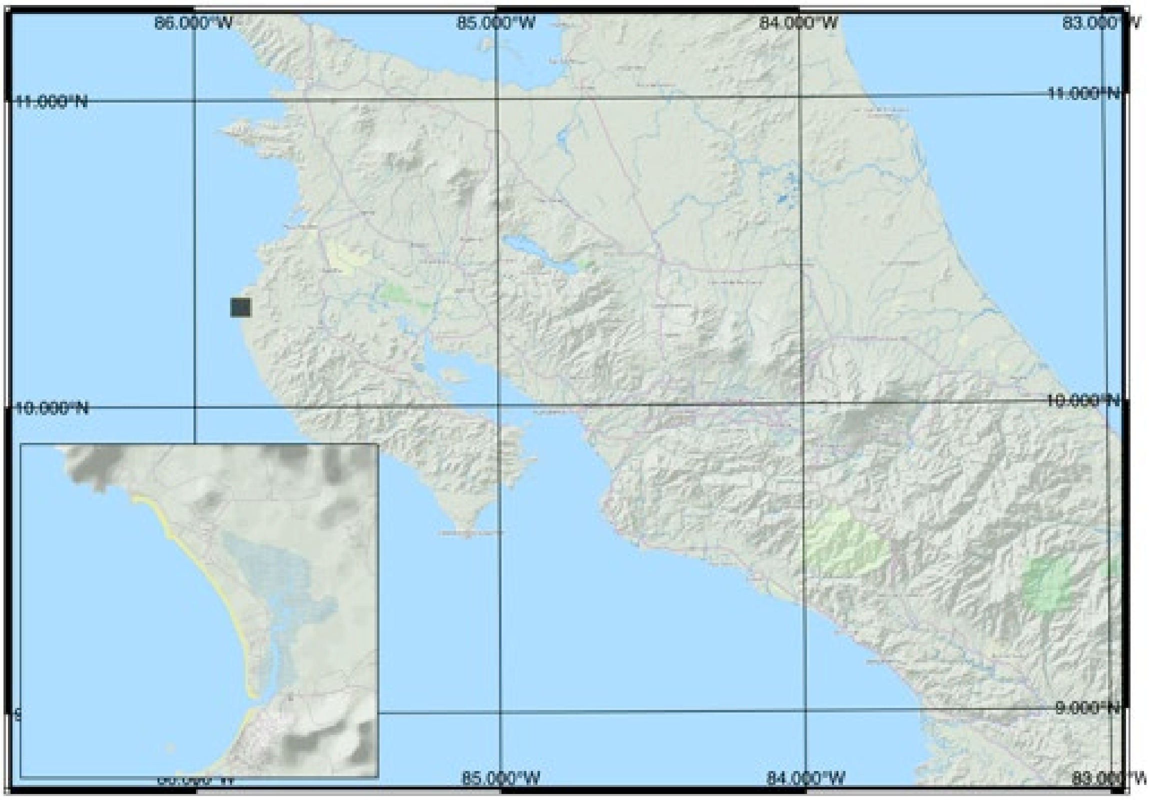


Figure 1: Map of Costa Rica. Black box and inset detailing Playa Grande, with the major water body of Tamarindo estuary in the southeast corner.

Figure 2: Satellite image of the Tamarindo estuary. Taken via google earth.

USE OF A DRONE FOR SURVEYING CROCODILE POPULATIONS

Introduction

Unmanned Aerial Vehicles (UAVs), more often called drones, have become a boon to wildlife researchers everywhere allowing the researcher to somewhat unobtrusively observe habitats and their flora and fauna. Drones have been used across a wide spectrum of applications in wildlife biology and conservation efforts, including and not limited to: census of raptor nests and rookeries of shorebirds, identifying nesting attempts by sea turtles, work in anti-poaching efforts, and survey of crocodilian nests (Bevan *et al.*, 2018; Olivares-Mendez *et al.*, 2015; Shaffer and Bishop, 2016). One of the best uses of drone technology is that it allows researchers to safely study wildlife in a variety of ecosystems that are often difficult to access, navigate, or otherwise assay (Koh and Wich, 2012). UAV's maneuverability, ability to overcome observer bias, and ability to allow multiple surveys to take place simultaneously while the operator remains in a safe location have made them a very powerful tool indeed.

Due to their use of high-resolution photography, drones have potential to survey crocodiles as efficaciously as diurnal helicopter surveys (Evans *et al.*, 2015). Drones are near silent when running, and have thus far have shown that they can monitor crocodiles without disturbance (*personal observation*). From the results of this study we will determine if crocodiles can be surveyed as effectively via drone as well as they are via helicopter, which could lead to better biologist safety. One of the greatest sources of accidental mortality to wildlife biologists is accidental death or severe injury in a plane or helicopter crash, which can be eliminated with a drone (Koh and Wich, 2012).

The expanding commercial availability of drones to average civilians is also leading to lowered expenses in planning for surveys thus reducing overall proposal costs for many research studies. With dramatic reductions in funding for the study of wildlife, this cost savings can allow researchers to continue critical conservation work they were otherwise unable to complete. Helicopter surveys can cost around $30,000-$40,000 per survey (Koh and Wich, 2012), but a commercially available quad copter can be purchased for $3,000 or less, and costs no fuel outside of a charged battery. Although post-hoc analysis of video needs to be accounted for in salary for man-hours, these analyses can be completed from the safety of behind a computer desk on the stipend of a graduate student or hourly salary of a researcher. It is also very likely that in the coming years it may be possible to run all videography through a computer program designed to look for specific research criteria.

My research focuses on the utility of a commercially available quad copter drone in surveys of the American crocodile in a natural mangrove estuary. I will conduct diurnal crocodile surveys and compare these results to diurnal boat surveys as well as compare this to the results of nocturnal surveys to determine if using a drone is a reliable method for surveying crocodilians in situ. Given that aerial surveys are one of the most cost effective and useful ways for studying crocodiles (Bayliss 1987) if a commercially available drone is able to effectively monitor crocodiles this can be a huge benefit to management practices of crocodilians.

Methods

Drone Survey Efficacy

The 10 surveys were completed over a year's time period, between 10-May-16 and 24-March-17. Five surveys were completed in each the wet and dry season. The wet season surveys occurred over one month between May and June, and the dry season surveys occurred over three months between December and March.

Drone surveys were conducted using a DJI Phantom 3 Professional and an apple iPad, and taken in tandem during a diurnal boat survey. The drone was flown 20m above the boat throughout the duration of each survey. This height is based on previous research by Bayliss in reference to aerial helicopter surveys (Bayliss, 1987). Two recorders on the boat would scout for crocodiles and when one was seen they would record the estimated size of a crocodile as well as its GPS location and whether it was out basking or in the water. They did this without making note to the person flying the drone, to keep things unbiased.

Typical battery life for this model is about 15 minutes, so when it needed to be changed the GPS location and time were noted. This was done to both ensure an accurate representation of crocodiles seen in time of surveying, as well as act as a backup for estimating drone location in case DJI software failed to record GPS data.

Post hoc analysis was conducted via videography using VLC, an open source cross platform multimedia player. When a crocodile was seen in a video, the snapshot feature of VLC was used to take a still image from the video and the relative location, size, and whether the crocodile was basking or in the water was recorded. After making a data table of all these observations, they were compared to the notes taken by the boat surveyors to see whether both methods had seen the same crocodile and to see if any crocodiles were missed by the boat observer or by the drone. This is how the comparison between diurnal boat surveys and drone surveys was calculated.

ImageJ (Fiji) Analysis

Analysis of crocodile size was conducted initially using ImageJ, and then using Fiji (an updated version of ImageJ). In order to create a scale in ImageJ/Fiji to estimate the size of crocodiles, the drone was flown 20m above ground level, with a transect tape laid flat and 2 colored rulers marking 2 meters apart. This image was used to create a scale in ImageJ/Fiji where 114.0312 pixels equated 1 meter. Still photos taken using the snapshot feature of VLC of crocodilians observed by the drone were then analyzed using the Segmented Line tool to create a line from the snout to tip of tail and then measured under the Analyze feature. Each measurement was taken in triplicate and averaged, and a standard deviation was calculated.

This same analysis was conducted for sharks and two species of ray. In total 10 crocodiles, 17 sharks, and 10 rays were measured. Crocodiles were measured from snout to tail tip. Sharks were measured snout to tail tip. Of the two species of ray, one (round in shape) was measured from the most cranial portion to the most caudal visible portion of the tail tip, and the other was measured at width from wing tip to wing tip. The reason for the difference in measurement is due to imaging. In the round rays, the stingers were clearly visible and marked for a good way to measure the animals for length. However in the other ray species, likely an Eagle ray had no visible stinger and was most easily analyzed for size wing tip to wing tip.

Results

Encounter Rates

The mean encounter rate of crocodiles was $0.44km^{-1}$, although there was variation between the wet season encounter rate ($0.67km^{-1}$) and the dry season encounter rate encounter rates ($0.20km^{-1}$)(Table 1). The mean shark encounter rate was higher than that of crocodiles

($0.81 km^{-1}$), and more sharks were encountered in the dry season ($0.81 km^{-1}$) than the wet season ($0.61 km^{-1}$). The ray encounter rate was overall similar to the crocodile encounter rate, with a mean encounter rate of $0.54 km^{-1}$ between both seasons. It differed in that more rays were observed in the dry season ($0.68 km^{-1}$) than in the wet season ($0.4 km^{-1}$).

Survey Efficacy

Sixteen crocodiles were observed in total over 29.72 km throughout 10 surveys (Table 2). Five of these surveys occurred during the wet season (May and June 2016), and the other five took place during the dry season (November 2016 and March 2017). Overall, encounter rates for both drone surveys and diurnal boat surveys were the same, with an encounter rate of $0.40 km^{-1}$. In comparison, night surveys for this area have an encounter rate between $1.2 km^{-1}$ – $2.9 km^{-1}$ (Cotroneo, 2010 and data collected from The Ministry of Environment and Energy (MINAE), unpublished).

The drone surveys were also able to observe sharks and rays, which were undetected by the observer. Encounter rates for sharks (0.84 km-1, $n=25$) and rays (0.64 km-1, $n=19$) were much higher than that of crocodiles.

The drone was able to identify animals as small as 0.35m in length, which was a small round ray that was measured from the most cranial region to the tip of the tail (Figure 3). The smallest crocodile observed was 0.65m in length (Figure 3). Table 3 shows the average size, standard deviation, and range for all four different animals.

Discussion

My goal with this study was to assess the utility of a commercially available quad copter drone in surveys of the American crocodile in a natural mangrove estuary through comparison of diurnal boat surveys and nocturnal eye shine surveys. I found that drones are suitable in spotting and sizing crocodiles in the waters of an estuary, and can be used as an effective tool for recording location and general size class. The drone was also suitable for spotting submerged crocodiles that were often missed via diurnal boat surveys. Crocodiles were missed by the drone surveys typically because they were obscured by vegetative overhang or crocodile proximity to covered banks. Crocodiles missed via the drone were generally between 1.0 and 1.5m in total length according to observer estimations taken during diurnal boat surveys. Post-processing analysis using Fiji from drone footage indicated those missed by the observer were between 0.87 and 2.71 meters in size (Table 4). This indicates that the drone is as efficacious at covering crocodiles within the same size classes as the diurnal boat surveys.

Ten surveys were taken between May 2016 and March 2017, covering both the wet and dry seasons. Of these surveys, the bulk of animals were observed during May and June, which is during the wet season. At this time, the water is much more turbid than that of the dry season. During the dry season, a secchi disk can be inserted to the bottom of the estuary and still be completely visible. This difference in animal observations between the two seasons is therefore unlikely a byproduct of poor water clarity during the wet season, as most of the animal observations were during this season.

Surveys taken by the drone had the same encounter rate as those taken by the diurnal boat observers, with an encounter rate of $0.40km^{-1}$. However, more crocodiles were observed during the wet season ($0.67km^{-1}$) than the dry season ($0.20km^{-1}$). At this time, the drone had a much higher encounter rate ($0.87km^{-1}$) than that of the diurnal boat surveys ($0.67km^{-1}$). During the wet season due to rain the water is noticeably more turbid. During the dry season the encounter rate between the diurnal boat surveyor and the drone was essentially equal, however, these are very limited conclusions to draw, as only three crocodiles were spotted between December and March of 2017.

Since crocodiles as small as 0.65 meters can be observed using this method, it accounts for most size classes of individuals, and only excludes that of the hatchling size class and some individuals in the juvenile category (Table 5). However, drone footage with the same drone has been used on our beach to identify sea turtle hatchling tracks, and in the video submitted to National Geographic by The Leatherback Trust of a leatherback returning to sea, could identify the hole in the right rear flipper of the leatherback that was no more than 10 centimeters in diameter. It is theoretically possible the drone could identify these smaller individuals, and they were unable to be observed, likely due to hiding in vegetation, or not present during the surveys. Image analysis using Fiji identified a round ray that was only 0.35m in length, so potentially the drone has the capacity to identify all size classes of crocodilians.

While some image distortion can come from deeper submerged individuals (Figure 3), the drone proves to be adept at capturing animals in-water during survey. In fact, in clear waters, the drone was able to identify schools of fish as well as two distinctly different species of ray. Larger rays and sharks were seen with throughout all surveys. This shows that the drone is able to identify animals both in and out of water, and makes it a useful tool for crocodilians, which occupy both types of area.

Drones allow for unobtrusive study of crocodiles in their natural environment from above. In an effort to capture a close high-resolution image of a crocodile, I flew the drone down to 2 meters above water before the crocodile submerged and swam away. Given that all surveys were taken at a height of 20 meters, this should not be disturbing or obtrusive to crocodiles. It’s important to note that in Australia, a study measured the behavioral responses of saltwater crocodiles in response to commercial drones. They found that crocodiles reacted to drones flown below 50m in altitude, most notably at 30m or below (Bevan *et al.,* 2018). However this study focused on Bare Sand Island and Cape Domett, which is notably more rural than Tamarindo. The Tamarindo estuary has multiple boats cruising the majority of its length daily as part of the local ecotourism industry and it is possible that the American crocodiles located here are probably more habituated to boat traffic and disturbance and less perturbed to audio or visual disturbances.

Unfortunately, there are some limitations to utilizing the drone for this study, but these can be found in other aerial surveys as well. Areas with high-density vegetation, such as a mangrove estuary, hide basking smaller crocodiles from view. Weather is also a strong limiter of drones, where drones should not be flown in excessive wind speeds (>10m/s). Current market drones also are not suited for use during a rainstorm, which is another large limiting factor during a rainy season. In Playa Grande, the rainy season is preceded by a “windy season” where the Papagayo winds increase in February, which precluded the ability to conduct surveys via drone. As well, the limited range of the drone from the remote control does not allow for a continuous sweeping of the estuary, but with time and rapid innovations, this should be a negligible factor. There are already computer programs on the market that are compatible with these drones that can be used to sweep along quadrants and out of range of the remote controller, although use of them often voids any form of warranty.

The drone proved that beyond the ability to study crocodiles, there are a large number of sharks, likely bull sharks, in the estuary. In fact, I observed more sharks in this study than I did crocodiles. Sharks were also encountered more frequently during the wet season than the dry season, and the average size of the sharks was 0.67m. The size of a bull shark pup is about 0.62 – 0.68 meters (Neer *et al.*, 2005) at birth. Bull shark females are known to enter riverine and estuarine habitats in order to give birth (Lea *et al.*, 2015). Pups and juveniles will then remain in these nursery habitats for up to four years (Tillett *et al.*, 2012). Juvenile bull shark habitat use has been well studied in the Gulf of Mexico, where it has been found that abiotic factors such as water temperature and salinity are quite important, in particular regard to salinity (Drymon *et al.*, 2014). Indeed, as the drone surveys break down nicely into two seasons where there is a wet and dry period, we can see there are potentially a larger number of shark sightings during the rainy season. Fifteen shark observations occurred during the wet season, and nine shark observations occurred during the dry season. It is possible that the seasonal change in salinity may be affecting the shark population as well.

It's also important to note that in showing the large number of sharks in Tamarindo estuary, which are most likely pups, Tamarindo estuary has potential to be a parturition and nursery site for sharks. Since protection of a species needs to encompass all aspects of a life cycle, this can aid us in conservation management strategies. Further, if the drone surveys are able to observe sharks so easily in the estuary, there is potential to use drone surveys along the rest of the coastlines in Costa Rica for discovery of other parturition sites.

Conclusively, use of a commercially available quad copter is an adequate means to surveying crocodile populations instead of diurnal boat surveys, while still remaining less efficacious than nocturnal spotlight surveys. It allows researchers to identify relative size of an animal from the safety of their desk. It opens up further avenues of research, particularly with crocodiles. The drone is able to identify data at a small scale, and suggested further studies would include evaluating for use in individual analysis based on colored cattle ear tags applied to the nuchal scute. This in and of itself may lead to a more inexpensive way of elucidating the kind of information that previous to this could only be determined by telemetric means.

Table 1: Encounter rate by season, where the wet season is May and June of 2016 and the dry season is late December through late March of 2016-2017.

	Wet Season	Dry Season	Total
Crocodile Encounter Rate	0.67	0.20	0.44
Shark Encounter Rate	1.00	0.61	0.81
Ray Encounter Rate	0.40	0.68	0.54

Table 2: Data from 10 surveys over 2 field seasons, indicating total number of crocodiles recorded by the drone and the percentage of these, which were also seen by the observer on the diurnal boat survey.

Survey	Date	Distance traveled (km)	Speed (km/hr)	# Crocodiles Recorded by Drone	% Seen by Observer
1	10-May-16	3.60	3.72	2	50.00
2	23-May-16	1.38	5.91	1	100.00
3	03-Jun-16	3.74	4.88	3	33.33
4	10-Jun-16	2.99	5.28	1	100.00
5	17-Jun-16	3.29	4.20	3	66.67
6	15-Nov-16	3.47	5.08	0	0.00
7	21-Dec-16	2.77	4.75	0	0.00
8	14-Feb-17	3.58	5.24	1	100.00
9	26-Feb-17	3.58	5.37	1	100.00
10	24-Mar-17	1.32	5.28	0	0.00

Table 3: Average size, standard deviation between measurements, and range of all four species of animals found in the estuary that were able to be measured in Fiji.

	Shark	**Crocodile**	**Round Ray**	**Winged Ray**
Number of individuals	24	10	3	4
Average Size (m)	0.67	1.66	0.54	0.71
Standard Deviation	0.16	0.69	0.17	0.11
Range (m)	0.4 - 0.98	0.65 - 2.71	0.55 - 0.86	0.35 - 0.66

Table 4: Average size and standard deviation from triplicate measurements of all 10 crocodiles that were able to be measured from drone footage using Fiji, and whether or not that crocodile was seen by the diurnal boat observer

Average Size (m)	**Standard Deviation**	**Seen by Observer**
1.87	0.04	Yes
0.65	0.01	Yes
0.87	0.01	No
2.71	0.10	No
2.16	0.01	Yes
1.91	0.01	Yes
2.30	0.02	Yes
1.39	0.06	No
1.94	0.01	Yes
0.85	0.02	Yes

Table 5: Crocodile size classes defined by size ranges

Size Class Name	Size Range (m)
Hatchlings	< 0.5
Juveniles	0.5 - 1.25
Subadults	1.25 - 2.25
Adults	> 2.25

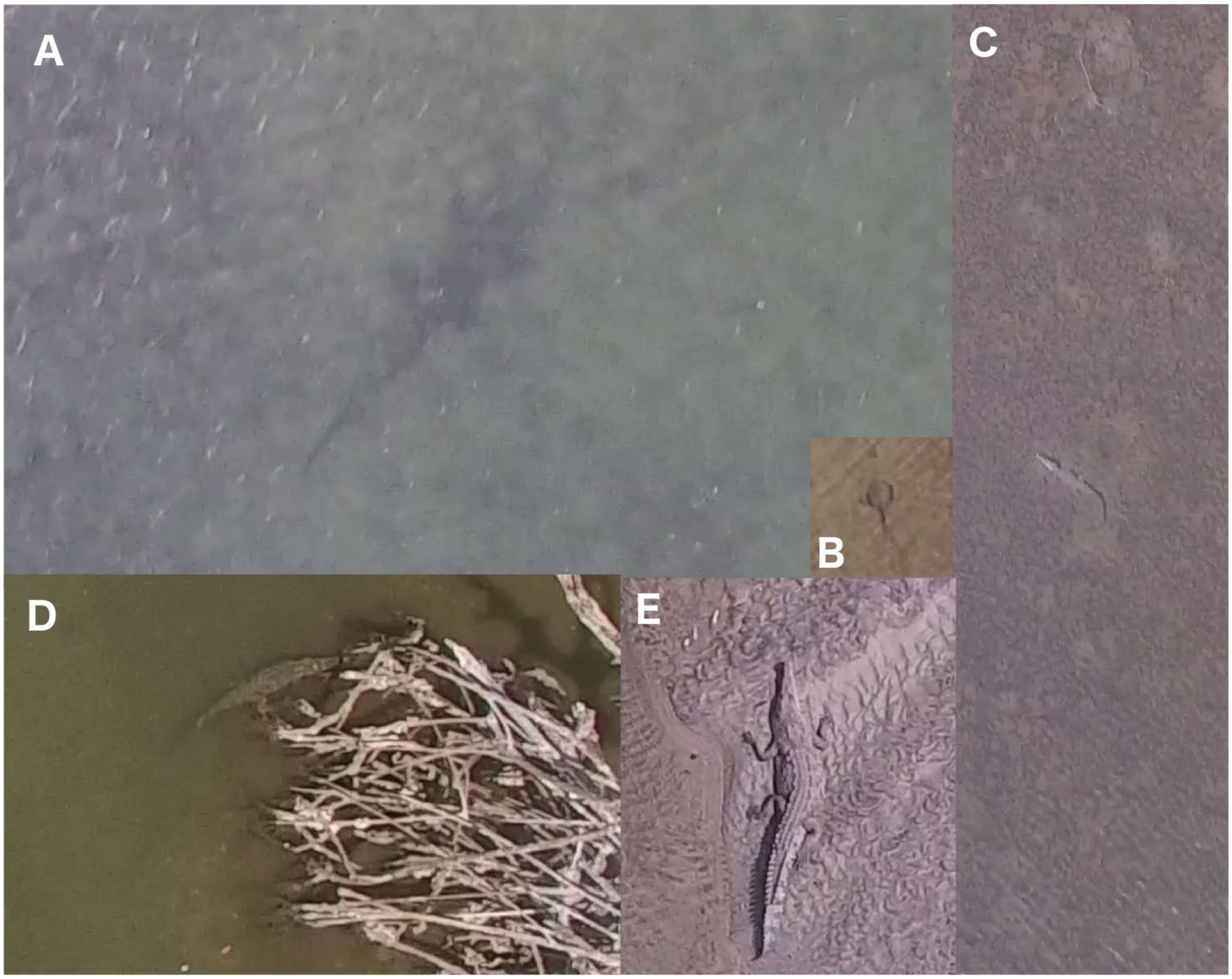


Figure 3: Animal observations from the drone. Figure has been manipulated to show animals best and is not uniformly accurate in size between all. A: fully submerged crocodile visual during a drone survey. While blurry, it is still clearly a crocodile and body size estimation is able to be made. B: small round ray, measuring 0.35m from most cranial portion to most caudal tip of tail. C: the smallest observed crocodile by the drone was 0.65m in length and was seen basking in shallow waters. D: submerged crocodile, 1.3m in length. E: basking crocodile, 1.9m in length. A, D, and E were all missed by diurnal boat observer.

SEASONAL EFFECT OF SALINITY ON HABITAT SELECTION OF THE AMERICAN CROCODILE

Introduction

C. acutus is a euryhaline species, meaning it has the ability to tolerate a large range of salinity in their habitats; this allows this species to span over a diverse range of aquatic ecotones. Most often, this species can be found in mangroves of coastal estuaries and lagoons; and although they prefer a brackish environment, they can be found in freshwater rivers or hypersaline landlocked lakes (Thorbjarnarson *et al.*, 2006). Beyond these areas, there have also been numerous sightings of the American crocodile in the open sea. It is thought that this species uses the open ocean to travel between estuaries, possibly due to shifts in territories related to mating, or to go in search of food.

When discussing the life history of American crocodiles, salinity is a very important factor to take into consideration due to its impact on the osmoregulation within the animals, in particular that of smaller individuals, and as such is one of the most well studied aspects of their life history. Notably, tolerance for salinity varies between species of crocodilians as well as life stage within each species (Mazzotti and Dunson, 1989; Ellis 1981, Mazzotti and Dunson, 1984). *C. acutus* is one of the most salt tolerant of all crocodiles, alongside both *C. niloticus* and *C. porosus* in terms of salt tolerance. Interestingly, while *C. porosus, C. niloticus,* and *C. acutus* all possess lingual salt glands, there has been no real evidence showing any significant role it plays regarding salt excretion in American crocodiles. (Platt *et al.*, 2013). However, a study in Florida indicated that roughly 80% of water ingested by *Crocodylus acutus* is marine in origin (Wheatley *et al.*, 2012). Data from this study indicates that behavioral osmoregulation via drinking fresh water was unnecessary for *C. acutus* (Wheatley *et al.*, 2012). Unfortunately, one very real limitation of this study was that all the American crocodiles were adults. Neonates are unable to tolerate high salinity conditions, given that they have a surface area to body mass ratio that is lower than adults and leaves them unable to properly osmoregulate (Ellis, 1981). For example *C. acutus* weighing <200g were unable to grow when kept in 35 ppt saline laboratory conditions (Mazzotti and Dunson, 1984). Smaller individuals between 73-124g could not maintain weight in 25%-50% seawater (Mazzotti and Dunson, 1984). There have been very few studies designed to investigate how salinity impacts hatchlings and/or juveniles of this species in situ. Those that exist have all taken place in Florida. Despite the experimental evidence showing difficulty with salt tolerance for smaller animals, juveniles have been found in hypersaline environments in Florida (Gaby *et al*., 1985), and there was an observation of a 10-day-old hatchling in 43ppt salinity (Dunson, 1982). While this may seem contradictory, there was a study conducted on *C. niloticus* that exposed neonates to progressively increased concentrations of salt water which showed that hatchlings are actually able to adapt to a higher saline environment if given the opportunity to acclimate (Leslie and Spotila, 2000).

So, although controlled studies show that hatchlings of *C. acutus* are relatively intolerant of seawater (Ellis, 1981; Evans and Ellis, 1977; Grigg *et al.*, 1980; Mazzotti and Dunson, 1984), there are still observations of small crocodiles in high saline conditions. It is also known that salinity tolerance changes with increases in size and age. Therefore we might expect that spatial separation between size-classes will change in relation to salinity. Neonates stay together in pods during the first few weeks after development, but social aggression emerges in juveniles and may contribute to spatial organization based on size class (Brien *et. al,* 2012). A study done by Rodda demonstrated that 10-month-old juveniles stay within 300 meters of the nest, but that this distance doubled for a 22 month old (Rodda, 1984); however one study noted a juvenile in

Florida that moved 13.1 km away from its nest within the first 18 months of its life (Gaby *et al.,* 1985). Crocodiles are also cannibalistic, and smaller crocodiles may move away from larger ones to avoid predation (Thorbjarnarson, 1989).

Additionally, there exists a paucity of information regarding the spatial ecology of the American crocodile at any life stage. Limited studies have shown that the average movement distance of an adult is 372m, where females have a greater average movement than the males (Balaguera-Reina *et al.*, 2016). This is likely due to the need to find suitable nesting environments in their home range. There have only been two studies that focused on calculating home range in crocodiles, and they have shown a very different use of territory by each sex. A study in Florida calculated a home range of 5.6km +/- 3km, in contrast to a study on Coiba Island, Panama, that found a home range of 0.6km +/- 1km (Balaguera-Reina *et al.,* 2016; Mazzotti, 1983). The difference in home range is very likely due to the large difference in the overall size of the available habitats for the two different areas, with one on a small island and the other a much larger coastal mainland. What has been found is that the amount of crocodile movement increases in the dry season, and decreases in the rainy season (Balaguera-Reina *et al.,* 2016). The study in Coiba Island, Panama has been the largest telemetry study of the American crocodile as of 2016, and also found that average movement distance correlates with life stage of crocodiles, where juveniles have much lower movement distances than sub-adults, which have a smaller movement distance when compared to adults (Balaguera-Reina *et al.,* 2016). It would be expected that crocodiles in Tamarindo estuary, which is about 4km in length, are locked in a rather small sized area which may potentially result in overlapping areas of use which could result in size class separation for this population. In further support of the notion of spatial separation occurring due to size class, it has been hypothesized that size segregation may become more readily apparent in low population areas with diverse microenvironments (Thorbjarnarson, 1989).

It is critical to aid in management of these populations, specifically aiming to protect potential nesting and nursery habitats to increase the probability of survival among hatchlings and juveniles (Cotroneo, 2010). In particular, since *C. acutus* hatchlings occupy lower salinity water than their adult counterparts (Richards *et al.*, 2004; Mazzotti *et al.*, 1986), it is imperative to maintain large estuaries that allow such access to fresh water. Estuaries already face a number of stresses both anthropogenic and through naturally occurring events such as river flooding, of which climate change is also a threat (Elliott and Whitfield, 2011).

My goal with this study is to examine if there is size-class separation in American crocodiles along a salinity gradient within a mangrove estuary in Costa Rica. In addition to this I will study seasonal changes of water physiochemistry associated with the local dry season, specifically salinity and water temperature. I hypothesize that in periods of drought, a size class arrangement will arise along the estuary where areas larger crocodiles occupy will have the absence of hatchling and juvenile crocodiles.

In order to test this hypothesis, I will document American crocodile (*Crocodylus acutus*) distribution by boat and unmanned drone surveys and assess water chemistry of the estuary. The data on salinity and animal distribution obtained during this study should provide information that should be essential for development of management practices of crocodiles for mangrove estuaries, especially those areas that experience a dry and rainy season throughout Central America.

Methods

Season Data & Rain Data

The Pacific Coast of Costa Rica experiences a dry season, with no rain between December and April, and a wet season, with plentiful rain, between May and November (Jiménez, 2001). Crocodile location data as well as water chemistry data were primarily collected October through March to demonstrate the changes in estuary physical properties in response to seasonality. The data collection schedule accounted for the shifts in seasonality, and data collected between January 2016-June 2016 and October 2016-March 2017 encompassed conditions with both wet and dry seasons. It should be noted that there was a very strong El Niño 2015-2016, and these normally wet months were uncharacteristically devoid of rain during the rainy season of 2016. This abnormally dry period was reflected in the data collected during this season.

Water chemistry was measured *in-situ* using an YSI Model 30, which records salinity in ppm based off of conductivity and temperature. Using the methods described in (Government of Western Australia, 2009), salinity was recorded using a 2-point system when possible. To use the 2-point system, a measurement (A) is taken just above the sedimentary bottom, and a second measurement (B) is taken just below the surface of the body of water. It is also acceptable practice to take one measurement from the middle of the water column when a rapid measurement is necessary. Similarly, I spot-checked the middle of the water column in addition to the 2-point measurement to ensure I wasn’t missing a freshwater layer in the water column.

Measurements were recorded in 200-300 meter intervals, and whenever a crocodile was spotted during a diurnal or nocturnal survey. Water sample measurements were recorded a minimum of twice a month during the time I was working in Costa Rica, with the exception of

June 2016 where only 1 survey took place and December 2016 and January 2017 when there were no specific surveys taken. I took 34 diurnal surveys across the three field seasons. Data were combined and organized and separated by Dry Season (December-April) and Rainy Season (May-November).

Distance Analysis

I created a linear shapefile of the Tamarindo estuary in qGIS, consisting of 8 segments (Figure 4). This was then ported into RStudio and used in the package, riverdist. Riverdist creates vertices in 100m intervals along the various segments and can calculate the shortest route to the estuary mouth by running along these vertices. All GPS points were ported into RStudio, then "snapped" to the nearest vertex along the estuary. The differences in distance between actual GPS points and the nearest snapped vertex are assumed to be negligible (Figure 5). When I ported Figure 5 into Fiji, the average distance between the blue squares (nearest snapped vertices) and red circles (actual GPS points) was 18.42 ±10.32 meters (n=12).

Plotting and statistics with regard to abiotic factors

I plotted all data points in RStudio, with Salinity and Water Temperature as the response variables to distance from the mouth of the estuary (Figure 6). When looking at this scatterplot I observed a fork shape in the wet season data for salinity. To investigate the cause of this divergence I used qGIS and I mapped out all of the data points and then used the select tool to differentiate them along a left and a right branch of the estuary (Figure 7). I then ported all of this information back in to R and replotted the data to account for the left branch and right branch (Figure 8), each including the common points seen in yellow in Figure 7.

Four abiotic factors were considered when looking at seasonal variation and association with crocodile distribution. Before being able to discern influence on crocodiles, it was necessary to look for and understand any colinearity in the independent variables. These four variables were distance from the mouth of the estuary (hereby referred to simply as distance), water temperature, salinity, and season.

I hypothesize that season will have a significant effect on water temperature and salinity. I used MANOVA to analyze seasonal affect on salinity and water temperature. I found that both salinity and water temperature differed significantly between the wet and dry season throughout both branches of the estuary (Table 6). Further univariate analysis with ANOVA showed that while season has a statistically significant effect on salinity ($p=2.2e^{-16}$), it has no effect on water temperature (Table 7).

I also used MANOVA in RStudio to analyze whether or not salinity and water temperature differed significantly with distance from the mouth of the estuary. (Table 8). It was run for each branch of the estuary. After this, ANOVA was used as a univariate analysis to determine significance with regard to each variable category (salinity and water temperature), as seen in Table 9.

Using GLM to determine abiotic effects on crocodiles

Using data obtained with regard to seasonal variation in the water chemistry of Tamarindo estuary from the previous MANOVA analyses, I used a generalized linear model (GLM) to investigate the possible correlation of water chemistry with regard to crocodile presence. GLM is used for predicting a categorical outcome, in this case looking at whether or not these abiotic factors had a possible correlation to crocodile presence. Crocodiles were grouped into two categories based on size class. The first category, HJ, was comprised of hatchling and juveniles. The second category, SbA, was comprised of subadults and adults. Since multivariate analysis showed an overall significant effect of distance from the mouth of the estuary on salinity and water temperature in both branches (Table 8) and season only affected salinity (Table 6), the GLM included salinity as an interaction with season and water temperature as the coefficient.

Creating Maps in GIS

Generalized linear models (GLM) are able to discern whether or not salinity, season, or water temperature have a statistically significant correlation with crocodile presence, but maps were necessary to determine crocodile location during the changes in these seasons. Two maps were created with regard to salinity and season. Horizontal linear divisions segmented the reaches of the estuary. These portions were named 'Top', 'Middle', and 'Bottom' where top is the furthest reach of the estuary from the ocean mouth and bottom is nearest to the ocean. These portions were used to determine what percent of either HJ (hatchling and juvenile) or SbA (subadult and adult) were occupying the given area.

All maps were created using qGIS version 3.6. I created 200m intervals from the mouth of the estuary to the furtherst reaches of both branches, and used the distance analysis tool to find the data points of salinity values that were nearest to those locations. I then averaged the salinity for each location, to plot on the map where increasing darkness of the circles indicates increasing values in salinity. Crocodiles were grouped into HJ and SbA categories, and added to the maps.

Results

Seasonal variation in salinity and water temperature

The results show that salinity and water temperature differed significantly between the seasons ($F(1,461)=410.42$, $p=2.2e^{-16}$), with no regard to specifically the left or right branch of the estuary. Subsequent univariate analyses using ANOVA show that there is a significant difference in salinity between the two seasons ($F(1,461)=804.76$, $p=2.2e^{-16}$), but no difference in water temperature ($F(1,461)=1.07$, $p=0.3$). There was no statistical difference between the left branch and the right branch (Table 6).

In order to look at effect distance from the mouth of the ocean has on salinity and water temperature, the data set was broken into wet and dry seasons as well as the left and right branches and run using MANOVA. Salinity and water temperature differed significantly among the distance of the estuary from the mouth to the furthest reaches, and varied within the branches (Table 8). Further univariate analysis from the dry season found that salinity differs significantly with distance from the mouth of the estuary, but water temperature does not (Table 8). In the wet season salinity differed in the right branch of the estuary, but not in the left branch (Table 8). During the wet season, water temperature differed significantly with distance from the mouth of the estuary (Table 8).

Crocodile location

Using information obtained with regard to seasonal variation in the water chemistry of Tamarindo estuary, I used a generalized linear model (GLM) to investigate the possible correlation of water chemistry with regard to crocodile presence. Crocodiles were grouped into two categories based on size class. The first category, HJ, was comprised of hatchling and juveniles. The second category, SbA, was comprised of subadults and adults.

The results of this analysis show that salinity and season all had an effect on the presence of crocodiles from the HJ group ($p < .05$ for all) and water temperature was not a factor at all. This same model was implemented in regard to SbA crocodiles and it was found that there was no effect from any abiotic factor in regard to SbA presence or absence.

Crocodile location – Map interpretations

Figures 9 and 10 show average salinity values mapped along the estuary in grayscale circles, where the circle gets darker with an increase in salinity. HJ crocodiles are represented by red diamonds, while SbA crocodiles are represented by yellow diamonds.

In the dry season, HJ comprise 59.7% of all crocodile sightings and in the wet season only 33.3% of total crocodile sightings. When looking at percent of crocodiles utilizing these different areas of the estuary, we can see that HJ crocodiles occupy 100% of the Top portion of the estuary in both wet and dry seasons, and that the largest percentage of crocodiles in total is always in the middle portion of the estuary (64.5% dry season, 74.5% wet season). In both seasons the bottom division is mostly comprised of SbA crocodiles (77.8% dry season, 80% wet season).

Discussion

My hypothesis was that in periods of drought a size class arrangement will arise along the estuary where areas larger crocodiles occupy will have the absence of hatchling and juvenile crocodiles, as well as the halocline and thermocline would differ significantly between seasons in the estuary. The results of my research supported this hypothesis.

There have been a number of previous studies looking at how crocodile size related in terms of salinity, as discussed in the introduction of this chapter. Salinity turned out, predictably, to be the greatest indicator of crocodile presence during the research presented here. However, crocodile spatial use did not occur as would be predicted. In the dry seasons a larger number of the HJ class are in the furthest reaches of the estuary. It makes sense during the wet season that the smaller crocodiles would be in the further reaches of the estuary due to environments that are more suitable for hiding. Something that remains interesting to consider is the fact that there was a crocodile nest at the mouth of the estuary two years in a row (2016 and 2017), so we know that there was viable offspring produced in the mouth of the estuary. Taking into consideration the movement patterns and home range as discussed earlier in the introduction, one would assume that these neonates would remain relatively close to the nest site. However, it is a high saline environment and the maternal crocodile may be transporting these individuals deeper into the estuary to a potential nursery environment in order to mitigate the dangers associated with an abundance of larger crocodiles and other predators as well as the high saline environment. It is possible that these individuals remain there during the wet season and subsequent dry season, despite the abiotic changes in salinity associated with this difference. It is further possible given the previous study on exposing Nile crocodiles to increasing levels of salinity that these individuals are able to acclimate to the seasonal shift in salinity and survive it.

The seasonality between the wet and dry seasons in this region affect all biospheres, a portion of which is easily observed in the Tamarindo estuary, as well as other local smaller estuaries. The results of water chemistry in the Tamarindo estuary seem surprising at first glance. Typical estuarine water chemistry usually has fresh water leading into the ocean with a gradient of salinity that increases the closer this fresh water source gets to the ocean. And in fact, this is typically the case for the wet season in this estuary (Fig 3.1 and 4). However, when the dry season is occurring in the Tamarindo estuary, this gradient not only ceases to exist, but in fact slightly reverses (Fig 3.3 and 4). Chaves (2009) found a similar shift in salinity in Tamarindo during 2007-2008 (Chaves, 2009). Reasonably, during the dry season the lack of rains cause a cease of significant fresh water flow into these estuaries which when coupled with the hotter diurnal temperatures causing evaporation can lead to the marked increase in salinity seen here. This would also make sense as to why the distance of the estuary has no effect with regard to water temperature during the dry season. With no cooler freshwater input and stagnation, there would be little variation in water temperature. However, during the wet season there is a difference between water temperatures, with water temperature decreasing as distance increases.

There are insufficient data to predict the long-term effects this would have on these neontates in terms of survivability and viability. If they are unable to gain proper weight, as seen in previous research where individuals under 200g had no weight gain in 35ppt water (Mazzotti and Dunson, 1984), there be decreased viability in these animals. However we would need a longer term capture and release program to really see if this is the truth occurring here. It is possible there are negative implications associated with continued droughts in this area. We already see negative consequences in the leatherback sea turtles that share the same area and experience decreased hatching success and skewed sex ratios due to the increasing temperatures, which render sand too loose, too hot, and too dry. American crocodiles experience high mortality above 34.5°C in temperature, and male embryos occur between around 31.7°C – 32.8°C (Charruau *et al.*, 2017). These negative implications are in addition to other evidence stating that environmental pollutants are also effecting crocodile sex ratios (Murray *et al.,* 2017).

One of the most beneficial aspects of this research is the associated implication in management strategies and conservation ideas. With increasing incidences of human-crocodile interactions and a demand for removal of these crocodiles, the local rangers are trying to find ways to minimize these interactions while still keeping this protected animal safe in the protected area it lives. If knowing that changes in abiotic factors affect the distribution of crocodiles then it may help address area-use issues that are occurring. Further, if crocodiles continue to nest at the mouth of the estuary where there is high foot traffic, it may be putting people at risk as adult crocodiles guard their nest. It is important to understand what areas of the estuary the hatchlings and juveniles are utilizing, to possibly minimize human disturbances to those areas.

Table 6: Multivariate analysis of the affect season has on salinity and water temperature. The reason that n for left and right branches does not match both branches together is due to the common points they share prior to splitting.

	Left	**Right**	**Both Branches**
n	288	278	462
df	1,287	1,277	1,461
f value	86.23	119.26	410.42
p value	2.2 e^{-16}	2.2 e^{-16}	2.2e^{-16}

Table 7: Analysis of variance to see if salinity and water temperature differ significantly among season and between the branches of the estuary.

	Left		**Right**		**Both**	
	Salinity	**Water Temperature**	**Salinity**	**Water Temperature**	**Salinity**	**Water Temperature**
n	288	288	278	278	462	462
df	1,287	1,287	1,277	1,277	1,461	1,461
f value	96.27	1.18	149.55	.0006	804.76	1.07
p value	2.2 e^{-16}	0.28	2.2 e^{-16}	1.28	2.2 e^{-16}	0.3

Table 8: MANOVA results to see if salinity and temperature differ significantly with distance from the mouth of the estuary in the different seasons.

Branch and Season	N	F Value	P-Value
Left Branch [Dry]	215	103.67	2.2 e^{-16}
Left Branch [Wet]	43	9.99	2.8 e^{-4}
Right Branch [Dry]	201	6.28	2.3 e^{-3}
Right Branch [Wet]	47	21.23	2.95 e^{-7}

Table 9: ANOVA results for effect of distance on salinity and water temperature for both branches and during both seasons.

Abiotic Factor	N	DF	F Value	Pr(>F)
Salinity (Dry Season) Left Branch	215	1	207.92	$2.2e^{-16}$
Salinity (Wet Season) Left Branch	43	1	0.99	0.3242
Water Temperature (Dry Season) Left Branch	215	1	2.62	0.1073
Water Temperature (Wet Season) Left Branch	43	1	20.22	$5.168e^{-05}$
Salinity (Dry Season) Right Branch	201	1	12.49	0.0005065
Salinity (Wet Season) (Right Branch)	47	1	40.47	$7.609e^{-08}$
Water Temperature (Dry Season) Right Branch	201	1	0.20	0.6523
Water Temperature (Wet Season) Right Branch	47	1	23.9	$1.223e^{-05}$

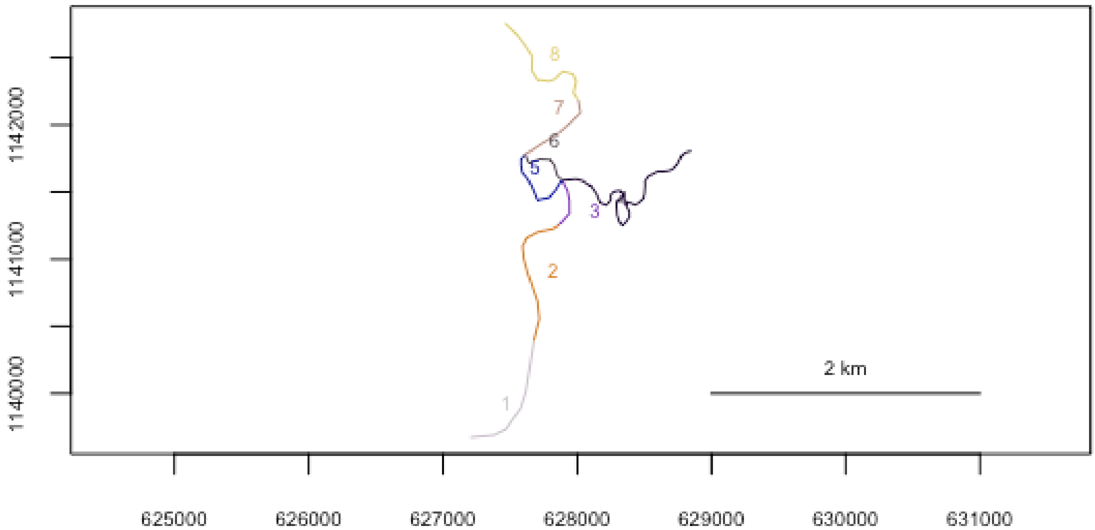


Figure 4: A linear shapefile of Tamarindo estuary consisting of 8 segments, with the most distal portion of segment 1 the mouth of the estuary.

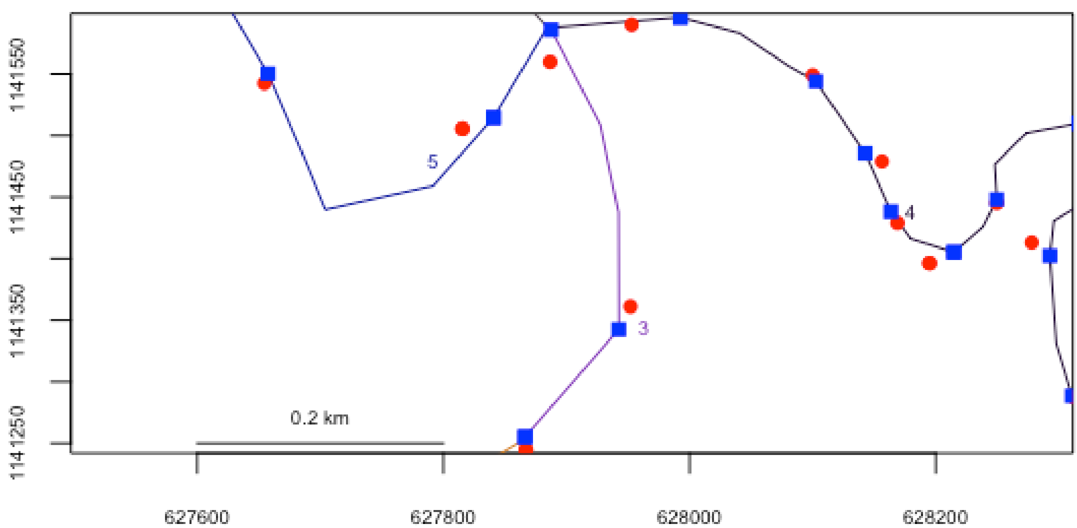
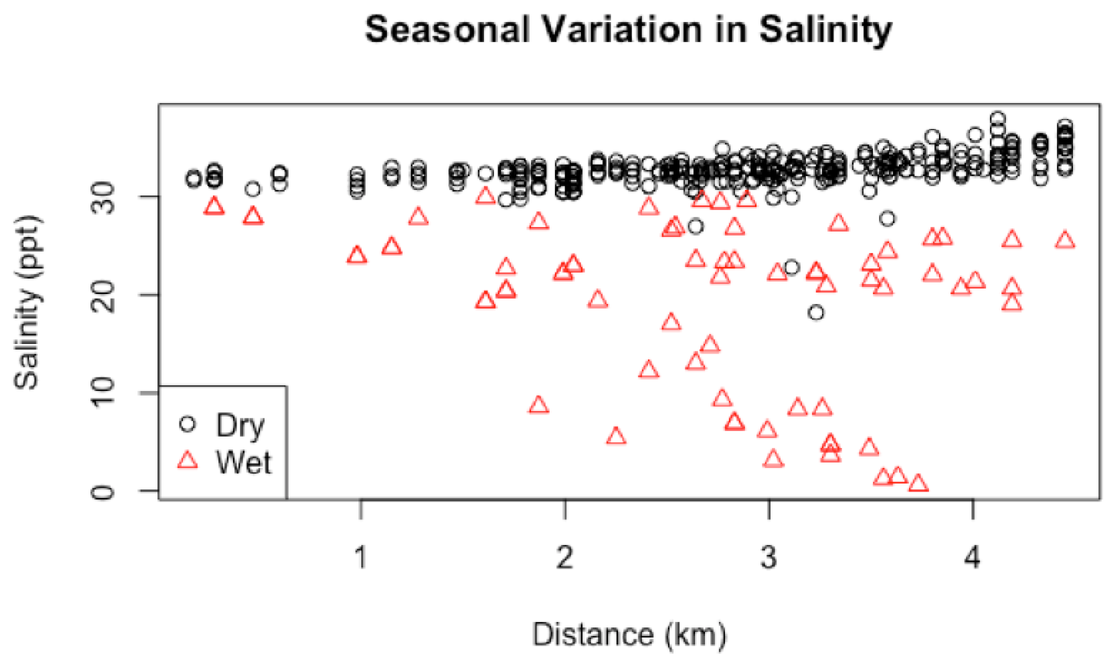


Figure 5: Actual GPS coordinates in relation to nearest snap vertices. Red circles indicate actual GPS coordinates, and blue squares represent nearest snapped vertex. The average distance between the blue squares and red circles in this image is 18.42 ±10.32 meters (n=12).



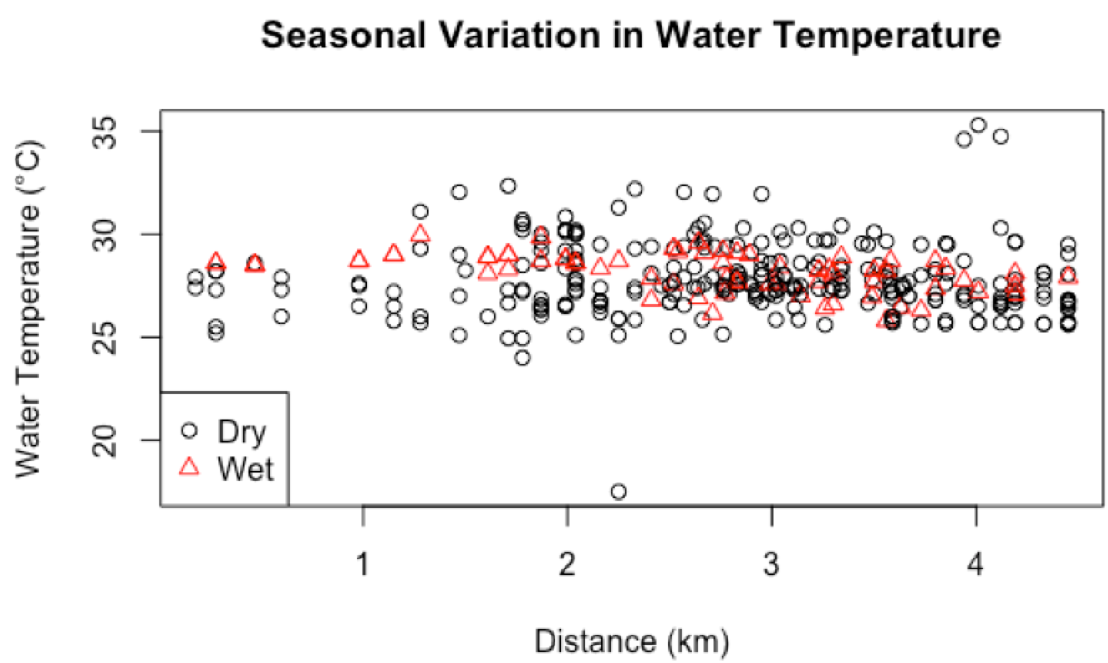


Figure 6: Seasonal variation in salinity and water temperature. Dry season is represented by open black circles, rainy season is represented with open red triangles. Distance is measured from the mouth of the estuary [0] to the furthest reaches accessible at the time. Top: Seasonal variation in salinity. Bottom: Seasonal variation in water temperature.

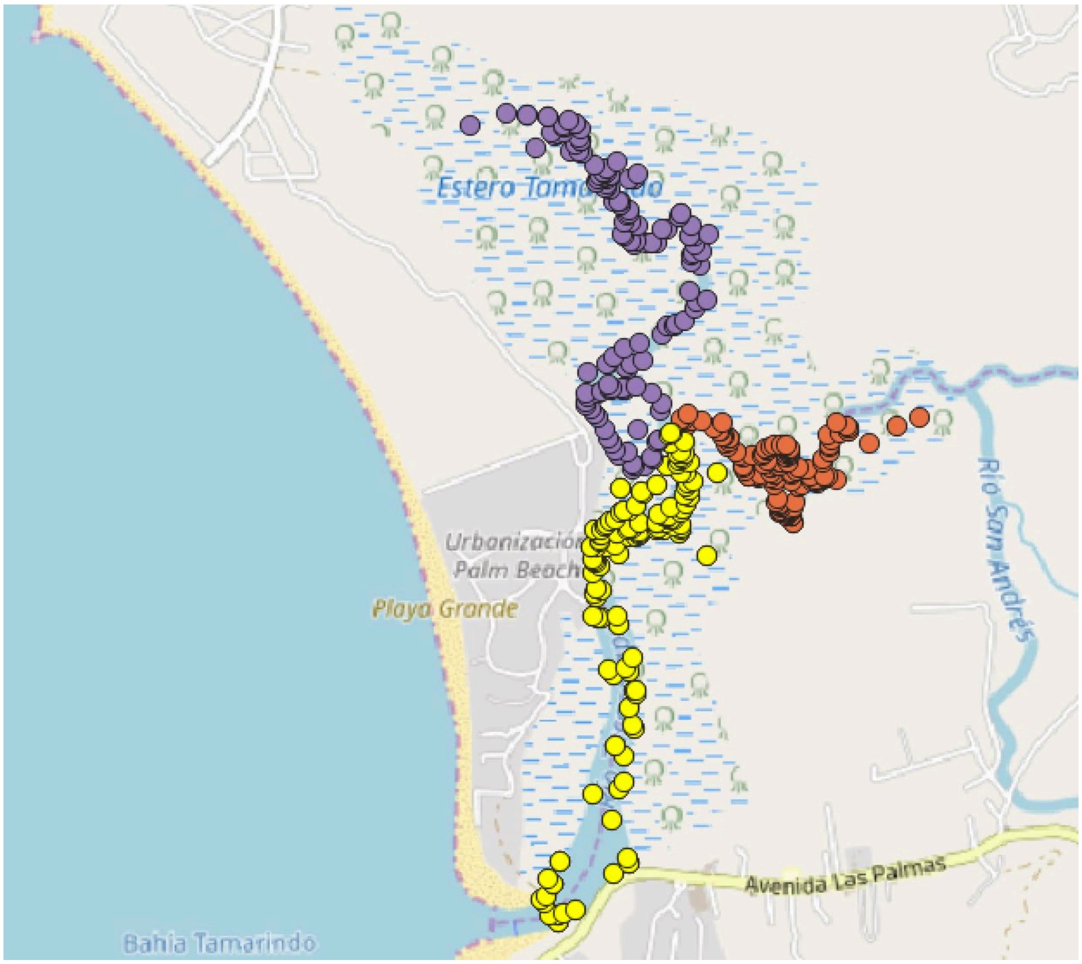


Figure 7: Separating data points by branches of the estuary. Yellow circles represent collected data points of water chemistry or crocodile location common to both left and right branches, orange circles represent data points specific to the right branch of the estuary, and purple circles represent data points specific to the left branch of the estuary.

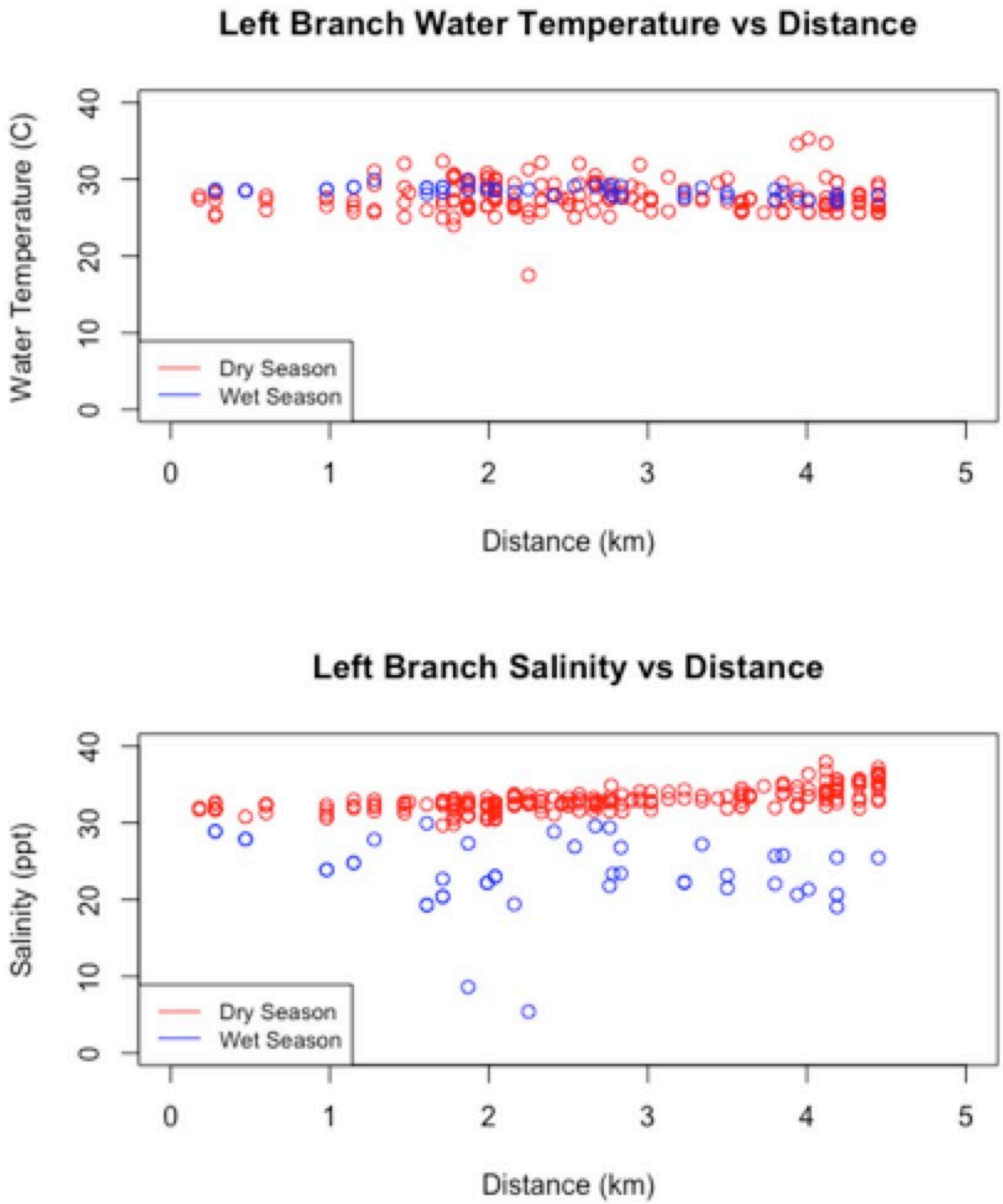


Figure 8: Variation in salinity and water temperature with regard to distance in the left branch of the estuary. Dry season is represented by open red circles, wet season is represented by open blue circles. All data is for the left branch of the estuary only. Distance is measured from the mouth of the estuary [0] to the furthest reaches accessible at the time Top graph is water temperature, bottom graph is salinity.

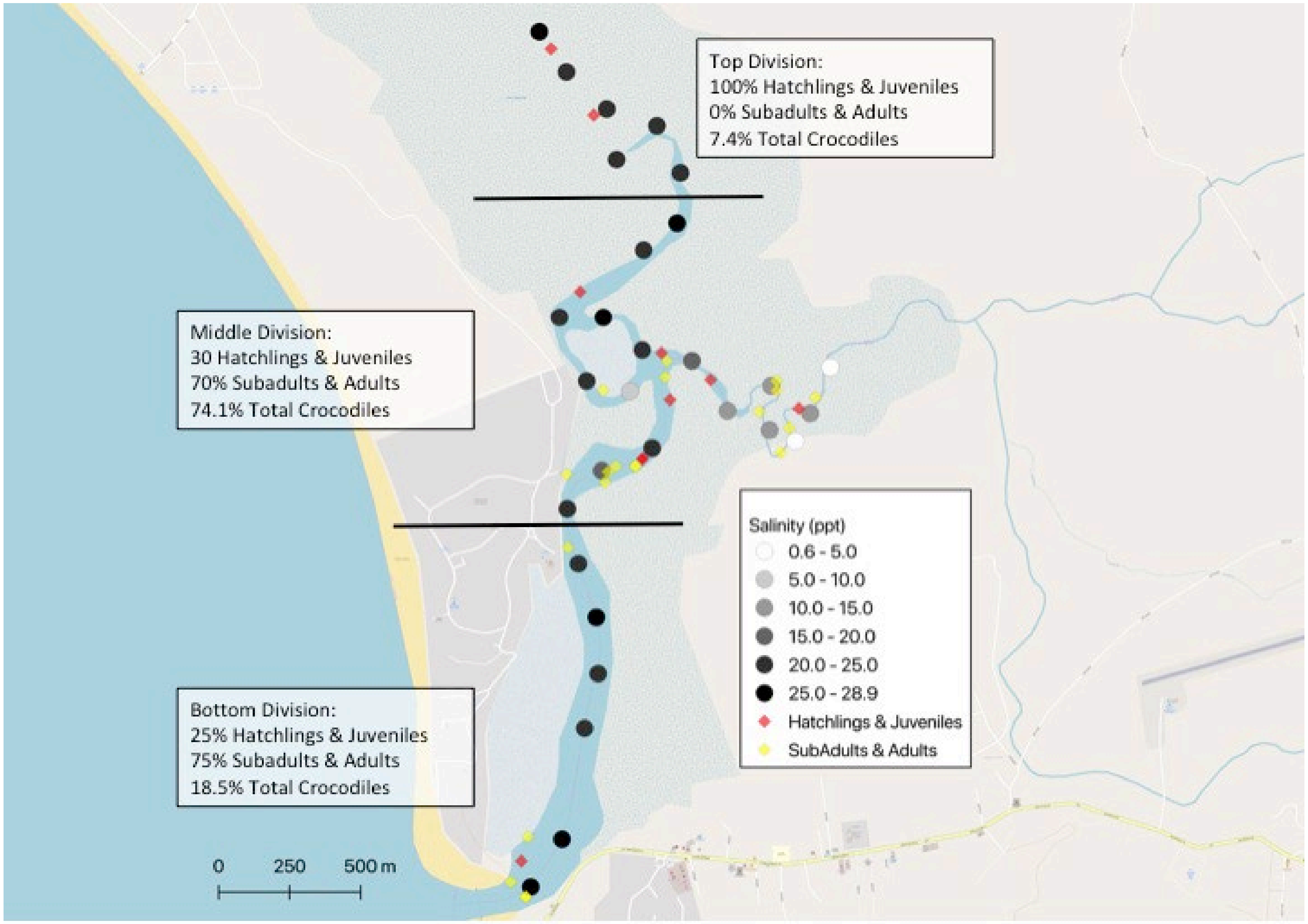


Figure 9: Crocodile locations and average salinity during the wet seasons. Average salinity values in the estuary for the wet seasons along 200m intervals. Data was collected in December 2015, June 2016, October 2016, and November 2016. Red diamonds indicate crocodiles in the hatchling and juvenile size classes, and yellow diamonds indicate crocodiles in the subadult and adult size classes.

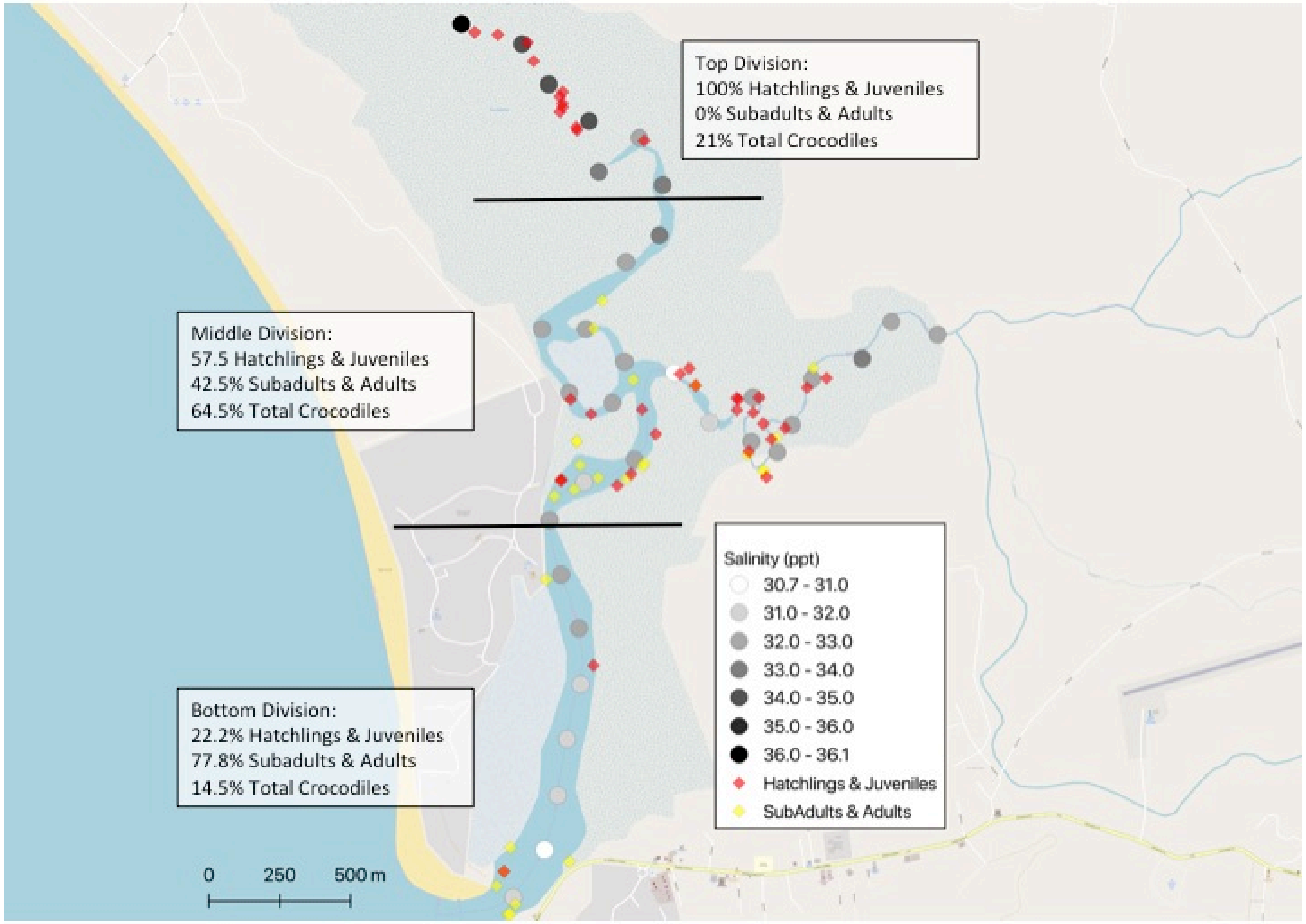


Figure 10: Crocodile locations and average salinity during the dry seasons. Average salinity values in the estuary for the dry seasons along 200m intervals. Data was collected in Jan-Feb 2015, Jan-May 2016, and Jan-Feb 2017. Red diamonds indicate crocodiles in the hatchling and juvenile size classes, and yellow diamonds indicate crocodiles in the subadult and adult size classes.

REFERENCES

Airoldi, L., Balata D, and Beck, M.W. (2008). The Gray Zone: Relationships between habitat loss and marine diversity nd their applications in conservation. *Journal of Experimental Marine Biology and Ecology*. *366(1-2)* 8-15.

Alfaro, A. C. (2010). Effects of mangrove removal on benthic communities and sediment characteristics at Mangawhai Harbour, northern New Zealand. *ICES Journal of Marine Science. 67(6)* 1087-1104.

Alongi, D.M. (2002). Present state and future of the world's mangrove forests. *Environmental Conservation. 29(3)* 331-349.

Balaguera-Reina, S. A., Venegas-Anaya, M., Sánchez A., Arbelaez, I., Lessios, H. A., Densmore III, L. D. (2016). Spatial Ecology of the American Crocodile in a Tropical Pacific Island in Central America. *PLoS ONE. 11(6)*.

Bayliss P (1987). Survey methods and monitoring within crocodile management programmes. *Wildlife Management: Crocodiles and Alligators*. 157-175.

Benfield, S.L, Guzman, H.M. and Mair, J.M. (2005). Temporal mangrove dynamics in relation to coastal development in Pacific Panama. *Journal of Environmental Management. 76(3)* 263-276.

Bevan, E., Whiting, S., Tuckery, T., Guinea, M., Raith, A., and Douglas, R. (2018). Measuring behavioral responses of sea turtles, saltwater crocodiles, and crested terns to drone disturbance to define ethical operating thresholds. *PLoS ONE.13(3)* e0194460.

Brien, M. L., Webb, G. J., Gienger C. M., Lang, J. W., Christian, K. A. (2012). Thermal preferences of hatchling saltwater crocodiles (*Crocodylus porosus*) in response to time of day, social aggregation and feeding. *Journal of Thermal Biology. (37).* 625-630.

Chabrek, R.H. (1963). Methods of capturing, marking and sexing alligators. Proceedings of the 17th Annual Conf. S.E. Assoc. Game and Fish Commiss, Hot Springs, Arkansas, 47-50 pp.

Charruau, P. (2012). Microclimate of American crocodile nests in Banco Chinchorro biosphere reserve, Mexico: Effect on incubation length, embryos survival and hatchlings sex. *Journal of Thermal Biology. 37* 6-14.

Charruau, P., Martínez G. Cantón, D.A., and Méndez de la Cruz, F.R. (2017). Additional details on temperature-dependent sex determination in *Crocodylus acutus*. *Salamandra. 53(2)* 304-308.

Chaves, F. M. B. (2009). *Determinación de la calidad de agua y sedimentos del Estero Tamarindo, Guanacaste.* Universidad Nacional, Heredia, Costa Rica.

Cotroneo, L. A. (2010). *Population Genetics and Conservation of the American Crocodile on the Pacific Coast of Costa Rica*. (Doctoral dissertation). Retrieved Drexel University Idea Library. (URI [http://hdl.handle.net/1860/3311])

Drymon, J. M., Ajemian, M.J., and Powers, S.P. (2014) Distribution and Dynamic Habitat Use of Young Bull Sharks *Carcharhinus leucas* in a Highly Stratified Northern Gulf of Mexico Estuary. *PloS ONE. 9(5)* e97124.

Dunson WA (1982). Osmoregulation of crocodiles: salinity as a possible limiting factor to *Crocodylus acutus* in Florida Bay. *Copeia*, *1982*. 374-385.

Elliott, M. & Whitfield, A.K. (2011). Challenging paradigms in estuarine ecology and management. *Elsevier. 94(4)* 306-314.

Ellis, T. M. (1981). Tolerance of Sea Water by the American Crocodile *Crocodylus acutus*. *Journal of Herpetology 15(2)* 187-192.

Evans, D. H., and Ellis, T. M. (1977). Sodium Balance in the Hatchling American Crocodile, *Crocodylus acutus*. *Comparative Biochemistry and Physiology. (58)* 159-162.

Evans, L.J., Jones, T.H., Pang, K., Evans, M.N., Saimin, S., and Goossens B. (2015). Use of drone technology as a tool for behavioral research: a case study of crocodilian nesting. *Herpetological Conservation and Biology. 10(1)* 90-98.

Fendt, L. (2015, March 11). *Crocodile attacks surfer in Tamarindo.* Retrieved from: http://www.ticotimes.net/2015/03/11/crocodile-attacks-surfer-in-tamarindo

Gaby, R., McMahon, M. P., Mazzotti, F. J., Gillies, W. N., Wilcox, J. R. (1985). Ecology of a Population of *Crocodylus acutus* at a Power Plant Site in Forida. *Journal of Herpetology. 19(2)* 189-198.

Mazzotti F.J. (1989). Factors affecting the nesting success of the American crocodile, *Crocodylus acutus*, in Florida Bay. *Bulletain of Marine Science. 44(1)* 220-228.

Getzin S. (2011). Assessing biodiversity in forests using very high-resolution images and unmanned aerial vehicles. *Methods in Ecology and Evolution. 3(2)* 397-404.

Gilman, E.L., Ellison, J., Duke, N.C., and Field, C. (2008). Threats to mangroves from climate change and adaptation options: A review. *Aquatic Botany. 89(2)* 237-250.

Government of Western Australia. (2009). Surface Water Sampling Methods and Analysis – Technical Appendices. Department of Water, Perth.

Grigg, G. C., Taplin, L. E., Harlow, P., Wright, J. (1980). Survival and Growth of Hatchling *Crocodylus porosus* in Saltwater Without Access to Fresh Drinking Water. *Oceologica. (47) 264-266.*

IUCN (2010, April 9). *Mangrove forests in worldwide decline*. Retrieved from: https://www.iucn.org/content/mangrove-forests-worldwide-decline

IUCN 2014. *The IUCN Red List of Threatened Species. Version 2014.3*. Retrieved from: http://www.iucnredlist.org

Jiménez, C. (2001). Seawater temperature measured at the surface at two depths (7 and 12 m) in one coral reef at Culebra Bay, Gulf of Papagayo, Costa Rica. *Rev. Biol. Trop.* 49(2) 153-161.

Koh, L.P. and Wich, S.A. (2012). Dawn of drone ecology: low-cost autonomous aerial vehicles for conservation. *Tropical Conservation Science. 5(2)* 121-132.

Krumholtz, M. (2016, October 25). *Crocodile that attacked U.S. surfer captured in Tamarindo.* Retrieved from: http://www.ticotimes.net/2016/10/25/tamarindo-calls-relocation-crocodiles

Lai, S., Loke, L. H. L., Hilton, M. J., Tjeerd, B. J., and Todd, P. A. (2015). The effects of urbanization on coastal habitats and the potential for ecological engineering: A Singapore case study. *Ocean & Coastal Management*. *103* 78-85.

Lea, J.S.E., Humphries, N.E., Clarke, C.R., and Sims, D.W. (2015). To Madagascar and back: long-distance, return migration across open ocean by a pregnant female bull shark *Carcharhinus leucas*. *Journal of Fish Biology. 87* 1313-1321.

Leslie, A. J. and Spotila, J. R. (2000). Osmoregulation of the Nile crocodile, *Crocodylus niloticus*, in Lake St. Lucia, Kwazulu/Natal, South Africa. *Comparative Biochemistry and Physiology Part A*. *(126)* 351-365.

Mauger, L. A., Velez, E., Cherkiss, M. S., Brien M. L., Boston, M., Mazzotti F. J., Spotila, J. R. (2012). Population assessment of the American crocodile, Crocodylus acutus (Crocodilia: Crocodylidae) on the Pacific coast of Costa Rica. *Journal of Tropical Biology. 60(4)* 1889-901.

Mazzotti F. J. (1983). The ecology of Crocodylus acutus in Florida. Ph.D Dissertation, Pennsylvania State University.

Mazzotti F.J. (1989). Factors affecting the nesting success of the American crocodile, *Crocodylus acutus*, in Florida Bay. *Bulletain of Marine Science. 44(1)* 220-228.

Mazzotti, F. J., Bohnsack, B., McMahon, M. P., Wilcox, J. R. (1986). Field and Laboratory Observations on the Effects of High Temperature and Salinity on Hatchling Crocodylus acutus. *Herpetologica. 42(2)* 191-196.

Mazzotti, F. J., and Dunson, W. A. (1984). Adaptation of *Crocodylus acutus* and *Alligator* for Life in Saline Water. *Comparative Biochemistry and Physiology. 79A(4)* 641-646.

Mazzotti, F., & Dunson, W. (1989). Osmoregulation in Crocodilians. *American Zoologist. 29*(3) 903-920.

Murray, C.M., Easter, M., Padilla, S., Garrigós, D.B., Stone, J.A., Bolaños-Montero, J., Sasa, M., and Guyer, C. (2015). Cohort-Dependent Sex Ratio Biases in the American Crocodiles (*Crocodylus acutus*) of the Tempisque Basin. *Copeia. 103(3)* 541-545.

Murray, C. M., Merchant, M., Easter, M., Padilla, S., Garrigós, D. B., Marin, M. S., and Guyer, C. (2017). Detection of a synthetic sex steroid in the American crocodile (*Crocodylus acutus*): Evidence for a novel environmental androgen. *Chemosphere. 180* 125-129.

Neer, J.A., Thompson, B.A., and Carlson J.K. (2005). Age and growth of *Carcharhinus leucas* in the northern Gulf of Mexico: incorporating variability in size at birth. *Journal of Fish Biology. 67* 370-383.

Olivares-Mendez, M.A., Fu, C., Ludivig, P., Bissyandé, T.F., Kannan, S., Zurad, M., Annaiyan, A., Voos, H., and Campoy, P. (2015). Towards an Autonomous Vision-Based Unmanned Aerial System against Wildlife Poachers. *Sensors (Base1). 15(12)* 31362-31391.

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

RAMSAR. (1990). Ficha Imformativa Sobre Humedales RAMSAR. Costa Rica, Estero Tamarindo, Playa Grande.

RAMSAR. (2014, September 11). *The List of Wetlands of International Importance*. Retrieved from http://www.ramsar.org/pdf/sitelist.pdf

Richards, P. M., Mooij, W. M., DeAngelis, D. L. (2004). Evaluating the effect of salinity on a simulated American crocodile (*Crocodylus acutus*) population with applications to conservation and Everglades restoration. *Ecological Modeling. 180(2-3)* 371-394.

Rodda, G. H. (1984). Movements of Juvenile American Crocodiles in Gatun Lake, Panama. *Herpetologica. 40(4)* 444-451.

Ross JP (1998). Crocodiles: an action plan for their conservation, IUCN/SSG Crocodile Specialist Group Publication. Oxford Press, Oxford.

Shaffer, M.J. and Bishop, J.A. (2016). Predicting and Preventing Elephant Poaching Incidents through Statistical Analysis, GIS-Based Risk Analysis, and Aerial Surveillance Flight Path Modeling. *Tropical Conservation Science. 9(1)* 525-548.

Tillett, B.J., Meekan, M.G., Field, I.C., Thorburn, D.C., and Ovenden, J.R. (2012). Evidence for reproductive philopatry in the bull shark *Carcharhinus leucas. Journal of Fish Biology. 80* 2140-2158.

Thorbjarnarson J. B. Ecology of the American Crocodile, *Crocodylus acutus*. Published in Crocodiles Their ecology, management, and conservation ISBN 2-88032-987-6, 1989 pgs 228-258.

Thorbjarnarson, J. B. (2010). American crocodile, *Crocodylus acutus*. Pp 46-53 in Crocodiles. Status Survery and Conservation Action Plan. Third Edition, ed. by S.C. Manolis and C. Stevenson. Crocodile Specialist Group: Darwin.

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

Wheatley, P.V., Peckham, H., Newsome, S.D., and Koch, P.L. (2012). Estimating marine resource use by the American crocodile *Crocodylus acutus* in southern Florida, USA. *Marine Ecology Progress Series. 447* (211-229).

Valiela, I., Bowen, J. L., York, J. K. (2001). Mangrove Forests: One of the World's Threatened Major Tropical Environments. *Bioscience. 51(10)* 807-815.