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Determining Critical Internesting and Foraging Habitats for the Conservation of Sea Turtles in Gabon, Africa Using Satellite Tracking and Stable Isotope Analysis

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DETERMINING CRITICAL INTERNESTING AND FORAGING HABITATS FOR THE

CONSERVATION OF SEA TURTLES IN GABON, AFRICA USING SATELLITE

TRACKING AND STABLE ISOTOPE ANALYSIS

by

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B.S. May 2014, Old Dominion University
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ABSTRACT

DETERMINING CRITICAL INTERNESTING AND FORAGING HABITATS FOR THE CONSERVATION OF SEA TURTLES IN GABON, AFRICA USING SATELLITE TRACKING AND STABLE ISOTOPE ANALYSIS

Tiffany M. Dawson
Old Dominion University, 2017
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Understanding the horizontal and vertical habitat of olive ridley sea turtles (*Lepidochelys olivacea*), a threatened species, is critical for determining regions of protection that may effectively reduce bycatch, the largest threat to this species. Satellite transmitters were used to determine the movement and dive behavior of 21 female olive ridley turtles tagged in Pongara National Park, Gabon during the 2012, 2013, and 2015 nesting seasons. A switching state space model was used to filter the tracking data and categorize the internesting and post-nesting movements. Gridded utilization distribution (UD) home range analysis of tracking data revealed that the entire core habitat occurred in the Komo Estuary and overlaps with an important area for artisanal fishing (79% overlap) and commercial shipping traffic (58% overlap). Dive data revealed turtles spent a majority of their time resting on the estuary seabed (20% of all dive time), indicating that bottom set gear may pose the greatest potential for fisheries interactions. The results from this study provide data that can inform the design and implementation of marine protected areas and fishery zones, currently underway. Additionally, post-nesting foraging sites are not well defined for this population. $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis of 149 epidermis samples from female nesting olive ridley turtles in Gabon, 38 of which also had satellite transmitters, was used to determine if distinct foraging locations exist for this population. Tracked turtles allow for visualization of foraging areas, which can then be correlated to their distinct isotopic signatures and further matched to untracked turtle signatures. Cluster analysis of the isotope ratios from all turtles produced two significant clusters with a 95% predictability rate. Track visualization of each cluster did not show any foraging patterns and a generalized linear model determined that nesting beach, sampling year, and average straight-line distance from

foraging location were not indicators for cluster assignment. Due to olive ridley turtles being opportunistic feeders and following a "looping" pattern to find new food sources, it is difficult to use stable isotope analysis to identify foraging habitat for non-tracked turtles in this population.

DEDICATION

       This thesis is dedicated to everyone who has helped me throughout my education and has been vital during my Master's Degree. I would especially like to acknowledge my parents, Ken and Marie Dawson; my sister, Kristen Dawson; and family and friends for their continuous support. I would also like to thank Dr. Sara Maxwell for her expertise as my advisor and unrelenting support as my mentor, Dr. Matthew Witt and Dr. Eileen Hofmann for their guidance as my committee members, and the faculty, staff, and fellow graduate students at Old Dominion University.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Olive ridley sea turtles (*Lepidochelys olivacea*) are considered the most abundant sea turtle species and inhabit tropical waters of the Atlantic, Pacific and Indian oceans (Abreu-Grobois & Plotkin, 2008). Despite being so numerous, research efforts are limited and concentrated in specific regions (Abreu-Grobois & Plotkin, 2008). For the East Atlantic population, one of the largest threats comes from the amount of olive ridley turtle bycatch in the shrimp trawl fisheries (Frazier et al., 2007). Bycatch is a major threat to these large charismatic megafauna because their wide distribution crosses multiple commercial and artisanal fishing hotspots as well as political boundaries, making conservation practices difficult (Wallace et al., 2013). In Gabon, a Central West African country, the previous and current presidents, Omar Bongo and Ali Bongo, created national parks that protect the beach nesting sites for these sea turtles but protection in the water is lacking. Sea turtles lay multiple clutches of eggs in a nesting season, and remain in the vicinity of nesting beaches for several weeks at a time, during which they are subjected to additional anthropogenic impacts. Currently, oil and timber are the main economic support for Gabon but the country is looking to shift toward ecotourism for revenue. Therefore, there is national interest in protecting this species, among others, to attract tourists. Also, in 2007 Gabon started plans for adding a large shipping port on the southeast coast. This building process includes dredging, heavy ship traffic, and seismic activity (Briefing: ICA 2007), all of which threaten the Gabonese nesting population of olive ridleys making conservation research a high priority. These marine reptiles are ideal candidates for conservation measures because they often congregate in the same waters to breed and females return to the same beaches to nest (Vanbuskirk & Crowder, 1994), such as Pongara National Park.

This study used satellite telemetry to track the movement and dive behavior of olive ridley sea turtles (*Lepidochelys olivacea*) from the East Atlantic population. This was done to better understand their habitat use and provide data to managers to create protective management practices in the area. Dive depth data collected from the satellite transmitters were used to determine what part of the water column the olive ridleys used during the internesting period, allowing us to distinguish where they used the benthos and where they surfaced. This accompanied the location data to better advise fishermen and fisheries managers of where and at what depths gear should be set at to avoid entanglement. We hypothesized that the females

remain in coastal waters close to the nesting beaches between clutches and overlap with fishing and commercial fishing areas and spend a majority of their time at bottom depths. If correct then they will be most at risk from bottom-set fisheries such as bottom-set gillnets.

An additional aspect of this project uses stable isotope analysis (SIA) to supplement the satellite tracking data to better define post-nesting foraging areas for olive ridley turtles. Epidermis samples were isotopically analyzed to determine if there are different foraging areas, differentiated by distinct isotopic ratios, being used by the olive ridleys after the nesting period. Carbon (δ^{13}C) and Nitrogen (δ^{15}N) isotope ratios were compared to the known telemetry tracks to determine if distinct groups were formed which could then allow the untracked sea turtles to be allocated to groups based on similar isotope ratios. We hypothesized that the tagged olive ridley turtles will group into two or more subsets based on similar isotopic ratios and a majority of the untagged turtles will identify with one of the subsets.

CHAPTER 1

INFORMING MARINE PROTECTED AREAS FOR SEA TURTLES IN GABON,

AFRICA USING SATELLITE TRACKING

INTRODUCTION

Protecting long-lived, highly migratory marine species is challenging since they encounter multiple threats across broad areas and in different life stages (Witt et al., 2011, Maxwell et al., 2013, Lascelles et al., 2014), often requiring multi-faceted and multi-national conservation efforts (Blumenthal et al., 2006, Gore et al., 2008, Maxwell et al., 2011, Croxall et al., 2012, Pikesley et al., 2013b, Doherty et al., 2017). Satellite telemetry is often used to characterize the horizontal movements of marine species (Godley et al., 2008, Block et al., 2011, Hazen et al., 2012, Yurkowski et al., 2016, Citta et al. 2017, Vaudo et al., 2017). There have been several studies that use the horizontal movement data of large marine vertebrates to determine overlap with anthropogenic threats such as fisheries (seabirds: Suryan et al.,2007, Bugoni et al., 2009, Zydelis et al 2011; sea turtles: da Silva et al., 2011, Witt et al 2011, Revuelta et al., 2015; marine mammals: Geschke & Chilvers 2009, Rosenbaum et al., 2013, sharks: Holmes et al., 2014), shipping (marine mammals: Mate et al., 1997, Schorr et al., 2009), and in-water habitat degradation (seabirds: Montevecchi et al., 2012; marine mammals: Johnson & Tyack 2003, Rosenbaum et al., 2013).   Regardless of the specific tag, satellite telemetry has been critical in evaluating threats for marine species (Witt et al., 2008, Maxwell et al., 2013, Lascelles et al., 2014) and assessing how efficient conservation boundaries, such as marine protected areas (MPA), are at encompassing the wide ranging habitat distribution of migratory species (Scott et al., 2012, Hart et al., 2010, Young et al., 2015, Maxwell et al. 2016).

Olive ridley sea turtles (*Lepidochelys olivacea*) are a vulnerable species as defined by the International Union for Conservation of Nature and Natural Resources (IUCN) Red List because of their slow growth rate, late reproductive maturity, and low hatchling survival rate (Abreu-

Grobois & Plotkin, 2008). These factors make it difficult to maintain sufficient populations in the face of anthropogenic influences (Abreu-Grobois & Plotkin, 2008). In the East Atlantic, one of the largest threats to olive ridley turtles is fisheries bycatch, including trawls and gillnets (Frazier et al., 2007, Parnell et al., 2007). Olive ridley turtles are distributed across multiple commercial and artisanal fishing hotspots as well as political boundaries, making conservation practices difficult (Godgenger et al., 2009, Wallace et al., 2013, Metcalfe et al., 2015b).

Gabon, in central West Africa, is home to the largest olive ridley rookery in the Atlantic (Metcalfe et al., 2015a), however, even with global decline and their vulnerable status this region remains data deficient. Despite an expansive network of national parks that protect 81% of sea turtle beach nesting sites (Metcalfe et al., 2015a), in-water protection for many nesting beaches is still lacking. Currently, the only in water protection occurs in southern Gabon via Mayumba National Park. Mayumba National Park is 900 km^2 and spans 60 km of the Gabonese coastline and extends 15 km into the Atlantic Ocean, protecting both the nesting beach and coastal waters of olive ridley and leatherback (*Dermochelys coriacea*) sea turtles (Witt et al., 2008, Maxwell et al., 2011). However, the government recently announced that it will implement a new network of MPAs that will consist of 10 marine parks covering 23% of the Gabonese Economic Exclusive Zone.

Olive ridley turtles, like other sea turtle species, lay multiple clutches of eggs in a nesting season (Plotkin 2007, Maxwell et al., 2011) and remain in the vicinity of nesting beaches for several weeks at a time (Whiting et al., 2007, Hamel et al., 2008, Da Silva et al., 2011, Maxwell et al., 2011), termed the internesting period, during which they are susceptible to additional anthropogenic impacts (Pikesley et al., 2013a). This internesting period also makes sea turtles ideal candidates for conservation measures because both sexes congregate in the same waters to breed and females return to the same beaches to nest within the internesting period (Vanbuskirk & Crowder, 1994). Still, there can be considerable variability in internesting movements among species at different nesting beaches and within the same nesting population (Godley et al., 2008). In most leatherback populations, females cover extensive areas between nesting events, though normally stay within the continental shelf (Eckert et al., 2006, Georges et al., 2007, Hitipeuw et al., 2007). Some loggerhead (*Caretta caretta*) and green sea turtle (*Chelonia mydas*) populations demonstrate females making oceanic loops during internesting periods (Blumenthal et al., 2006)

and an olive ridley population in Australia was seen to move offshore during the internesting period (McMahon et al., 2007). Flatback sea turtles (*Natator depressus*) demonstrated variable internesting movements in Western Australia, some staying within 10 km of shore and others moving offshore more than 25 km (Waaters et al., 2011, Whittock et al., 2014). However, most hard shelled turtles, (loggerheads (Godley et al., 2003a), greens (Hays et al., 1999, Craig et al., 2004, Troëng et al., 2005b, Fuller et al., 2008), hawksbills (*Eretmochelys imbricata,* Troëng et al., 2005b, Whiting et al., 2006, Hoenner et al., 2015), and Kemp's ridley sea turtles (*Lepidochelys kempii,* Seney & Landry 2008, Shaver & Rubio 2008)) tend to stay close to their nesting beaches and this intense use allows for more targeted conservation measures (Godley et al., 2008).

Effective management of large marine species requires a working knowledge of the study species as well as putative threats (Roberts & Hamann, 2016). The Gabonese government has collected olive ridley turtle bycatch data for several years, and a study on beached carcasses indicated that bycatch of sea turtles, particularly olive ridley turtles, is appreciable in Gabon (Parnell et al., 2007). In a 2005-2006 partnership between RENATURA and local fishermen in neighbouring Republic of Congo, 1,500 leatherback and olive ridley sea turtles were released alive from fishing gear, the distribution displaying higher olive ridley bycatch all year long (Godgenger et al., 2009). Similar to other Central African countries, Gabon has more small-scale fishing than industrial fishing, and only small-scale fishing vessels are permitted to fish within 6 nautical miles from shore (Metcalfe et al., 2015b). There are thirty times more small-scale fishing vessels than industrial vessels in Gabon; small-scale fishing vessels use purse seine, drift gillnet, and bottom set gillnet techniques (Metcalfe et al., 2015b). Through field surveys in 2013 and 2015, Metcalfe et al. (2015b) found that 62% of the 1,831 boats were working out of Libreville and the Komo Estuary, which is also adjacent to one of the largest olive ridley nesting beaches in Gabon (Metcalfe et al 2015a). Artisanal fishing was also found to occur in heavily aggregated areas in estuaries, and river and lagoon mouths (Metcalfe et al., 2015b). The Komo Estuary is also a major shipping region, however the impacts of shipping on sea turtles have not been well studied. Impacts may occur in the form of pollution from vessels via discharge of oil, fuel, organic pollutants, sewage, and debris, noise pollution, or through direct ship strikes (Abdulla and Linden 2008, Maxwell et al., 2013 and references within). Dredging is another evident threat within the Komo Estuary which can directly affect sea turtles through noise

pollution, vessel strikes, and change habitats and resource density (Whittock et al., 2017). Even when the magnitude of impacts is unclear, understanding where and when turtles and threats overlap is necessary to inform conservation strategies.

Horizontal movement and habitat use are important measures for conservation but dive behavior can also be critical to understanding in-water threats to marine species. Understanding water column use can influence fishing strategies such as gear set depth, soak time, and gear type (Lewison et al., 2013). For example, in the North Pacific, Polovina et al (2003) used turtle dive data to determine that banning shallower set longlines would decrease the amount of bycatch for sea turtles, specifically loggerheads and olive ridley turtles, because they spent most of their time in the upper water column. Presently, little is known about dive behavior of olive ridley turtles and even less is known about their dive behavior during the internesting period. The only olive ridley dive studies are from the West Atlantic and focus on post-nesting dive behavior (Plot et al., 2015). Understanding how olive ridley turtles use the water column is crucial to anticipating interactions with fishing gear and vessels along the Gabonese coast.

In this study, we collected location and dive depth data from female olive ridley turtles nesting in Pongara National Park to better understand their internesting habitat use along the Gabonese coast. Pongara National Park is adjacent to the Komo Estuary, the location of a major port, and proximate to Gabon's capital city, Libreville, one of the main anthropogenic use regions in Gabon and most populated city in the country with approximately 800,000 inhabitants. The Komo Estuary is also an important artisanal fishing region. We further consider the movements of olive ridleys in relation to boundaries of an artisanal fishing closure, shipping lanes and potential boundaries for future marine protected areas. Movement and dive data provide a basis for an understudied population and can be used to inform conservation measures for the area.

METHODS

ETHICS STATEMENT

All federal, international, and institutional guidelines were followed, and this study was approved by and carried out in accordance with the recommendations of University of Exeter's College of Life and Environment Sciences Ethics Committee and the Institutional Animal Care and Use Committee at Old Dominion University (IACUC Permit 15-016). Permissions to work within park boundaries and with the study species were issued by the Gabon Agence Nationale des Parcs Nationaux (AEl5025, AR0010/12, AE140003).

SATELLITE TRANSMITTER DEPLOYMENT

The internesting movements of 21 olive ridley sea turtles were monitored from Pongara National Park, one of the largest nesting beaches in Gabon (Metcalfe et al., 2015a), over three nesting seasons (2012, 2013, 2015). Satellite transmitters were attached to nesting females early in the nesting season to capture internesting movements (Maxwell et al., 2011). SPOT-293A transmitters (Wildlife Computers, Redmond WA, USA) were deployed in between 26 October and 5 November 2012 (n=6) and 12 October and 13 October 2013 (n=5). SPLASH10-351C (Wildlife Computers, Redmond WA, USA) were deployed between 27 October and 31 October 2015 (n=10). Attachment followed techniques described in Maxwell et al (2011), and did not start until the female began oviposition and care was taken not to disturb the nest following attachment. Transmitters were attached to the flattest portion of the two front most vertebral scutes of the carapace using either T308+™ Epoxy (Powers Fastners, Brewster NY, USA) in 2012 and 2013 or Superbond 1:1 Expoxy Adhesive (FiberGlass Coatings, Inc. St. Petersburg FL, USA) in 2015. First the carapace was cleaned of algae and smoothed using sandpaper and sea water; once cleared the area was dried with acetone. The transmitter was placed with the antennae pointing towards the head and perpendicular to the water and the epoxy was molded to ensure attachment around the edges. To minimize stress and handling, a wooden box was placed around the female after she finished covering the nest. Once the epoxy set, the female was released and monitored to make sure she returned to the water. Before tag attachment curved carapace length (CCL) and curved carapace width (CCW) were measured, metal flipper tags

Monel 1005-62 (National Band and Tag Co., Newport, KY) were attached to the front flippers. Satellite data were collected via the Argos Satellite System.

TRACK ANALYSIS AND STATE-SPACE MODEL

All Argos location quality points except for 0 and Z were used in the movement analysis (i.e. location classes 3, 2, 1, A, B, Witt et al., 2010) and a hierarchal state-space model (hSSM) with a time interval of 3.5 hours was used to filter the location data and determine internesting behavior (Breed et al., 2009, Jonsen et al., 2013). SSMs use parameters associated with an animal's position (Jonsen et al., 2003) and determine a behavioral state for each point in time (Jonsen et al., 2013, Breed et al., 2009). Through the use of a two part model, an equation is used to determine how behavioral states change randomly in time and previous locations are used to estimate the next location based on an independent random walk model and biological constraints of the animal, such as how far they can travel in a day (Jonsen et al., 2013). SSMs are used to draw inferences from data on behavioral changes through time and how it can be related to actual biological processes (e.g. foraging, breeding, or migrating). The model also accounts for observation error between the satellite produced location and the unseen true location and credible limits for the predicted locations (Breed et al., 2009). A hSSM structure was used since we had multiple tagged animals and wanted to focus on population level dynamics instead of individual movement. A short track, run individually through the model, could not produce accurate behavioral states but when run with other tracks in the hSSM, behaviors can then be categorized (Breed et al., 2009). The hSSM is ideal when true behaviors cannot be observed throughout the track, such as with the olive ridley turtles, therefore data such as speed and turn angle are used to delineate behavior (Jonsen et al., 2013).

We used the hSSM model to run 2 Markov Chain Monte Carlo simulations for 10,000 iterations with a burn-in of 15,000 to generate 10,000 posterior samples in the "bsam" package in R (Jonsen et al., 2005, Jonsen 2016). We used a thinning factor of 10 to minimize the with-in chain sample autocorrelation. The time step of 0.15, creating a 3.5-hour time interval between points, was determined by finding the average time between existing points, following Maxwell et al (2011). Trace plots were examined for autocorrelation and to ensure that they converged on a mean density, and resulting tracks were examined to determine if tracks made biological sense, the most important validation metric (G. Breed, pers. comm.). Because of our coastal and

estuarine study area, resulting hSSM locations occasionally occurred on land. These locations were shifted, such that previous and post locations were used to determine directionality of movement. The inland location was then manually moved to the closest in-water location. If a relatively confident location could not be estimated then the location was discarded from analysis.

The hSSM also assigned a binary behavioral estimate between 1 and 2 to each location point based on the mean turn angle (θ) and the autocorrelation between speed and direction (γ). We defined the "transiting" behavior state as behavioral estimates between 1.0-1.39 and "internesting" behavioral state as 1.6-2.0, and only internesting locations were used for the remainder of the analyses. Behavioral estimates between 1.4-1.59 were considered behavioral transitions from internesting behavior to transiting; comprising only 6.8% (1,268 locations) of the data, the transiting and transition locations were not considered in the analysis.

COMPARISON OF SEA TURTLE DISTRIBUTION, BOUNDARIES, AND COMMERCIAL SHIPPING

Home range analysis, in the form of a gridded utilization distribution (UD), was used in the software R to determine the core area used during the internesting period (Maxwell et al., 2011). Using custom scripts in R 3.0.1 (R Core Team, 2003) we visually determined a base grid size of 0.015° (approx. 1.5 km^2) which allowed for the best spatial resolution within the restricted area of the Komo Estuary without oversmoothing as a turtle moved between cells (Maxwell et al., 2011). The core use area, the area 50% or more of the locations occurred, was compared to a current seasonal artisanal fishing closure to determine the overlap between the internesting movements and the closure. To aid in future zoning of the Komo Estuary, we quantified the percent of the internesting tracks inside of the Komo Estuary. Additionally, we divided the estuary into 6 zones based on current political boundaries (i.e. National Parks) and nautical landmarks, and then calculated percentage of internesting movements that fell within each zone.

The UD was also compared to a UD of commercial shipping location data. Under Regulation 19 of the Safety of Life at Sea: Chapter V by the International Maritime Organization, location data for shipping vessels are recorded globally for all vessels greater than 300 tonnes if traveling internationally and greater that 500 tonnes if localized (IMO 2001). We

used available vessel location data along the Gabonese coast from October and November 2012 to mirror the internesting period of the olive ridley turtles. All anchored or mooring data points were removed from analysis (1.6% of all data points), such that only transiting data were used. The shipping UD was created using the latitude and longitude points and on the same base grid as the olive ridley UD. The UDs were then compared to determine the percent of overlap within the internesting habitat.

DIVE DEPTH ANALYSIS

Dive depth was collected in 2015 only (n=10), with depth recorded every 2.5 minutes. The transmission of dive data, however, was variable and dependent on satellite availability. Additionally, the transmitted dive data did not necessarily correspond to known locations. To determine dive behavior near known locations, dive depths within a one-hour window before and after a high-quality Argos location (location quality 3, 2 and 1 only) were extracted. The maximum dive depth within this 2-hour window was compared to a gridded bathymetric map of the Komo Estuary to determine if turtles likely reached the estuary bottom. The gridded bathymetric map was created from *Bahia de Corsico to Estuaire du Gabon, Nautical Chart 1356*, a nautical chart of the estuary and rasterized using the Raster package in R with the same resolution and extent as the grid used for the UDs.

Using all of the dive data available from the 2-hour windows, regardless of location quality, we determined the portion of the water column used by the olive ridley turtles. In this analysis, we included all depth locations that were recorded along the course of the dives. We normalized the dive data from each 2-hour window previously described by dividing each depth by the maximum depth of that window creating a proportion between 0 (representing surface use) and 1 (representing bottom use). This allowed us to determine where in the water column turtles spent time throughout the internesting period across all dive data.

RESULTS

SATELLITE TRANSMITTER DEPLOYMENT

Twenty-one satellite transmitters were attached to nesting female olive ridley turtles in 2012 (n=6), 2013 (n=5), and 2015 (n=10) in Pongara National Park (Appendix 1, Figure 1A-D). The overall average track duration was 102.8 days (±58.4, min=29, max=203) with an average duration of 82.4 days (±53.9, min=29, max=151) in 2012, 169 days (±36.4, min=112, max=203) in 2013, and 79.9 days (±45.5, min=34, max=184) in 2015. The average internesting period was 15.6 days (±12.2). Turtle M from the 2015 season stopped transmitting before switching from internesting to post-nesting movement but remained in the Komo Estuary for 43 days and was therefore retained in the analysis. Turtle B from the 2012 season was discarded from further analysis because the Argos data returned improbable locations and movement patterns thought to be from transmitter failure. Turtle P had location points within the internesting period that demonstrated an unexpected full behavioral switch from internesting to transiting then back to internesting. During the switch, the turtle remained in the Komo Estuary and later we saw the anticipated long-term behavioral switch to transiting along with a southward movement toward the foraging grounds (Pikesley et al., 2013b). Therefore, we included the short behavioral change in our internesting period analysis.
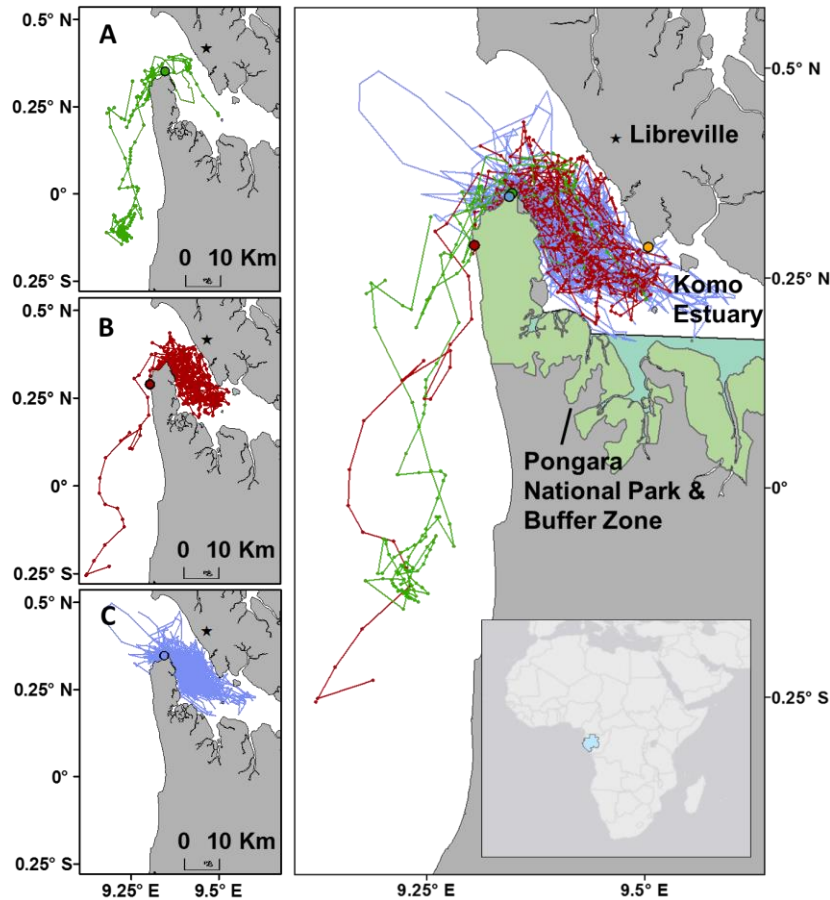
Figure 1: Olive ridley turtle internesting tracks from Pongara National Park. **(A)** State-space modeled internesting tracks of female olive ridley sea turtles tagged from Pongara National Park, Gabon from the 2012 field season (n=5). The green dot represents the tagging location for this season **(B)** Tracks from the 2013 field season (n=5). The red dot represents the tagging location for this season **(C)** Tracks from the 2015 field season (n=10). The blue dot represents the tagging location for this season D) Tracks from the 2015 field season (n=10). **(D)** Combined tracks from all three field seasons (n=20). Green, red, and blue tracks represent 2012, 2013, and 2015 data respectively along with the correlated colored dots representing the tagging locations. The orange dot represents the Owendo commercial shipping port in the Komo Estuary.
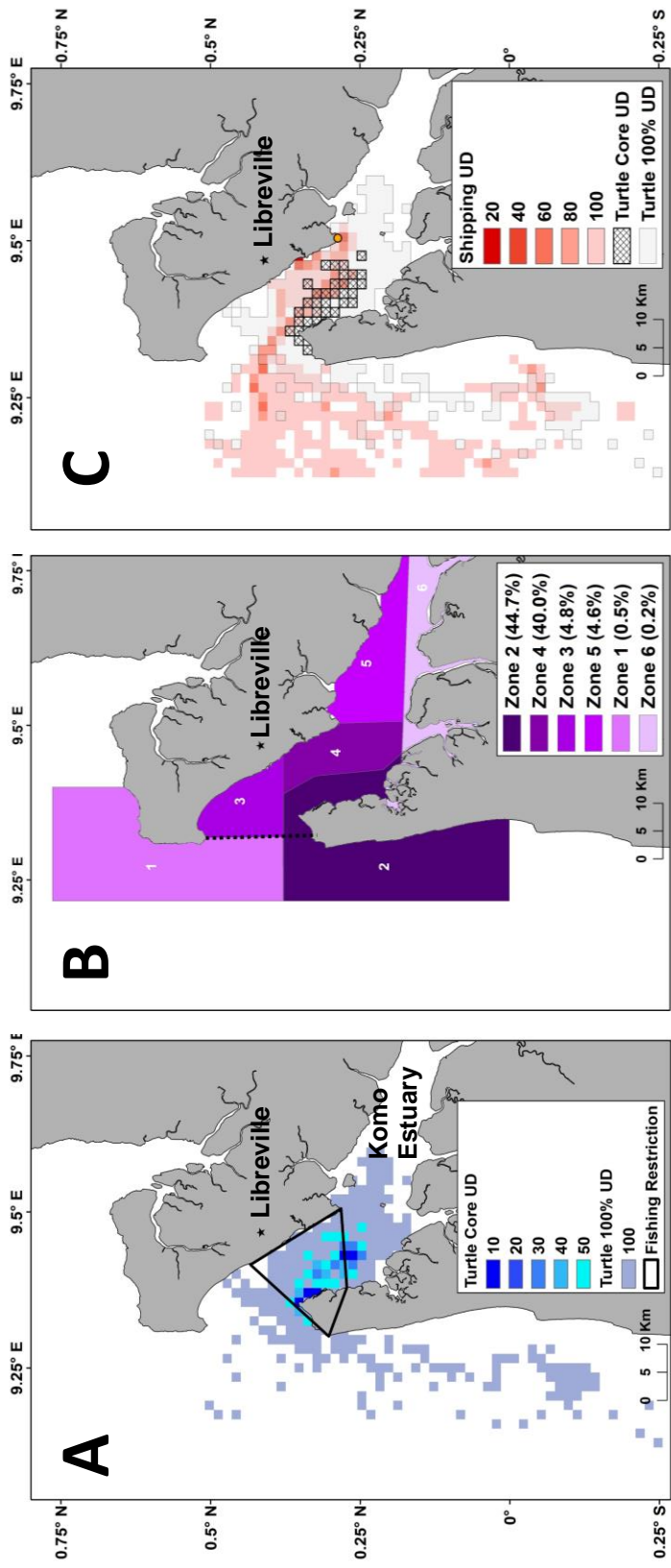
Figure 2: Sea turtle distribution, boundaries, and anthropogenic overlap in the Komo Estuary. (**A**) Utilization distribution (UD) of all female olive ridley sea turtles during the internesting period tagged in Pongara National Park. The turtle core UD represents the most used areas by the olive ridley turtles with 79% located within the seasonal fishing restriction. (**B**) Arbitrary zonation of the Komo Estuary based on current political boundaries and nautical landmarks. The zones are colored based on utilization by the tagged olive ridley turtles during the internesting period with Zones 2 and 4 being the most utilized areas, implying areas of conservation potential. The dotted line represents the boundary used to calculate density in and out of the estuary. (**C**) UD of October 1 – November 30, 2012 transiting shipping location data (red) compared to the olive ridley full UD (dotted) and the core UD (hashed) within the Komo Estuary. The organce circle represents the Owendo Port, the majoring shipping port in the Komo Estuary.

COMPARISON OF SEA TURTLE DISTRIBUTION, BOUNDARIES, AND COMMERCIAL SHIPPING DATA

The full (100%) UD demonstrated that olive ridley turtles remained in the Komo Estuary and within 20 km of the coast south of the estuary. This includes use of the waters off the nesting beach of Pongara National Park (Figure 2A). The core UD highlighted the high use areas to be entirely within the Komo Estuary and at the mouth of the estuary near to where much of the nesting occurred and where most tags were deployed. The current artisanal fishing decree in the estuary encompasses 79% of the core UD and 23% of the full UD (Figure 2A). Using the mouth of the estuary as an arbitrary divide, 89% of the hSSM internesting points were within the estuary and 95% of the points were in the mapped zones. Zones 2 and 4 had the highest density of points, with 45% and 40% respectively, and Pongara National Park, Zone 6, only encompassed 0.21% of the points (Figure 2B).

The UD of the commercial shipping data demonstrated the highest ship traffic in the mid estuary from the Owendo port and out along the Gabonese coast line. The full shipping (100%) UD overlapped with 34.1% of the olive ridley full UD and 48.5% of the core UD (Figure 2C).

DIVE DEPTH ANALYSIS

The dive depth data from the ten satellite tags deployed in 2015 provided an average of 185.3 hours of data (±136.3, min=32.0, max=403.4) during the internesting period. The mean maximum depth recorded was 33 meters (±10, min=19, max=51) and we found the range of mean dive depths to be between 7 meters and 12 meters (mean= 9±2) (Appendix 1).

Of the transmitted dive data that correlated with high quality locations, 100% of the maximum dive depths were found to be at the seabed when compared to the benthic grid of the estuary (Figure 3). Normalized depths, proportion of time spent at depths within each dive (2.5 min interval), normalized across all dives, demonstrated that 19.7% of the recorded depths occurred on the bottom and 7.8% were at the surface, with the remainder in the water column (Figure 4).
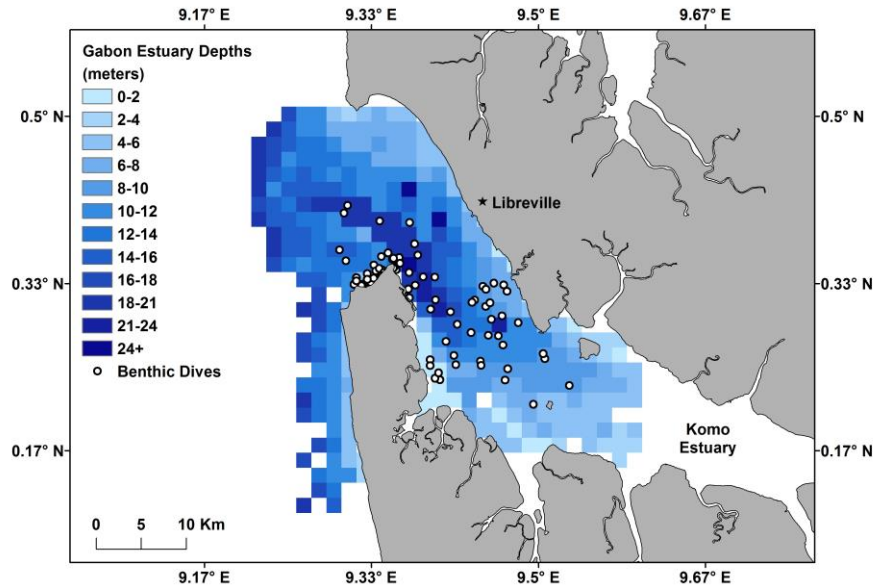
Figure 3: Benthic grid of the Komo Estuary highlighting locations where olive ridley turtles location data correlated with dive depth data providing a comparison between max dive depth and the estuary bottom.
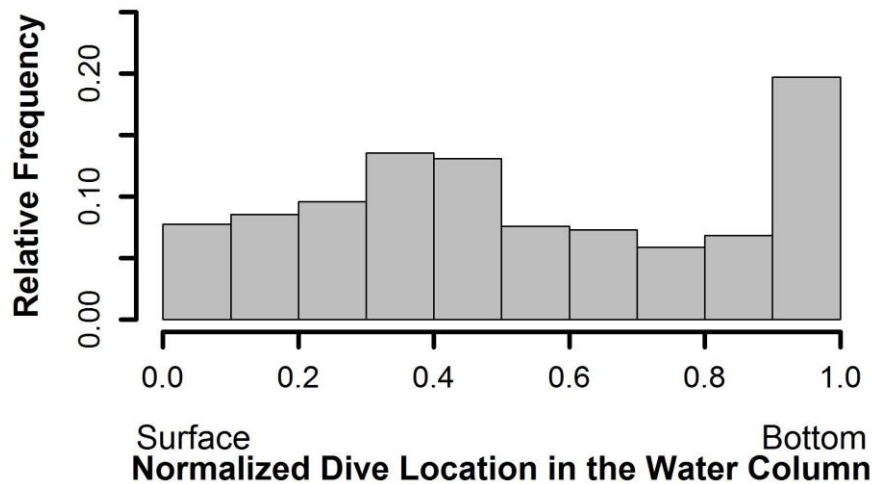
Figure 4: Dive Depth Analysis. Proportion of time spent at depths within each dive at each 2.5 min interval, normalized across all dives. Dive data extracted from the 2-hour window surrounding each location where dive data was transmitted and normalized based on the maximum depth of the respective window.

DISCUSSION

Marine vertebrates in all ocean basins are under threat throughout their range as seen by large-scale declines in recent decades (Baum et al., 2003, Heithaus et al., 2008, Myers et al., 2003). Red List Status might be changing for some species (Balazs & Chaloupka 2004, Dutton et al., 2005, Simpfendorfer & Dulvy 2017), highlighting that improved knowledge can lead to better management and conservation outcomes. Threats are highest where species aggregate to breed (Edgar et al., 2008, Hays et al., 2010) and where species and humans frequently overlap; for marine species this is often in coastal zones (Maxwell et al., 2013). Quantifying specific threats can be difficult due to limited data, largely because of the feasibility of collection, but quantitative data is necessary to undertake robust assessments and gauge impacts on marine vertebrates. With robust data on human impact and species distribution it becomes possible to accurately gauge threats in space and time, and then appropriately manage human activities for the preservation of species of conservation concern. Here we were able to quantitatively determine fine-scale movements of olive ridley sea turtles and compare them with anthropogenic activities, as well as potential mitigation via a fishing closure, in order to highlight where management measures are likely successful, as well as areas where it can be improved.

INTERNESTING ESTUARINE MOVEMENTS

The female olive ridley turtles that nest in Pongara National Park are a prime example of a marine vertebrate population whose habitat use is understudied and are at risk from fishing pressures in the adjacent estuary. As the first study to look at the internesting movements from this site, the telemetry tracks illustrate that females remain in the vicinity of the nesting beach (<20 km), and spend a majority of their time within the Komo Estuary (89% of locations, Figure 1A-D). Some of the females remained in the area for over a month before switching to the transiting phase and moving south, likely to known foraging grounds off the coast of Angola (Pikesley et al., 2013b). This is the first noted occurrence where olive ridley turtles near exclusively used estuarine habitat during their internesting period. Green sea turtles nesting in French Guiana similarly used the Maroni River Estuary during their internesting period (Chambault et al., 2016) but additional occurrences are not known. In the Indian Ocean, female olive ridley turtles tagged on nesting beaches in Oman display consistent coastal use (Rees et al.,

2012) but other populations of olive ridley turtles have been seen to move offshore to neritic waters adjacent to the nesting beaches or immediately end the internesting period and migrate towards foraging grounds after their last nesting event (Whiting et al., 2007, Maxwell et al., 2011, Plot et al., 2015).

Within the estuary both the shallower shorelines and deeper mid-estuary were used, highlighting the importance of this enclosed area to the internesting females. There are several hypotheses for estuarine use. Shallower depths in the estuary may result in reduced energy expenditure by turtles coming to the surface to breathe as they wait for egg development between clutches. During their time in the estuary, the olive ridley turtles appear to be utilizing the bottom habitat with U-shaped dives which are related to resting or foraging (Figure 3 and Figure 5, Plot et al., 2015). In addition, turtles may find increased foraging opportunities in the estuary; whether olive ridley turtles forage during the internesting period is unclear, but some turtles remained in the estuary long after nesting was completed, suggesting that they were likely taking advantage of available resources (Rees et al., 2012).
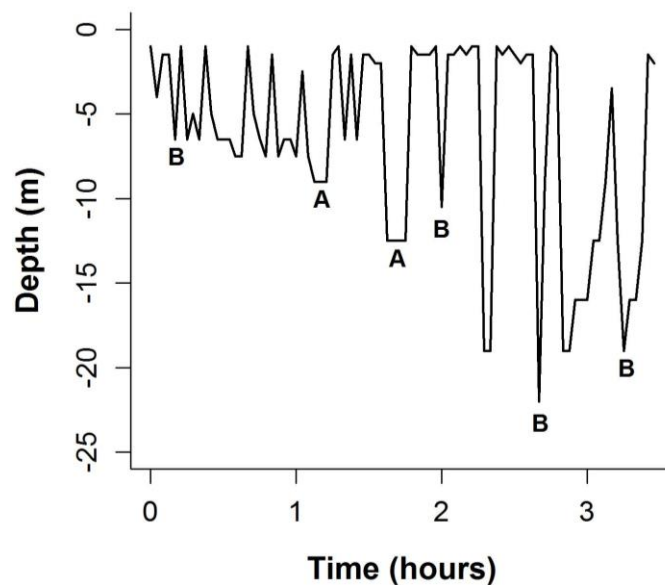
Figure 5: Dive profile of Turtle U during the internesting period. "A" represents a U-shaped dive indicative of resting or foraging and "B" represents a V-shaped dive indicative of exploratory dives.

TURTLE-THREAT OVERLAP AND CONSERVATION MEASURES

Here, using a multi-use human area, we showcase an example where data collected on a marine vertebrate species overlaps with threats in the habitat. We further augment with diving data, a behavioral component, to evidence where bycatch and boat interaction risk might be high and where management interventions may lead to improved conservation outcomes. The current artisanal fishing closure exemplifies conservation boundaries that protect a high-use area during the internesting period, encompassing 79% of the core UD (Figure 2A). The decree boundaries could be expanded on the southern boundary, deeper into the estuary, to fully incorporate the most highly used turtle areas. While this decree protects the sea turtles from artisanal fisheries, it does not eliminate the risk from ship strike. With a major port located in the Komo Estuary, heavy ship traffic occurs throughout the year in addition to coinciding with the internesting period (Figure 2C, Figure 1S). The overlap between the core UD and the transiting ship data in the same time period creates an opportunity for conservation management, such as speed restrictions or designated shipping lanes, during the internesting months.

In 2014, the President of Gabon announced plans to create a network of marine protected areas (MPAs) in Gabonese waters. The planning process is currently underway and habitat utilization data of sea turtles, including olive ridley turtles, is being explicitly incorporated into the planning process. To examine estuary use and inform the process, we created zones similar to those being considered within the planning process (Figure 2B). Our results indicate that the area around the mouth of the estuary, Zone 2, is a critical area in need of conservation strategies. This is the most heavily used nesting beach for olive ridley turtles in this region, hence where most tagging occurred. As a result, close to 45% of the tracking locations occurred within this zone and another 40% in the adjacent zone, Zone 4. With protection of these areas, particularly during the nesting season, over 80% of the turtles' use of the estuary could be encompassed, which is more beneficial than the current park boundaries (Zone 6) encompassing only 0.2% of the locations. This reaffirms the benefit of the new network of marine parks in development. Additionally, dive data indicated that olive ridley turtles spent a large portion of their time on the estuary bottom (Figures 3 and 4). Bottom set gillnets, which make up approximately 30% of gear in the estuary (Metcalfe et al., 2015b), pose a direct threat to sea turtles resting on the bottom and studies have highlighted the depth at which fishing occurs can influence the bycatch rates of sea

turtles (Gilman et al., 2006, Polovina et al., 2003). As turtles must surface to breath, midwater or surface gear also has the potential to result in interactions with turtles.

CONCLUSIONS

While the insights gained from this study greatly improve knowledge on the movement of female olive ridley turtles during the internesting period there are still critical data gaps for this population. We know very little about male olive ridley turtle movement in this area and they could also be at risk from fishing and boat strikes if they aggregate in the area to breed. Coastal waters could be even more of a conservation concern if the males are shown to utilize coastal waters such as zone 2, as has been demonstrated in other regions (James et al., 2005, Shaver et al., 2005, Hays et al., 2010), but this is entirely unknown. Additionally, we are lacking data on the seasonality of males in the region. As they are likely to arrive at breeding grounds prior to females (Plotkin, 2007), they are potentially exposed to threats before the internesting period highlighted in this paper. Understanding this seasonality is critical to ensuring that management measures align with when turtle abundance occurs across sexes. Furthermore, while our data spans three nesting seasons, looking at long-term interannual data could reveal different patterns and habitat use of this area. There may be interannual variability in habitat utilization as well as variability in the distribution of threats such as fishing and shipping. More information on threats would also better aid in designing effective conservation measures. While there is the potential for sea turtle-ship interactions, little is known about how likely these interactions are to occur, or what the impact is on either individuals or populations. More detailed fishing data on the bycatch rate of sea turtles, specifically within the artisanal fisheries, in the estuary or off the coast could guide conservation strategies for restrictions or gear modifications in the area that best suit both the species and fishermen. Though more knowledge is needed, the current data we provide can lead to beneficial conservation strategies for the nesting females from Pongara National Park, Gabon, and provide a framework for studies in other regions.

CHAPTER 2

INVESTIGATING FORAGING HABITAT FOR OLIVE RIDLEY SEA TURTLES (*LEPIDOCHELYS OLIVACEA*) USING STABLE ISOTOPE ANALYSIS AND SATELLITE TELEMETRY.

INTRODUCTION

Satellite telemetry is a vital tool for developing an understanding of sea turtle habitat, behavior, and movement patterns, especially for conservation management. As highly migratory species, sea turtles occupy multiple habitats at varying spatial scales including breeding and internesting habitat, nesting beaches, migratory routes, and foraging habitats. While satellite tracking provides significant insights into these behaviors the technique often does not record the full transition from nesting to post-nesting foraging habitat and back to breeding waters (Hays et al., 2007, Godley et al., 2008).The attachment of satellite transmitters has mostly been completed on female sea turtles while they use nesting beaches due to ease of accessibility and for the potential of recording both the internesting period and migratory route to subsequent foraging grounds (Godley et al., 2008). However, monitoring the entire foraging period and tracking the turtle for more than one breeding cycle (i.e. multiple years) is ideal for identifying distinct foraging habitat and site fidelity. Identification of a discrete area that is used consistently for foraging by a population provides a potential region for the implementation of conservation measures.

Satellite telemetry has advanced the field of marine spatial ecology over several decades but the addition of forensic techniques can supplement this advancement. Isotopes are biogeochemical markers of natural elements that are found in living tissues via trophic enrichment (Rubenstein & Hobson, 2004). Isotopic values normally represent primary producers in an area that have been moved up the food web, which give individuals unique isotopic ratios produced from stable isotope analysis (SIA) based on patterns of consumption and foraging

locations (Post, 2002, Vander Zanden et al., 2015, Seminoff et al., 2012). This technique has been used to determine a species' trophic level (Kurle & Worthy, 2002, Estrada et al., 2003, Layman et al., 2012, Peavey et al., In Review), diet determination (DeNiro & Epstein, 1978, Reich et al., 2007, Cherel et al., 2008, Dodge et al., 2011, Logan et al., 2011, Layman & Allgeier, 2012), morphological studies (Kusche et al., 2014), and for movement ecology (Hobson, 1999, Burton & Koch, 1999). ^{13}Carbon and ^{15}Nitrogen are two of the most common isotopes analyzed because of their accessibility, easy sample preparation, and relative ease of analysis. Carbon ratios (δ^{13}C), the ratio of ^{13}C to ^{12}C in a sample, from marine phytoplankton are driven by changes in temperature, dissolved carbon dioxide levels, and carbon dioxide uptake. Carbon patterns change with inshore-offshore movement as a means of changing depth. Shallower water has higher algal growth with sea grass dominated environments creating higher carbon values than phytoplankton (Vander Zanden et al., 2015). North-south latitudinal movement can also affect carbon ratios (Burton & Koch, 1999, Ceriani et al 2012). Nitrogen ratios (δ^{15}N), the ratio of ^{15}N to ^{14}N in a sample, are influenced by the type of nitrogen source used by the phytoplankton such as nitrogen fixing or denitrification (Vander Zanden et al., 2015) and therefore identifies the trophic position of the consumer (DeNiro & Epstein 1978). For example, the East Pacific has higher baseline nitrogen levels because of the de-nitrification processes by producers that dominates in that basin (Hood et al., 2004, Tucker et al., 2014), where the Northwest Atlantic experiences nitrogen fixation by cyanobacteria in the Sargasso Sea which lowers the relative nitrogen baseline (Tucker et al., 2014).

The type of animal tissue sample gathered for SIA determines the relative time period of insight that might be gained. Metabolically active tissues such as plasma have fast metabolic turnover (hours to days), and therefore only reveal information of recent foraging activities (Rubenstein & Hobson, 2004). Metabolically inert tissues, those with slow growth factors or keratin, capture and store isotopic ratios over extended periods of time (months to years) and do not become replaced with new growth (Rubenstein & Hobson 2004). For example, Ceriani et al. (2012) used red blood cells to determine previous months foraging activity of loggerhead sea turtles (*Caretta caretta*) prior to the migration to the breeding waters and nesting events. Therefore, to investigate pre-nesting foraging behavior and putative regions in the ecosystem, sea turtle epidermis and subdermal tissues are ideal for SIA as they are believed to have a 4-6 month turnover rate (Seminoff et al., 2007).

In the past decade, SIA has proved to be a useful supplement to satellite tracking in determining putative foraging habitats (fish species: Cunjack et al., 2005; pinnipeds: Hückstädt et al., 2012, Baylis et al., 2016; seabirds: Knoche et al., 2007, Jaegar et al., 2010, Votier et al., 2011). To determine foraging area, SIA relies on four assumptions: 1) the isotopic signature in the sample represents the foraging time frame, 2) prey consumption remain relatively similar during the study period, 3) habitat baseline values do not fluctuate, and 4) sea turtles do not feed in multiple areas with different baselines (Tucker et al., 2014). These assumptions have been adopted by other SIA studies involving sea turtles (loggerheads: Hatase et al., 2002, Zbinden et al., 2011, Ceriani et al 2012, Pajuelo et al., 2012a, Pajuelo et al., 2012b Tucker et al 2014, Vander Zanden et al 2015; leatherbacks: Seminoff et al 2012; greens: Hatase et al., 2006), yet each species and geographic population differs in habitat and movement patterns so success of the technique may vary. Additionally, SIA is an attractive option based on its relatively low cost (Seminoff et al., 2012) because it allows a small sample size of tracking data to be supplemented with large datasets of untracked individuals by matching isotopic signatures of tracked individuals with untracked individuals (Vander Zanden et al., 2015, Seminoff et al., 2012, Ceriani et al., 2012). In terrestrial systems, pre-defined isotopic ratios of a region called isoscapes are well defined across continents due to the availability of predictable δ^2H and δ^{18}O gradients from precipitation (Hobson et al., 2010), providing baseline indicators to match isotopic ratios with potential foraging areas. However, in the marine realm tracking data are crucial to this process since there are few baseline samples to create isoscapes. This shortage of isotope baseline references necessitates the use of foraging location provided by the satellite tags (Vander Zanden et al., 2015, Ceriani et al., 2012).

Satellite telemetry and SIA data from olive ridley sea turtles (*Lepidochelys olivacea*) in the East Atlantic were combined to determine if distinct foraging habitats exist or if olive ridley turtles disperse widely throughout the region as found by Pikesley et al. (2013). In this study, satellite telemetry tracks and epidermis samples collected from nesting females in Gabon, Africa, the largest rookery for this species in the Atlantic (Metcalfe et al., 2015), were combined with the objective of identifying the location of distinct foraging habitats for this population. We hypothesize that tracked turtles have distinct isotopic signatures that correlate with foraging areas, and that isotopic signatures from untracked turtles can be used to identify these areas.
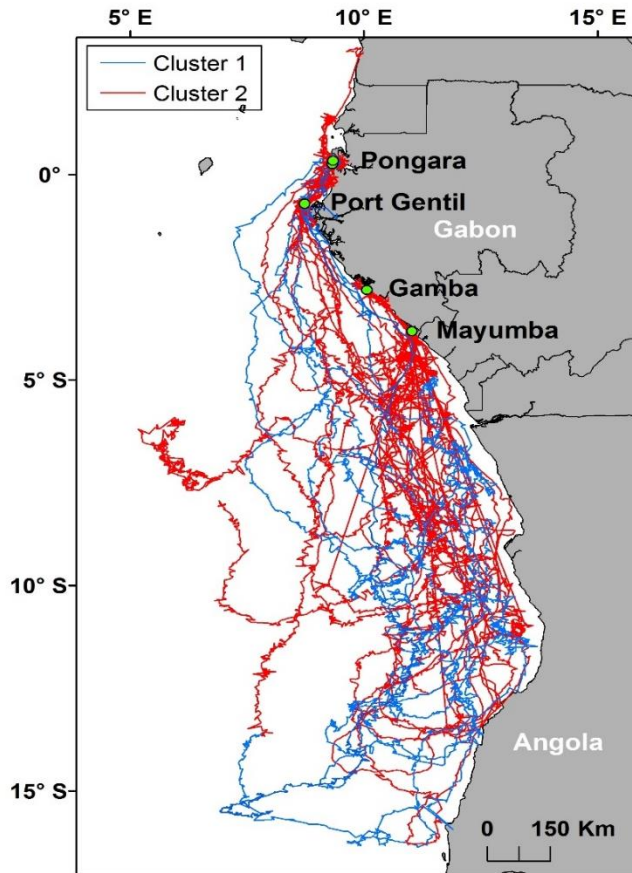
Figure 6*: Satellite tracks for nesting female olive ridley sea turtles (n=38) by cluster assignment. Cluster 1 tracks are blue and cluster 2 are red. Nesting beaches are denoted by green circles with black labels and the countries with white labels.*

METHODS

STUDY SITE AND SAMPLE COLLECTION

Epidermis samples were collected from nesting females along the Gabonese coast during the nesting season, primarily between December and October of respective years. From 2007 to 2015, 149 nesting females were sampled from five nesting beaches along the coast of Gabon.

Starting with the northern most beach, 32 turtles were sampled from Pongara National Park, 3 from Port Gentil, 1 from Liambissi-omboue, 42 from Gamba, and 71 from Mayumba National Park (Figure 6) and satellite transmitters were deployed from each location (Table 1). Samples were taken from the elastic skin on the upper shoulder of the front flipper. The skin and equipment were sterilized using ethanol. Forceps pinched a small skin sample approximately 0.5 cm in length and surgical scissors were used to detach the sample which was stored in ethanol.

From 2008 to 2015, 38 nesting females were fitted with satellite telemetry transmitters (KiwiSat 101, Sirtrack Ltd, Havelock North, New Zealand and Telonics ST20, Model A1010, Mesa, AZ, USA (2007-2008), SPOT-293A, Wildlife Computers, Redmond WA, USA (2012, 2013, 2014), SPLASH10-351C, Wildlife Computers, Redmond WA, USA (2015)). Attachment procedures followed techniques described in Maxwell et al. (2011) and Dawson et al. (In Review) and using one of several epoxy brands depending on availability (Sika Anchorfix 3 epoxy, Lyndhurst, NJ, USA (2007-2008), T308+™ Epoxy Powers Fastners, Brewster NY, USA (2012, 2013, 2014), Superbond 1:1 Expoxy Adhesive, FiberGlass Coatings, Inc., St. Petersburg FL, USA (2015)). Sea turtle tracking data were collected via the Argos Satellite System and managed using STAT (2008, 2012, 2013, 2014, Coyne & Godley, 2005) or Wildlife Computers Portal (2015).

Table 1: Data for each satellite tagged nesting female sea turtle. Data includes turtle name, satellite tag number, cluster assignment, sampling and tagging year, nesting beach where tagging occurred, length of the satellite track, curved carapace length (CCL), and average straight-line foraging distance from the coast.

Turtle	Tag Number	Cluster	Year Sampled	Nesting Beach	Track duration (days)	CCL (cm)	General Foraging Area	Mean Offshore Foraging Dist. (km) *
A	122955	1	2012	Pongara	120	71	Angola	205.65
B	122957	1	2012	Pongara	151	69	Angola	136.45
C	122958	1	2012	Pongara	31	72	Angola	218.05
D	132997	1	2013	Pongara	159	70	Angola	534.65
E	133000	1	2013	Pongara	203	70	Angola	192.25
F	133003	1	2013	Port Gentil	22	67	Angola	--
G	141641	1	2014	Port Gentil	61	66.5	Gabon	--
H	152859	1	2015	Pongara	100	69.5	Angola	416.2
I	13361	2	2008	Mayumba	79	72	Angola	63.4
J	13362	2	2008	Mayumba	276	69	Angola	162.2
K	14750	2	2008	Mayumba	312	70	Angola	240.2
L	23098	2	2008	Mayumba	62	70	Angola	165.35
M	23101	2	2008	Mayumba	230	69	Angola	16.02
N	23102	2	2008	Mayumba	4	69	Angola	--
O	26340	2	2008	Mayumba	170	72	Angola	81.05
P	28600	2	2008	Mayumba	117	70	Angola	205.35
Q	57253	2	2008	Mayumba	110	66	Angola	48.2
R	122956	2	2012	Pongara	90	73	Cameroon	22.5
S	122959	2	2012	Pongara	81	71.5	Angola	188.4
T	122960	2	2012	Pongara	29	68	Angola	263.1
U	122960	2	2012	Pongara	29	68	Angola	263.1
V	132998	2	2013	Pongara	174	73	Angola	189.25
W	132999	2	2013	Pongara	112	73	Angola	60
X	133002	2	2013	Pongara	197	74	Gabon	22.3
Y	133004	2	2013	Port Gentil	99	64	Angola	--
Z	133714	2	2013	Gamba	70	68	Angola	--
AA	133715	2	2013	Gamba	123	72	Angola	--
BB	133716	2	2013	Gamba	134	71	Angola	--
CC	133718	2	2013	Liambissi	53	72.5	Angola	--
DD	141642	2	2014	Gamba	21	75	Gabon	--
EE	141643	2	2014	Gamba	65	69	Angola	--
FF	141644	2	2014	Gamba	28	66	Gabon	--

Table 1 continued

Turtle	Tag Number	Cluster	Year Sampled	Nesting Beach	Track duration (days)	CCL (cm)	General Foraging Area	Mean Offshore Foraging Dist. (km) *
GG	152853	2	2015	Pongara	112	66	Angola	523.5
HH	152854	2	2015	Pongara	43	68.5	Gabon	4.94
II	152855	2	2015	Pongara	96	68	Angola	275.05
JJ	152856	2	2015	Pongara	69	72	Angola	139.9
KK	152857	2	2015	Pongara	48	68	Cameroon	18.9
LL	152861	2	2015	Pongara	43	71	Angola	250.8

*Some tracks were not long enough to determine a foraging area

SAMPLE PREPARATION AND MASS SPECTROMETRY

Each epidermis sample was rinsed with 70% ethanol before being frozen and dried at -90°C for 24 hours (CoolSafe Freeze Dryer, 9 litre, Labogene, Lynge, Denmark). The dried samples were ground using a mortar and pestle and 0.7 mg ($\pm$ 0.1 mg) was folded into a tin capsule for SIA. Skin $\delta^{13}C$ and $\delta^{15}N$ isotope ratios were determined via the elemental analyzer isotope ratio mass spectrometer (EA-IRMS, Sercon Integra 2, Sercon Limited, UK) at the University of Exeter's Environmental Sustainability Institute Stable Isotope Laboratory. The main furnace operated at 1000°C, the reduction furnace at 600°C, and the gas chromatography column at 65°C with the helium carrier at 99.9% purity. Reference samples were included in each run of 48 samples to assess accuracy over time. Stable isotope ratios were presented in the standard format of delta (δ) parts per thousand (‰) as:

$$(1) \qquad \delta X = \left[\left(\frac{R_{sample}}{R_{standard}} \right) 1 \right] x\ 1000$$

where δX represents either ^{15}N or ^{13}C and R represents the isotope ratio of $^{15}N{:}^{14}N$ or $^{13}C{:}^{12}C$.

CLUSTER ANALYSIS

     To determine whether distinct 'groupings' existed within the SI data for nesting female olive ridley turtles we used the "Mclust" package in R (Fraley et al., 2012, Fraley & Raferty, 2012) to produce unique clusters. This method was particularly useful because it does not require a priori groupings and is programed to choose the best model and number of clusters for the data based on maximum likelihood estimation and Bayesian Information Criteria (BIC, Baylis et al., 2016). The cluster classification is based on hierarchical methods for a Gaussian distribution. Data met the assumptions for normality and homogeneity of variance based on a Kolmogorov-Smirnoff test and Levene's test, respectively conducted in R. A linear discriminant function analysis (LDA, "MASS" package in R, Venables & Ripley, 2002) was used on the clusters to test for the accuracy of the cluster model. Additionally, a generalized linear model (GLM) was used to determine the relationship between clusters and $\delta^{15}N$, $\delta^{13}C$, nesting beach location, and sampling year.

     Satellite derived tracks of marine turtle movement gathered during the internesting and post-nesting period from each cluster were visually examined for putative geospatial separation. Additionally, to determine if there was a geospatial relationship between isotopic ratios and foraging location, a second GLM was used determine the relationship between clusters and the tagged turtles based on $\delta^{15}N$, $\delta^{13}C$, and average straight-line distance from foraging location to land. Foraging locations were determined using a Bayesian state-space model (ssm) in the R package "bsam" (Figure 7, Jonsen et al., 2005, Jonsen, 2016), which provides a system of binary behavioral estimates representing either a transiting or foraging state, as described in Dawson et al. (In Review) and Maxwell et al. (2011). Straight-line distances (kilometers) were measured in ArcGIS. Lastly, we used the "Mclust" model and a standard prediction function with non-satellite tagged data as a training set and the satellite tagged values as a test set to determine the predictive accuracy of the cluster model.
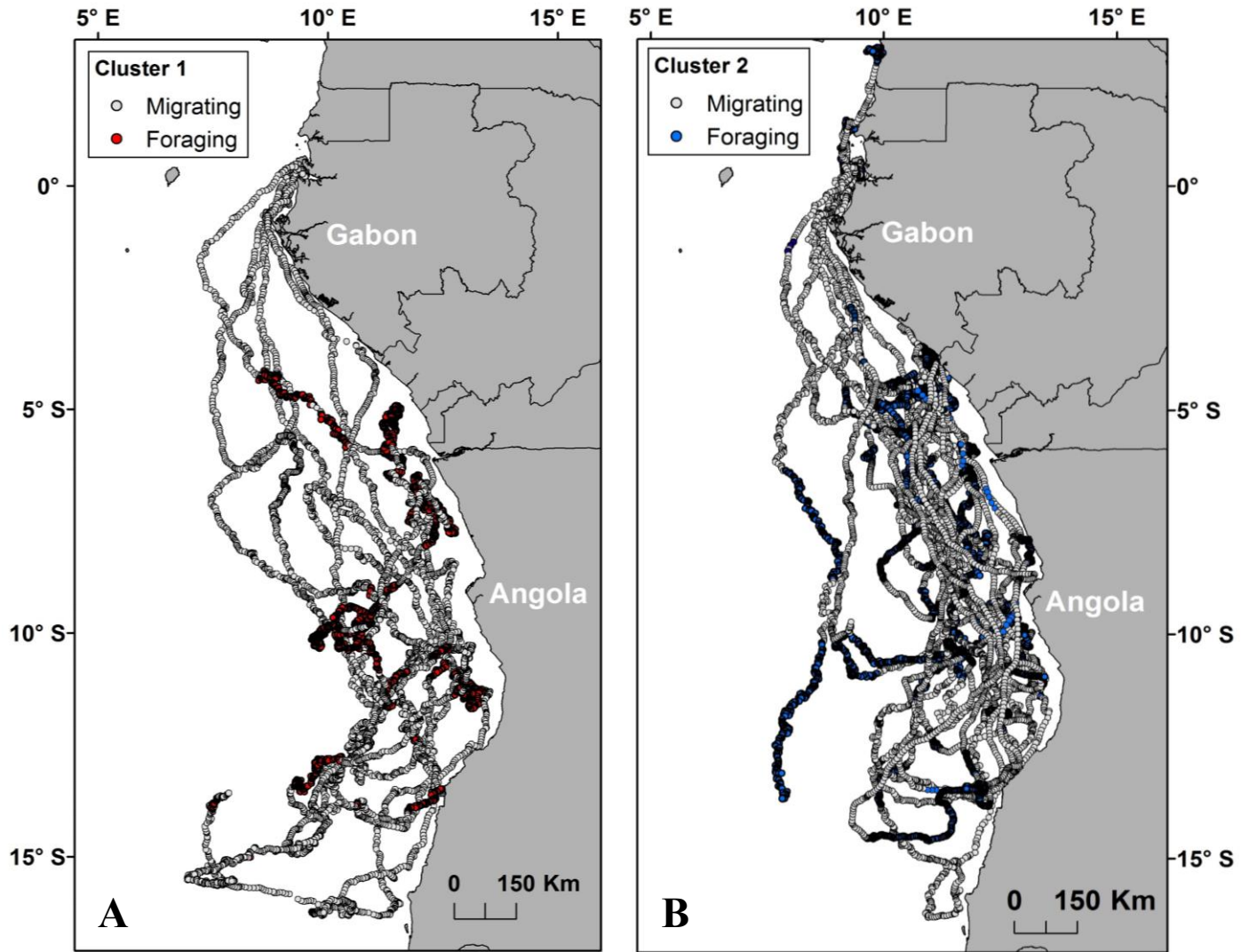
Figure 7: Foraging locations determined by a Bayesian state-space model. **A)** Satellite tracks in Cluster 1 segmented based on behavioral states. The gray points represent migratory movements and the red points represent foraging activity. **B)** Satellite tracks in Cluster 2 segmented based on behavioral states. The gray points represent migratory movements and the blue points represent foraging activity.

RESULTS

STABLE ISOTOPE RATIOS AND SATELLITE TELEMETRY

All 149 epidermis tissue samples from the nesting females were subject to EA-IRMS and $\delta^{13}C$ values ranged from -19.8% to -14.5% (Figure 8A) and $\delta^{15}N$ values from 9.1% to 13.7% (Figure 8B). The average CCL of the sampled turtles was 70.4 cm ($\pm$ 3.2, n=148, min=60, max=79).

The mean tracking duration for the 38 satellite-tracked female olive ridley turtles was 103.2 days ($\pm$72, min= 4, max= 312). Eleven tracking datasets were excluded from the foraging location analysis as they had inadequate data for the SSM, either the track duration was too short or there were gaps in the transmitted data, and therefore the model could not differentiate between behavioral states. This resulted in a total sample of 26 tracked turtles but the excluded turtles were still included in the non-tracked nesting female data set for the remaining analyses. Thirty one sea turtles migrated south towards Angola (Figure 6), initially described in Pikesley et al. (2013) [of which this reports uses 9 turtles], 2 turtles migrated North towards Cameroon, and 5 turtles remained in Gabon. Turtles that remained in Gabon provided short tracking durations (21-61 days), except for turtle X, whose track was 197 days. Of the 26 tracks with viable foraging locations, the average straight-line offshore foraging distance was 181.7 km ($\pm$142.3, min= 4.9, max=534.7).
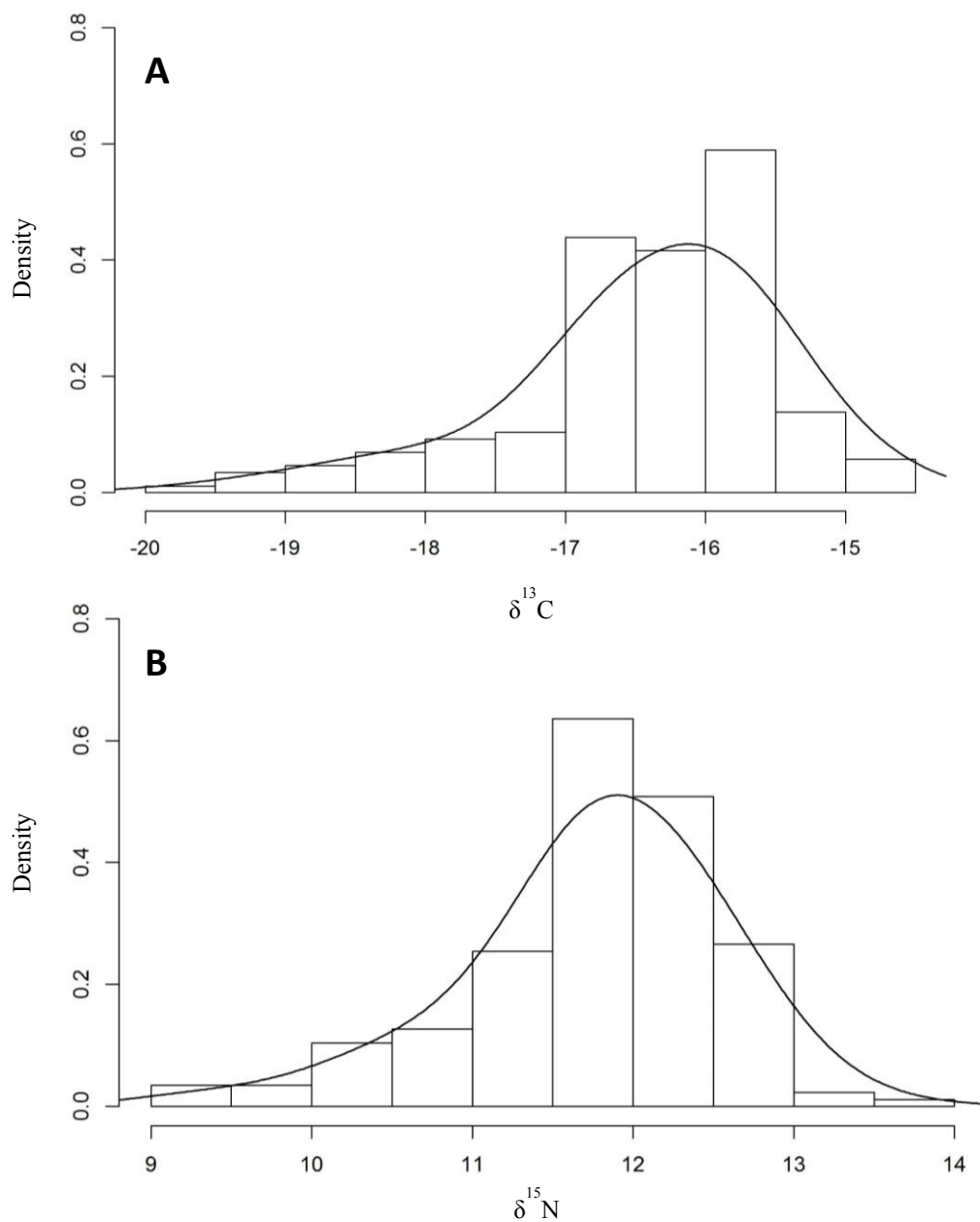
Figure 8: Isotope Density Histograms **A)** Density Histogram of the Carbon isotope ratios for the nesting olive ridley sea turtles. **B)** Density Histogram of the Nitrogen isotope ratios for the nesting olive ridley sea turtles.

CLUSTER ANALYSIS

The results of the Bayesian Information Criteria model in the cluster analysis selected a multivariate mixture vector with a spherical shape and unequal volume (Model VII). The model therefore produced two unequal sized clusters (Figure 9); cluster 1 contained 31 turtles, 8 of which were satellite tagged, and cluster 2 contained 118 turtles, 30 of which were satellite tagged (Figure 6, Table 1). Cluster 1 had an average $\delta^{15}N$ value of 10.86% ($\pm0.98\%$) and range of 9.12% to 13.34%, an average $\delta^{13}C$ value of -17.77% ($\pm0.97\%$) and a range of -19.80% to -15.60% (Table 2). Cluster 2 had an average $\delta^{15}N$ value of 11.98% ($\pm0.57\%$) and range of 10.41% to 13.66%, an average $\delta^{13}C$ value of -16.04% ($\pm0.58\%$) and a range of -17.23% to -14.51% (Table 2). Based on the predictive capabilities of the DFA, which removes one point from the data at a time and re-assigns it to a cluster using the remaining data as a training set, the model was 95.3% accurate at re-assigning turtles, 75.8% accurate for cluster 1 and 100% accurate for cluster 2.
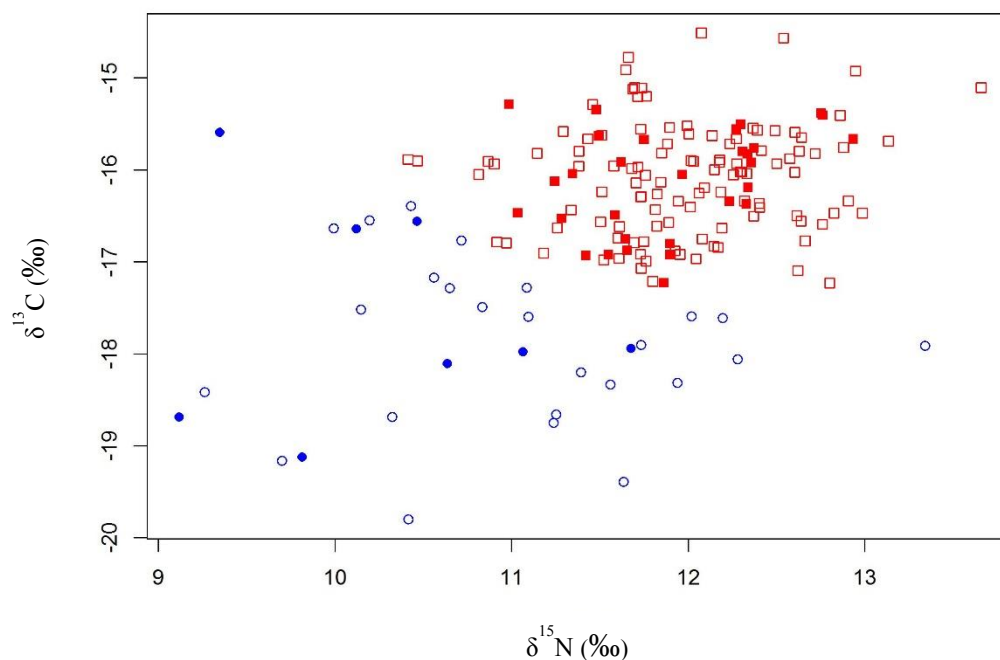
Figure 9: Scatter plot of $\delta^{15}N$ and $\delta^{13}C$ for the 149 olive ridley sea turtles sampled from the nesting beaches of Gabon, Africa. Cluster 1 is denoted by blue circles and cluster 2 by red squares. The filled shapes represent satellite tagged individuals.

Table 2: Cluster values. Data includes the mean $\delta^{15}N$, $\delta^{13}C$, Curved Carapace Length (CCL), and straight-line foraging distance from the coast for each cluster.

	$\delta^{15}N$	$\delta^{13}C$	CCL (cm)	Offshore Foraging Dist. (km)
Cluster 1	10.86% ($\pm$0.98%)	-17.77% ($\pm$0.97%)	70.6 cm ($\pm$ 3.8)	283.9 km ($\pm$155.6)
Cluster 2	11.98% ($\pm$0.57%)	-16.04% ($\pm$0.58%)	70.3 cm ($\pm$ 3.0)	159.9.9 km ($\pm$126.1)

To determine if clusters differed in biological or ecological traits, a GLM was used to investigate isotopic signatures, nesting beach where the turtles were sampled, and sampling year. Only $\delta^{15}N$ (F= 77.76, p < 0.0005) and $\delta^{13}C$ (F=175.13, p < 0.0005) were significantly different between the two clusters, indicating nesting beach and sampling year were not adequate predictors for cluster assignment. Additionally, there was no significant difference in average offshore foraging distance between cluster (GLM, Z = -0.775, p = 0.438). When 111 of the turtle samples (without satellite tags) were used as the training set, the predictive model correctly assigned all satellite tagged turtles (n=38) to their respective clusters. When the reverse was done, using the 38 satellite tagged turtles as the training set, the predicative model initially placed 4 of the satellite tagged turtles in the wrong cluster (89% accurate assignment), due to the satellite tagged turtles having a less encompassing range of isotope values. When 74 of the turtles were randomly selected to be in the training set and predict the assignments of the remaining 75, the model correctly assigned 36 of 37 turtles, incorrectly assigning a single individual.

DISCUSSION

This is the first study to use $\delta^{15}N$ and $\delta^{13}C$ stable isotopes analysis to supplement satellite telemetry data for this East Atlantic olive ridley sea turtle population. The satellite tracks of the nesting female olive ridley sea turtles were indicative of the commonly observed "looping pattern", where they do not necessarily feed in one concentrated area but instead move in loop like patterns until they find places to forage (Figure 6, Pikesley et al., 2013). This may also be representative of opportunistic feeding, which is observed in this species (Beavers & Cassano, 1996, Peavey et al., In Review). Both the looping and opportunistic feeding behavior did not allow for the turtles to be grouped based on coastal or oceanic foraging or any other spatial segregation based on the tracks.

Even though two significant clusters were derived from the $\delta^{15}N$ and $\delta^{13}C$ ratios, the mixture of turtles in each cluster complement the opportunistic feeding behavior. The lower $\delta^{15}N$ and $\delta^{13}C$ values in cluster 1 indicate less enriched waters potentially influenced by nitrogen fixing producers, and lower nutrient levels demonstrated by being overall further offshore. Similarly, the higher $\delta^{15}N$ and $\delta^{13}C$ of cluster 2 represent higher enrichment potentially from de-nitrification via producers and nutrient rich in-shore waters (Peterson & Fry 1987, Graham et al., 2010, Newsome et al., 2010). Also, seasonal and interannual variation in subsurface water temperature and nutrient fluxes occur within the South Atlantic near the olive ridley turtle's foraging areas (Doi et al., 2007). An oceanographic feature, known as the Angola Dome sits off the coast of West Africa. The Angola Dome is made up of two domes, a weaker one at 6°S, 1°E offshore from Gabon and a stronger one at 16.5°S, 10.5°E closer to the Angolan coastline (Doi et al., 2007). Seasonal temperature variation occurs in the upper 120m and though the domes exist throughout the year they have the coldest waters due to increased upwelling from May to September, coincidentally during the olive ridley foraging season. Interannual variation associated with the Atlantic Niño introduces warm water from the Angola Current into waters near the Angola-Namibia border. This influx changes the size of available upwelling zones and olive ridley turtles have been shown to rely on upwelling zones for foraging (Pikesley et al 2013). The interannual variability may mean that some years ideal foraging grounds may span the coast of Angola (~1000+ km North-South, ~ 500km East-West) and in others they may be

limited to smaller coastal regions (~500-1000 km North-South, ~300 km East-West, Doi et al., 2007). Interannual changes in upwelling areas could explain the mixing of wider versus more constrained looping demonstrated by the olive ridley turtles tagged in different years. The inconsistent foraging locations and nutrient levels in the water could clarify the lack of differentiation in the satellite tracks within clusters. Nutrient level variations could also influence the baseline values for the area and therefore influence both $\delta^{15}N$ and $\delta^{13}C$ in the olive ridley turtles.

CONCLUSIONS

In this study, we assumed that the sea turtles exhibit foraging site fidelity and repeated similar foraging behavior once in the area. While repeated tagging has not been done *en mass*, one turtle tagged in 2012 was retagged in 2015 and though tissue for stable isotopes were not collected this second year, it returned to the same foraging region off Angola and displayed "looping" behavior. The satellite tracks observed were collected after epidermic sampling and therefore the isotopic ratios represent the previous foraging season. If sea turtles do not return to similar foraging habitats or feed in the same way year to year, then isotopic discrimination may not be to useful to supplement tracking. Additionally, satellite tracks are not all encompassing of the turtle's movement, and could have missed later foraging locations as tags fail typically prior to females commencing their pre-breeding migrations to key areas.

The lack of isotopic signature across a latitudinal gradient was unexpected because of the inclusion of turtles with mostly northward migrations (turtles R and KK, table 1) and those with southward migrations patterns. The small latitudinal change represented in the data set is either not sufficient to reveal a significant shift in patterns of nitrogen in coastal zones or both foraging areas have similar enrichment levels. Alternatively, foraging site fidelity may be low and these two northward migrating turtles may not have used northern foraging grounds in the year prior to tagging. Another potential issue is that the return migration from foraging grounds to nesting beaches might be too long compared to the turnover rate of the skin samples resulting in mixed samples. Regardless, the satellite tracks highlighted that both costal and offshore waters of Angola are important foraging habitat for this olive ridley sea turtle population (Pikesley et al., 2013), and SIA results reinforce the cosmopolitan nature of this species.

The lack of a clear pattern in the isotopic data may also be a result of laboratory techniques. When working with epidermis samples of sea turtles, some studies have separated the hard keratinized epidermis layer from the softer skin tissue below to further insure isotope ratios correspond to foraging time (Seminoff et al., 2012). For this study, both epidermis layers were used together in the samples due to the small size of the tissues taken and quantifying the effect of using individual layers on the isotopic ratios is not possible. However, the skin samples were not collected using biopsy punch which often results in the collection of deeper tissues (Reich et al., 2007, Vander Zanden et al., 2010), so both layers may have a similar turnover rate and subsequently similar isotopic values.

While there are many successful examples of stable isotope analysis being used to supplement satellite tracking (loggerheads: Hatase et al., 2002, Zbinden et al., 2011, Ceriani et al., 2012, Pajuelo et al., 2012a, Pajuelo et al., 2012b, Tucker et al., 2014, Vander Zanden et al., 2015; leatherbacks: Seminoff et al., 2012; greens: Hatase et al., 2006), this method did not distinguish further spatial separation in foraging sites for this specific population. The olive ridley turtle's opportunistic feeding style, looping foraging pattern, and environmental fluctuations in $\delta^{13}C$ and $\delta^{15}N$ make it difficult to isolate distinct foraging groups or even foraging habitats. Their satellite tracks highlight movement in both coastal and offshore waters eliminating the ability to visually group the turtles and makes explaining cluster assignments in a spatial or ecological context unclear.

CONCLUSION

This research focuses on understanding the habitat use and conservation potential during two life stages of the female olive ridley sea turtles in the East Atlantic. First, females nesting in Pongara National Park, Gabon Africa face threats from the trawl fisheries and commercial shipping lanes in the waters surrounding the nesting beach. We found that during the internesting period, these females intensely use the Komo Estuary, which creates a high overlap between the turtle movement and the above mentioned anthropogenic threats. The concentrated movements within the estuary allow for implementation of marine protected area boundaries and seasonal fishing restrictions in the area, which are currently underway.

After the internesting period the female olive ridleys migrate to another critical habitat, the foraging grounds. For this population, a major foraging location is off the coast of Angola, yet specific areas have not been determined. In an effort to study a larger subset of the population and determine distinct foraging habitats, we used stable isotope analysis to supplement satellite telemetry data. Due to olive ridley turtles being opportunistic feeders, searching for prey sources in a "looping" pattern, and fluctuations in baseline nutrient levels we were not able to determine distinct foraging habitats. This led to the conclusion that the Angolan coast and shelf waters as a whole are a critical foraging habitat for this population.

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APPENDIX 1

Table A1: Satellite Transmitter Deployment Table. "IN" represent the internesting period, "CCL" and "CCW" are the curved carapace length and curved carapace width measurements in cm, respectively, taken before transmitter application.

Year	Tag	Tagging Date	CCL (cm)	CCW (cm)	Days in IN	Deployment Duration (days)	Total Dive Data (hours)	Max Depth (m)	Average Depth (m)
2012	A	10/26/2012	71	71	9	120	--	--	--
	B	10/28/2012	73	72	--	--	--	--	--
	C	10/29/2012	69	69	3	151	--	--	--
	D	10/31/2012	72	71	3	31	--	--	--
	E	11/1/2012	71.5	72	22	81	--	--	--
	F	**(11/5/2012)	68	68	1	29	--	--	--
2013	G	10/13/2013	70	72	20	159	--	--	--
	H	10/13/2013	73	72	20	174	--	--	--
	I	10/12/2013	73	72	6	112	--	--	--
	J	10/12/2013	70	70	16	203	--	--	--
	K	10/13/2013	74	73	16	197	--	--	--
2015	L	10/27/2015	66	69	24	112	221.8	-29	-9.5
	M	10/27/2015	68.5	68	43	43	403.4	-39.5	-8.7
	N	10/28/2015	68	70	22	96	238.7	-32	-8.9
	O	10/29/2015	70	67	4	69	32.8	-26	-11.7
	P	10/29/2015	72	69	24	48	311.1	-50.5	-12.0
	Q	10/29/2015	69.5	69	34	34	194.9	-36.5	-6.6
	R	10/29/2015	70	70	4	100	42.9	-19	-7.5
	S	10/30/2015	68	67	6	184	60.8	-33.5	-8.1
	T	10/30/2015	71	71	2	43	32.0	-19	-7.2
	U	10/30/2015	67	69	32	70	315.1	-44.5	-10.4

** Attachment date for Turtle F was not recorded in situ, date was extracted from tracking data.
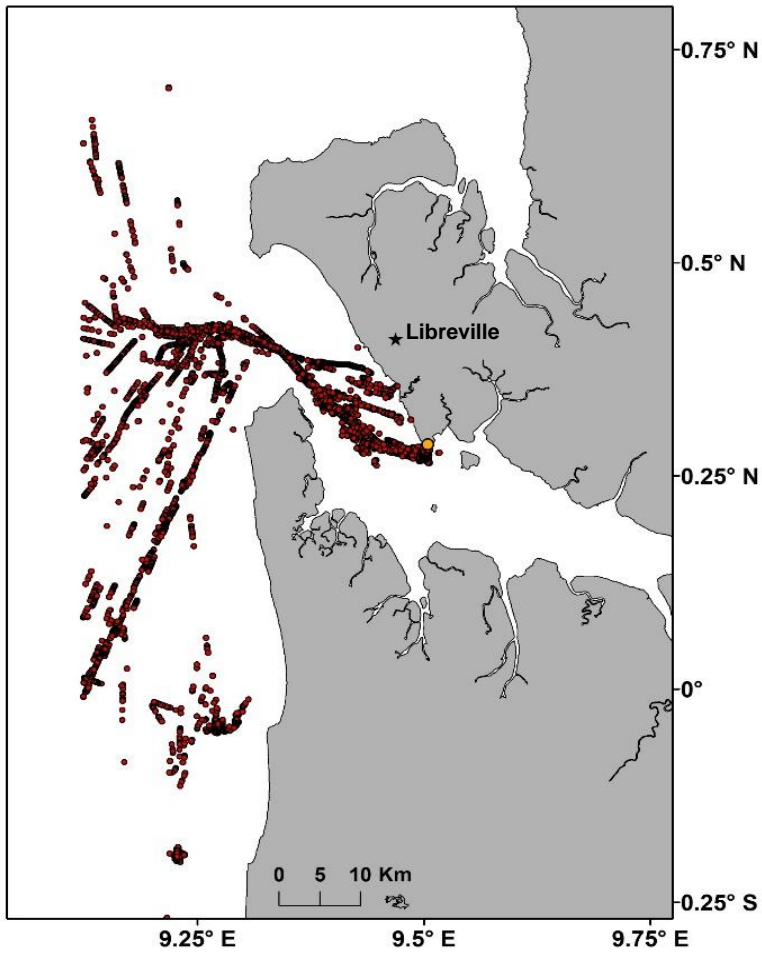
Figure A1: AIS location points of the transiting ships from October 1- November 30, 2012 illustrating the shipping channel within the Komo Estuary. The blue circle represents the Owendo Port, the majoring shipping port in the Komo Estuary.

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Dawson TM, Agamboue PD, Asseko GM, Boussamba F, Cardiec F, Chartrain E, Doherty P, Du Plessis P, Fay JM, Formia A, Godley B, Lambert F, Mabert BDK, Manfoumbi JC, Metcalfe K, Miton G, Ndanga I, Nzegoue J, Oliwina CK, Sounguet GP, Tilley D, Witt MJ, Maxwell SM (In Review). Informing MPAs and gear modifications for a threatened Central West African sea turtle species using satellite tracking. *Frontiers in Marine Science* . (Special Issue on 'Advances in the Biology and Conservation of Marine Turtles').

Maxwell SM, Conners MG, Sisson NB, and **Dawson TM.** (2016). Potential Benefits and Shortcomings of Marine Protected Areas for Small Seabirds Revealed Using Miniature Tags. *Front. Mar. Sci*. 3:264.

Presentations

International Sea Turtle Symposium: Oral Presentation   April 2017
  Las Vegas, Nevada
Biology Graduate Student Organization Symposium: Oral Presentation   April 2017
  Norfolk, Virginia
Animal Nutrition BIOL 395: Guest Lecture   April 2017
  Norfolk, Virginia
4th International Marine Conservation Congress: Poster Presentation   August 2016
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Extracurricular, Honors, and Awards

College of Sciences Outstanding M.S. Thesis Research Award   2017
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Biology Graduate Student Organization (President)   2016-2017
Fellowship of Women in Science (Secretary)   2016-2017
American Academy of Underwater Sciences Certification   April 2016