

MIGRATORY CONNECTIVITY AND CARRY-OVER EFFECTS IN NORTHWEST
ATLANTIC LOGGERHEAD TURTLES (CARETTA CARETTA, L.)

by

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ABSTRACT

Migration is a widespread and complex phenomenon in nature that has fascinated humans for centuries. Connectivity among populations influences their demographics, genetic structure and response to environmental change. Here, I used the loggerhead turtle (*Caretta caretta*, L.) as a study organism to address questions related to migratory connectivity and carry-over effects using satellite telemetry, stable isotope analysis and GIS interpolation methods. Telemetry identified foraging areas previously overlooked for loggerheads nesting in Florida. Next, I validated and evaluated the efficacy of intrinsic markers as a complementary and low cost tool to assign loggerhead foraging regions in the Northwest Atlantic Ocean (NWA), using both a spatially implicit and spatially explicit (isoscapes) approach. I then focused on the nesting beaches and developed a common currency for isotopic studies based on unhatched eggs, which provide a non-invasive and non-destructive method for more extensive sampling to elucidate isotopic patterns across broader spatiotemporal scales. Lastly, I found that intra-population variations in foraging strategies affect annual and long-term reproductive output of loggerheads nesting in Florida. Understanding geospatial linkages is critical to the fostering of appropriate management and conservation strategies for migratory species. My multi-faceted approach contributes to the growing body of literature exploring migratory connectivity and carry-over effects.

To my parents for their unconditional love and support.

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CHAPTER 1: INTRODUCTION

Animal Migration

Migration, the regular seasonal movement of individuals, often from a breeding location to a nonbreeding location and back, is a widespread phenomenon in nature (Webster et al. 2002). Many species travel across thousands of kilometers in regular movements that constitute some of the most spectacular natural phenomena on the planet (e.g. Arctic tern, monarch butterfly, salmon, sea turtles, wildebeests). The study of animal migration is challenging as migratory species often traverse vast distances and are often elusive. Despite the difficulties, identifying linkages between habitats used by migratory species during their lifecycle is necessary to understand their ecology, demography and evolutionary biology. There is an urgency to understand migratory connectivity because it is unknown how imperiled migratory species will respond to threats posed by climate change and habitat loss and degradation (Hobson and Norris 2008), which typically differ between foraging and breeding areas. Nonetheless, our understanding of animal migration has seen tremendous improvements over the last two decades thanks to advances in genetics, stable isotope applications and tracking device technology.

Migrants spend different periods of their annual cycle in widely separated and ecologically different locations. These periods are linked through so-called carry-over effects such as physical condition or date of arrival (Marra et al. 1998, Bearhop et al. 2005, Norris 2005). The idea is that individuals carry-over effects from one season to the next, and that these residual effects can explain important variation in reproductive success and/or annual survival (Webster et al. 2002).

Thus, what we observe at one location is the result of a complex set of interactions occurring over this space and time continuum. To understand the biology of any animal, we need to consider how events in different stages of the lifecycle interact and influence subsequent events at the level of the individual and eventually the population (Webster et al. 2002).

The Loggerhead Turtle (*Caretta caretta*) as a Model Organism to Study Migratory Connectivity in Marine Systems

The loggerhead turtle is a long living, late maturing and highly migratory organism with a complex life cycle where different life stages occupy different ecological environments. Loggerheads typically switch from an initial oceanic juvenile stage to at least one in the neritic zone, where maturity is reached. Breeding migrations are subsequently undertaken every one to four years (Schroeder et al. 2003) between spatially distinct foraging grounds and nesting areas. The loggerhead turtle is classified as endangered by the IUCN Red List (2013) and listed as having 9 distinct population segments (4 of which are threatened and 5 endangered) under the U.S. Endangered Species Act (USFWS & NMFS 2011). My research focuses on the threatened Northwest Atlantic Ocean distinct population segment (NWA DPS). Within the NWA DPS, I focus on the NWA Peninsular Florida Recovery Unit, the largest loggerhead nesting population in the western hemisphere and one of the two largest in the world (Ehrhart et al. 2003). I have chosen the NWA loggerhead turtle as a model organism to study migratory connectivity in the marine realm because its spatial ecology is better understood than many other marine species, making NWA loggerheads good candidate organisms for the development of new methodologies

to study migratory connectivity in marine systems. Moreover, my choice was driven by conservation and management needs. When I started developing my research ideas (spring 2008), Florida's long-term loggerhead nesting trend indicated a 43% reduction from 1999 to 2007 but the reasons for the observed decline in nest numbers were unclear (NMFS & USFWS 2008, TEGW 2009, Witherington et al. 2009). Over the last 6 years (2008-2013) nest numbers have shown a strong increase suggesting a reversal of the post-1998 decline (FWC 2013).

Goals of This Study

I used a combination of satellite telemetry and stable isotope analysis to unravel migratory connectivity and explore carry-over effects in the NWA loggerhead DPS. I first identified key foraging areas used by loggerheads nesting at the Archie Carr National Wildlife Refuge (ACNWR), the largest loggerhead nesting aggregation in the Atlantic Ocean, using a combination of satellite telemetry and stable isotope analysis (Chapter 2, Ceriani et al. 2012). Inferences based on satellite telemetry are limited and may be misleading, as telemetry studies are very expensive and generally based on a small sample size. Thus, I focused on validating and evaluating the efficacy of intrinsic biomarkers as a complementary and low cost tool to assign loggerhead foraging regions in the Northwest Atlantic Ocean. With collaboration, I increased the number of satellite tags deployed and collected tissue samples for stable isotope analysis from tracked and untracked loggerheads at several foraging areas in the Northwestern Atlantic. This allowed me to conduct an external validation of the isotopic approach as a tool to assign foraging region use by adults and large juveniles in the Northwestern Atlantic, using a spatially implicit

framework. Next, I developed loggerhead specific isotopic base maps (isoscaples) to visualize isotopic geographic patterns and explored whether a spatially explicit approach could be used to gain further insight on the ecology of this highly migratory species (Chapter 3, Ceriani et al. in review). In Chapter 4, I investigated the relationship among four tissues that have been used to assign foraging grounds in order to develop a common currency for stable isotope analysis studies on nesting beaches that could allow future meta-analysis aiming to elucidate isotopic patterns across broader spatiotemporal scales (Ceriani et al. in review). In Chapters 2-4, I demonstrate that stable isotope analysis of several slow-turnover rate tissues is a reliable tool to infer foraging areas used during the non-breeding season. In Chapter 5, I used stable isotope analysis to examine the link between foraging ecology and reproductive output in order to investigate carry-over effects on loggerheads nesting at the ACNWR over a six-year period.

The implications of my research extend beyond sea turtle conservation and further the study of migratory connectivity and carry-over effects. This research also contributes to the growing body of literature studying migration and foraging ecology of migratory species using stable isotope analysis of naturally occurring elements. In particular, this series of studies supported the validity of stable isotope analysis to infer origin in the marine realm using a spatially implicit approach and laid the foundation for the use of spatially explicit isotopic methods to assign geographic origin in marine systems. Many marine organisms move across broad geographic areas and are difficult to track with conventional methods (e.g., banding, surveys). Populations of marine predators (e.g., sharks, sea turtles, marine mammals) and most commercially-exploited fish have declined significantly in the last century and the consequences of these declines on marine ecosystems are not fully understood (Baum et al. 2003, Heithaus et al. 2008); thus, there is an

urgency to better understand their spatial ecology and migratory connectivity in order to develop effective conservation strategies.

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CHAPTER 2: INFERRING FORAGING AREAS OF NESTING LOGGERHEAD TURTLES USING SATELLITE TELEMETRY AND STABLE ISOTOPES¹

Introduction

The movement of organisms in space and time defines their interaction with the environment and, thus, constitutes a central aspect of their ecology and evolutionary biology (1). How, where, and when organisms move also defines the array of resources they encounter, the range of threats they experience (predators, environmental conditions, anthropogenic hazards), and the degree to which they interact with other organisms. Migration, the regular seasonal movement of individuals, often from a breeding location to a nonbreeding location and back (2), is widespread in nature. Many species travel across thousands of kilometers in regular movements that constitute some of the most spectacular natural phenomena on the planet (e.g. Arctic tern (3), monarch butterfly (4), salmon (5), sea turtles (6), humpback whales (7)). Migratory connectivity describes the movement of individuals between breeding and nonbreeding areas. For many species the latter areas have not been identified (2).

Conserving migratory species has become a profound issue in the twenty-first century as habitats worldwide are being reduced in size or quality (1) (e.g. Nearctic migrant birds (8), Golden-cheeked Warbler (9), songbirds (10), monarch butterfly (11), salmon (12)). Thus, it is crucial to

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understand key migratory linkages in order to develop appropriate management and conservation measures in a rapidly changing world.

Our understanding of the ecology and evolution of migrating organisms has been impeded by the inability to observe directly their long distant movements. However, recent advances in satellite telemetry, genetic analysis and stable isotope analysis are unraveling geographical origin, movement patterns and foraging behavior of individual organisms. Until recently, tracking migratory animals involved the use of passive extrinsic markers (e.g. banding, patagial tags, numbered neck collars, streamers, flipper tags). In the last decade, stable isotope ratios have been increasingly used as intrinsic markers to trace foraging habits and movements of wildlife populations. Individuals that use geochemically different habitats, or feed on different resources, can be differentiated through use of stable isotope analysis because the isotopic profile of consumers reflects that of their prey in a predictable manner (13). Consumers are typically enriched in $\delta^{15}\text{N}$ relative to their food and, consequently, $\delta^{15}\text{N}$ measurements serve as indicators of a consumer's trophic position (given knowledge of prey species' or baseline $\delta^{15}\text{N}$ values), while $\delta^{13}\text{C}$ values vary little along the food chain and are mainly used to identify location (14-16). Moreover, the timescale over which dietary information is represented by stable isotope ratios (i.e., residence time) varies with tissue type and depends largely upon metabolic turnover (17).

Isotopic signatures may be influenced by diet, habitat type and geographic location. Differences among and within oceanic regions in nutrient cycling at the base of the food web produce geographical gradients in carbon and nitrogen isotope composition (13). For example, both

carbon and nitrogen stable isotope ratios can provide information on foraging latitude because phytoplankton have higher $\delta^{13}\text{C}$ and lower $\delta^{15}\text{N}$ values in temperate than in higher-latitude ecosystems (18). Despite the widespread use of this technique in marine systems, geographic variation in stable isotope ratios at the base of the food web have been described only at very coarse scales (13). Few regional maps of marine isoscapes (spatially explicit regions of stable isotope ratios) are available, thereby limiting the use of isotopic methods in the marine realm. However, another way to interpret the carbon signature of top predators is to calibrate isoscapes using top predators themselves (Pacific humpback whales (7), Pacific bigeye and yellowfin tuna (13), albatrosses (19)).

Loggerhead turtles (*Caretta caretta*, L.) are highly migratory organisms with a complex life cycle. Loggerheads exhibit weak connectivity (*sensu* Webster (2)); that is, individuals at a breeding area may travel to different foraging grounds and individuals at a foraging ground may return to different breeding areas. Only some key foraging grounds have been identified so far using satellite telemetry. In the last decade, stable isotope analysis and satellite tracking have provided insight into loggerhead feeding ecology and migration. Hatase et al. (20) demonstrated that some adult female loggerheads nesting in Japan inhabit oceanic zones rather than neritic habitats, which differs from the accepted life-history model for this species (21). Likewise, McClellan and Read (22, 23) described a behavioral dichotomy among immature loggerheads that alternate between neritic and oceanic habitat. More recently, Zbinden et al. (24) used a combination of satellite telemetry and stable isotope analysis to assign foraging areas of untracked loggerheads nesting in Greece, and Pajuelo et al. (25) used a combination of the two techniques to investigate post-mating destinations of male loggerheads from a breeding

aggregation in Florida. Using stable isotope analysis and epibionts from loggerheads nesting on the east coast of Florida, Reich et al. (26) found a bimodal distribution of $\delta^{13}\text{C}$ that could reflect a bimodal foraging strategy that the authors interpreted as a nearshore/offshore dichotomy or – because of the potential for confusion among four gradients of $\delta^{13}\text{C}$ in marine environments - a polymodal foraging strategy. Reich et al. (26) called for integrated studies in which sufficient numbers of individuals are fitted with satellite transmitters and passive tags and are sampled for stable isotope analysis, epibionts and other biomarkers to evaluate further the foraging strategies and foraging habitats of Florida loggerheads. While there has been extensive tracking effort on loggerheads nesting along the Florida west coast (27, 28) (Tucker unpublished), a paucity of tracking studies has focused on loggerhead nesting on the Florida east coast, despite the fact that the latter accounts for approximately 80% of all the nesting activity in the United States (29). Furthermore, few studies have measured stable isotope ratios in marine megafauna in the western North Atlantic (sharks (30), Atlantic Bluefin tuna (31), leatherback turtles (32), loggerheads (25)).

In this study using a combination of satellite telemetry and stable isotope analysis, we (1) identified key foraging grounds used by female loggerheads nesting in Florida and (2) examined the relationship between stable isotope ratios and the location of nonbreeding foraging areas. This is the first study integrating satellite telemetry and stable isotope analysis to investigate migratory strategies used by loggerhead females in the Atlantic Ocean. If loggerhead isotopic signatures from distinct foraging areas differ significantly, stable isotope analysis may be considered a viable alternative to satellite telemetry for denoting migratory patterns in the NW Atlantic, as found elsewhere (33, 34). Knowledge of foraging grounds and migratory

connectivity for loggerheads in the NW Atlantic is crucial to develop appropriate conservation measures and help managers define and protect loggerhead critical habitat.

Methods

Ethics Statement

The animal use protocol for this research was reviewed and approved by the University of Central Florida Institutional Animal Care and Use Committee (IACUC protocol #09-22W). Procedures were approved under the Florida Fish and Wildlife Conservation Commission (Marine Turtle Permit #025).

Biology and Conservation Status of Loggerhead Turtles

Loggerheads are highly migratory organisms with a complex life cycle in which different life stages occupy different ecological environments. They typically switch from an initial oceanic juvenile stage to one in the neritic zone, where maturity is reached. Breeding migrations are subsequently undertaken every two to three years (21). Loggerheads are largely carnivorous during all life history stages (35, 36). The loggerhead turtle is classified as endangered by the IUCN Red List (37) and listed as 9 distinct population segments (4 of which are threatened and 5 endangered) under the U.S. Endangered Species Act (38) (2011). The Northwest Atlantic Ocean distinct population segment is classified as threatened under the U.S. Endangered Species Act. In

2008, the U.S. National Marine Fisheries Service (NMFS) and the U.S. Fish & Wildlife Service issued a revision of the North West Atlantic (NWA) loggerhead recovery plan. Five Recovery Units (management subunits of a listed species that are geographically or otherwise identifiable and essential to the recovery of the species) have been identified based on genetic differences and a combination of geographic distribution of nesting densities and geographic separation (39). The NWA Peninsular Florida Recovery Unit, which comprises loggerheads nesting from the Florida/Georgia border through Pinellas County (Florida), is the largest loggerhead nesting population in the western hemisphere and one of the two largest in the world (29). Florida's long-term loggerhead nesting trend indicates a nesting decline of 16% from 1998 to 2011 (40) but the reasons for the observed decline in nest numbers are unclear (41). In a recent analysis of nesting trends in Florida, Witherington et al. (42) argued that the reduction in annual nest numbers could be best explained by a decline in the number of adult female loggerheads in the population. Although multiple stressors are likely responsible for the decline in adult females, fishery by-catch ranked first in the analysis of threat factors for adult females (42) and has been identified as a major threat for the recovery of the Northwest Atlantic loggerhead population (43). Only some key foraging grounds for the NWA Florida Peninsular Recovery Unit population have been identified so far using satellite telemetry: the Bahamas, Cuba, the West Coast of Florida, the Yucatán Peninsula of Mexico and the Gulf of Mexico (27, 28, 44). A recent paper on the global priorities for sea turtle conservation in the 21st Century highlights the need to identify key foraging grounds and oceanic hotspots to develop informed management plans for the recovery of the species (45).

Study Site and Sampling

Blood samples were collected for stable isotope analysis from turtles nesting within the 21 km stretch of beach of the Archie Carr National Wildlife Refuge (hereafter Carr NWR) located in southern Brevard County on Florida's east-central coast. This area hosts the most important loggerhead rookery in the western hemisphere and accounts for approximately 25% of all the loggerhead nests in Florida (29). Here, all nesting activity is monitored and a subsample of females is encountered and tagged using both Inconel flipper tags and passive integrated transponders during night surveys. A total of 71 females, 14 of which were equipped with a satellite tag, were included in this study.

Tracking Analysis

Between 2008 and 2010, we attached satellite transmitters (Wildlife Computers MK10-A and MK10 AFB, Redmond, Washington, USA and SIRTRACK KiwiSat 101 K1G 291A, New Zealand) to 14 female loggerheads and tracked their post-nesting migration (Table 2.1). Half of the units were deployed at the beginning of the nesting season on turtles previously marked (with Inconel flipper tags) as part of a different project investigating clutch frequency, movements and foraging activity during the inter-nesting period. The remaining seven tags were deployed at the end of July of each year in collaboration with the Sea Turtle Conservancy, a Florida based non-profit organization. Transmitters were affixed to the turtle's carapace (between the first and second vertebral scute) using two cool-setting two-part epoxies (Power Fast and Sonic Weld). Females were kept in a wooden box during attachment and released at the capture location a few

hours later. Satellite tags were programmed to transmit daily over a 24 h period during the nesting season (beginning of May to end of August) and every other day outside of the nesting season to extend battery life. Service Argos, Inc provided position estimates and associated location accuracy. To reject implausible locations, we employed a customized script in the R package software that was based on a two-stage filtering algorithm (land/sea and Freitas' speed-distance-angle filters (46)). Sea turtle movements were reconstructed by plotting the best location estimate per day of the filtered location data using ArcGIS version 10.0. If two or more high quality locations were received, we only used the first received for that day. Migratory destination was classified as 'oceanic' if a turtle moved off the continental shelf, as defined by the 200 m isobath, or 'neritic' if it remained on the shelf.

To investigate the relationship between foraging areas identified by telemetry and isotopic signatures of female tissues, we calculated average latitude and longitude of foraging grounds. We define foraging ground as the area where an individual loggerhead resides during the nonbreeding season and migration as the movement between foraging areas (if more than one foraging area is used, Figure 2.1A) or between foraging area and nesting area (Figure 2.1A, B). Migration, summer and winter foraging phases were determined by plotting displacement from deployment site (Figure 2.1). Migration was considered to have ended when displacement began to plateau. Likewise summer and winter foraging phases were considered to have ceased when displacement values started to change again (47). To calculate mean latitudes and longitudes of summer and winter foraging areas, we averaged the locations of all filtered data (best estimate/day) from each plateau. If a tag transmitted for more than one year and the individual made multiple seasonal movements (Figure 2.1A: winter 2009-summer 2009-winter 2010-

summer 2010-winter 2011), we averaged all filtered data from the summer plateaus (summer 2009 and 2010) and the winter plateaus (winter 2009, 2010, 2011) in order to obtain a unique latitude and longitude value representing the overall turtle summer and winter foraging area. We then used mean latitude and longitude to calculate the distance to the nearest coastline (distance from shore, km).

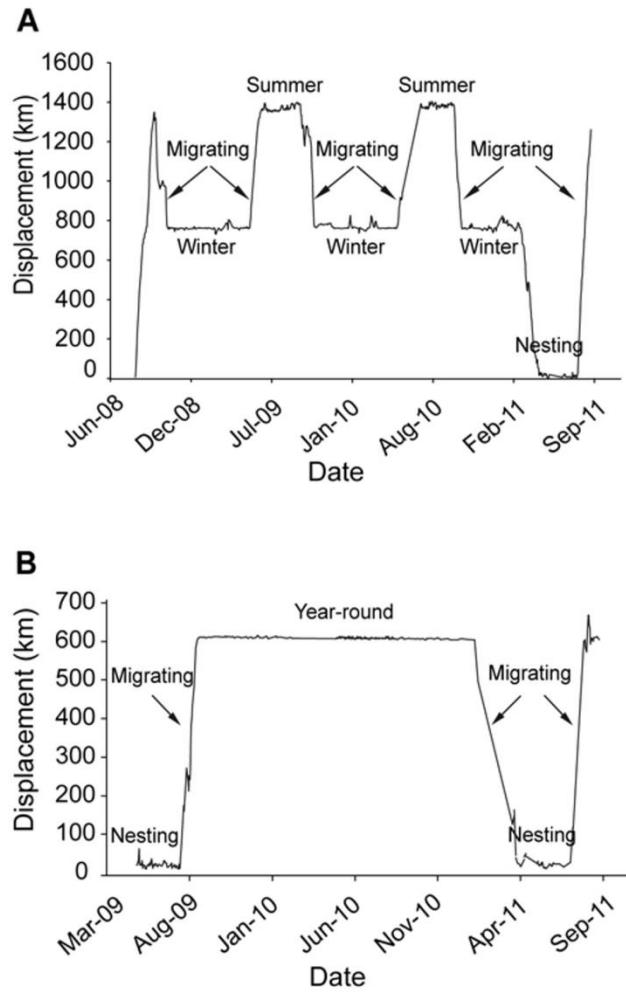


Figure 2.1. Displacement from release site plot of loggerheads equipped with satellite tags. (A) Displacement pattern of a turtle that followed the northern strategy and migrated between summer and winter foraging areas (turtle a, see Table 2.1 for details). Females following the northern strategy moved between summer foraging grounds in the Mid-Atlantic Bight (MAB) off the Delmarva Peninsula and winter foraging grounds located in the waters off North Carolina. (B) Displacement pattern of a turtle that took up year-round residence in the Great Bahamas Bank and did not show seasonal migration (turtle l). Phases of migration are represented by rapid changes in displacement distance; summer, winter and year-round foraging areas can be seen where displacement values plateau.

Stable Isotope Sampling and Analysis

Blood samples (4 ml) were collected from the cervical sinus with a 20-gauge needle and syringe (48) as soon as the turtle began to cover her nest. Blood was transferred to a non-heparanized container and separated into serum and cellular components by centrifugation (5000 rpm x 10 min), then frozen at -20° C until analysis. To address our objectives, we measured the stable isotope ratios of red blood cells (RBC), a tissue assumed to have a long turnover rate that should reflect an integration of diet and habitat at the foraging ground prior to breeding migration.

Tissue turnover rate for RBCs in adult sea turtles is unknown but it has been estimated to reflect the foraging habits of the 4-7 months prior sampling (49, 50) (Ceriani et al. unpublished). We assumed females exhibit site fidelity to foraging grounds (pre-nesting foraging area = post-nesting foraging area). This assumption is commonly used in studies combining telemetry and stable isotope analysis (20, 24, 51, 52) and is supported by the data available for individual marine turtles that have been equipped repeatedly with satellite tags (47, 53, 54) and by long-term studies at foraging grounds (55). Recently, site fidelity in female loggerheads has been indicated by the long-term consistency in isotopic signatures of scute layers, a tissue that incorporates several years of dietary history and habitat use (54). Moreover, if our analysis finds concordance among individual turtle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ groupings and distinct post-nesting migratory destinations, our study will provide further evidence supporting foraging ground philopatry in most adult loggerhead females.

Sample preparation was done at the Biology Department of the University of Central Florida. Samples were prepared following standard procedure. RBC samples were freeze-dried for 48 h before being homogenized with mortar and pestle. Lipids were removed using a Soxhlet apparatus with petroleum ether as solvent for 12 h. Approximately 0.5 mg of each sample was weighed and sealed in tin capsules. Prepared samples were sent to the Stable Isotope Core Laboratory at Washington State University, where they were converted to N₂ and CO₂ with an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA) and analyzed with a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen). Isotopic reference materials were interspersed with samples for calibration. Stable isotope ratios were expressed in conventional notation as parts per thousand (‰) according to the following equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where X is ¹⁵N or ¹³C, and R is the corresponding ratio ¹⁵N:¹⁴N or ¹³C:¹²C. The standards used for ¹⁵N and ¹³C were atmospheric nitrogen and Peedee Belemnite, respectively. Precision was 0.07‰ for δ¹³C measurements and 0.11‰ for δ¹⁵N.

Statistical Analysis

Relationships between δ¹³C and δ¹⁵N and mean latitude of foraging ground and distance from shore were explored through multiple regression analysis. Akaike's Information Criteria (AIC) was used to determine the best fitting regression (56). We included distance from shore in the multiple regression analysis to take into account differences in coastline shape and female differential use of the continental shelf (inner, mid or outer shelf). Because some females

undertook a seasonal migration and it is unknown whether RBC isotopic signatures reflect the diet and geographic location occupied during the summer or winter months, we performed two distinct multiple regression analyses. In one we used mean latitude and distance from shore of summer areas identified from telemetry, while in the other we used mean latitude and distance from shore of winter areas. The remaining females did not exhibit a seasonal migration and, therefore, we calculated only one average latitude and distance from shore.

To test for significant differences in isotopic signatures among foraging areas, we used multivariate analysis of variance (MANOVA) with the Pillai's trace test. Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnov and Levene's test, respectively. Data were normal but did not meet the equal variance assumption even after transformation. We chose the Pillai's trace test because it is the most robust of the tests when the assumption of similar-covariance matrix is not met (57). We used post hoc Games-Howell (GH) multiple comparison tests (which assumes unequal variance) to identify groups responsible for statistical differences (58). We used discriminant function analysis (DFA) to examine how well $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ predict the post-nesting foraging grounds used by loggerheads. We used $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the 14 females equipped with satellite tags as training data set (with equal priors for the classification) to develop the discriminant functions and the untracked turtles as test data set for the discriminant classification. Untracked turtles are defined as females that were sampled for stable isotope analysis but that were not equipped with satellite tags. Data were analyzed using program R (R Development Core Team 2009), SPSS v. 19, Sigma Plot 10.0 and ArcGIS 10.0. Alpha level was set to 0.05 for all statistical analyses.

Results

Satellite Telemetry & Post-nesting Migration Destinations

Loggerheads moved across a wide range of latitudes spanning from the Great Bahamas Bank (23°N) to the offshore waters of Virginia and Delaware (38.6°N). Satellite telemetry identified three migratory pathways and associated foraging grounds (Figure 2.2): (1) a seasonal shelf-constrained North-South migratory pattern between waters offshore Virginia/Delaware and North Carolina (along the NE USA coastline), (2) a year-round residency in southern foraging grounds (Bahamas and SE Gulf of Mexico) and (3) a residency in the waters adjacent to the breeding area (eastern central Florida). We classified female loggerheads into three migratory strategies according to whether they migrated “north” (northern), “south” (southern) or stayed in central Florida (resident or central) and will follow this classification hereafter. Migratory destinations of the 14 females were classified as “neritic” since all individuals took up residency within the limits of the continental shelf (water depth < 200 m).

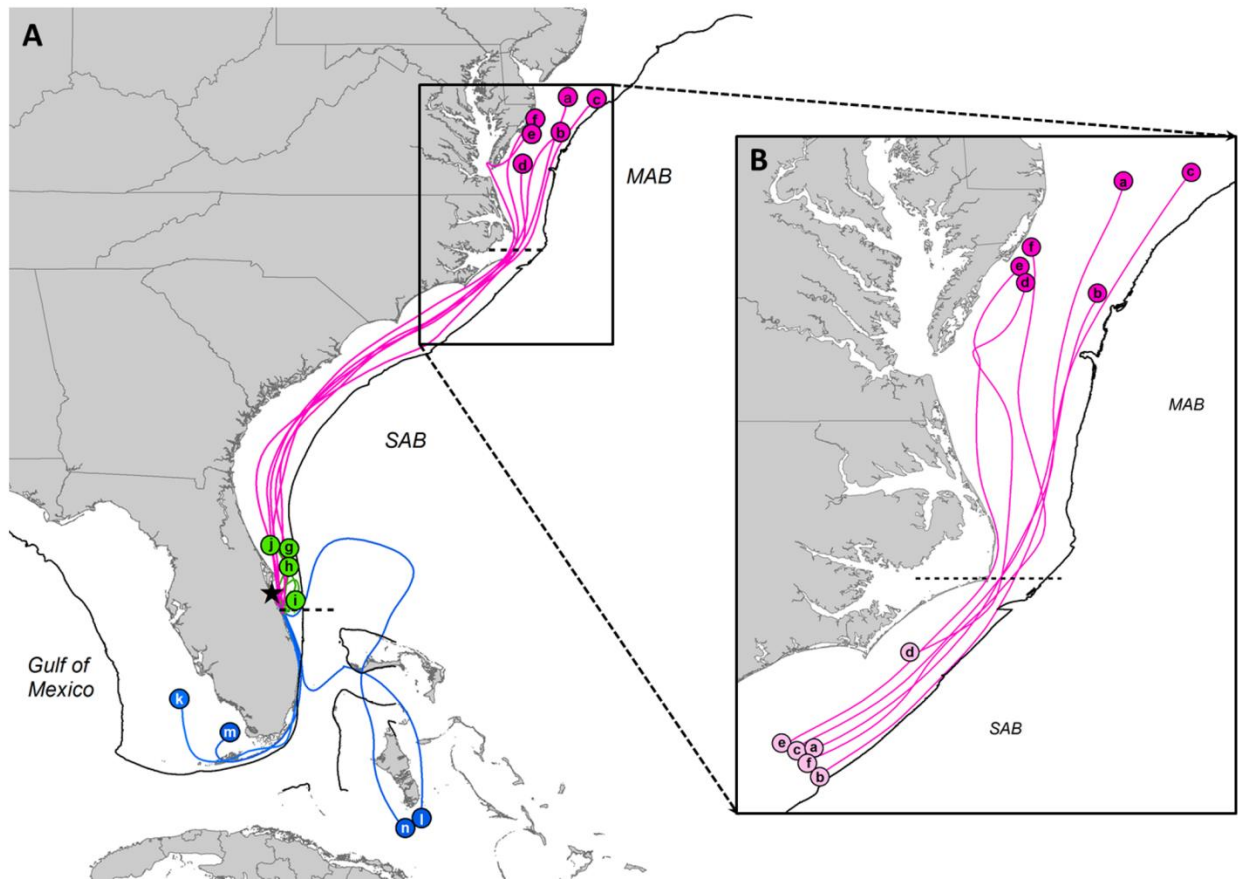


Figure 2.2. Reconstructed satellite tracks ($n = 14$) of loggerheads tagged after nesting at the Carr NWR. (A) Reconstructed route (pink, green and blue lines) to foraging areas (labeled circles) for individuals *a* to *n* from release location (black star). Loggerheads were classified into three migratory groups: northern (*a* to *f*), central Florida resident (*g* to *j*) and southern (*k* to *n*). Pink, green and blue reconstructed routes represent northern, resident and southern migratory groups, respectively. (B) Reconstructed route (pink lines) from summer foraging areas (darker pink-labeled circles) to wintering areas (lighter pink-labeled circles) for individuals that followed the northern strategy (*a* to *f*). The 200 m isobath is delineated (black line). Dotted line separates Mid-Atlantic Bight (MAB) and South-Atlantic Bight (SAB). A bight is defined as a long, gradual bend or recess in the coastline that forms a large, open bay. The MAB is defined as the region enclosed by the coastline from Cape Cod (MA), to Cape Hatteras (NC). The SAB extends from Cape Hatteras (NC) to West Palm Beach (FL).

At the end of the nesting season, six individuals departed eastern central Florida and migrated north to seasonal foraging grounds above 35°N in the Mid-Atlantic Bight where they spent the rest of the summer and beginning of fall (Figure 2.2A). By the end of October, these six

individuals left summer feeding areas and migrated south toward winter grounds located in North Carolina between Cape Hatteras and Wilmington where they stayed until the beginning of May (Figure 2.2B). Three of these six females, whose tracking lasted more than 1 year, exhibited the same seasonal displacement among years (Figure 2.1A, Figure S2.1). Four females that were equipped with tags at the end of the nesting season (Table 2.1, individuals g-j) did not leave the area of eastern central Florida but remained in the waters off Cape Canaveral (Figure 2.2A, Figure S2.2). Tracking data for these 4 individuals were limited since tags failed between 2 and 7 months from deployment. However, females that undertook long-distance post-nesting migrations (all but individuals g-j in Table 2.1) left the breeding area by mid-August, immediately after laying the last nest of the season, and traveled a minimum of 288 km during the first two months after deployment (northern: $1205 \text{ km} \pm 121 \text{ km}$; southern: $458 \text{ km} \pm 171 \text{ km}$). Therefore, since these 4 loggerheads did not lay additional clutches and did not depart from the area (displacement after 2 months at large: $89 \text{ km} \pm 52 \text{ km}$), we assumed eastern central Florida to be their final destination. The remaining 4 females headed to subtropical northwest Atlantic and southeast Gulf of Mexico foraging areas where they remained year-round until the next breeding migration (Figure 2.1B, Figure 2.2A, Figure S2.3). Two females took up year-round residency in the Great Bahamas Bank, just south of the Bahamian island of Andros, one female dwelled in the shallow waters of the Gulf of Mexico immediately west of the Florida Keys, while the last individual resided in the SE Gulf of Mexico off the SW Florida coast. Even though loggerheads that migrated south used two geographic regions (the Bahamas Great Bank vs. the Gulf of Mexico) with distinctive oceanographic regimes, we refrained from splitting the southern aggregation due to the small sample size of loggerheads equipped with satellite tags.

Table 2.1. Information on satellite tracking and foraging area of choice of 14 satellite-tracked loggerheads.

Turtle ID	PTT deployment date	Tracking duration (d)	Date of last location	Foraging area	PTT type
a	31 July 2008	1397	28 May 2012	North (MAB)	KiwiSat 101
b	05 May 2009	873	30 Sept 2011	North (MAB)	Mk10-AFB
c	12 May 2009	530	21 Oct 2010	North (MAB)	Mk10-AFB
d	19 May 2010	188	23 Nov 2010	North (MAB)	Mk10-A
e	19 May 2010	286	1 March 2011	North (MAB)	Mk10-A
f	20 May 2010	380	4 June 2011	North (MAB)	Mk10-A
g	1 Aug 2009	60	30 Sept 2009	Central (SAB)	KiwiSat 101
h	1 Aug 2010	204	21 Feb 2011	Central (SAB)	Mk10-A
i	31 Jul 2010	127	7 Dec 2010	Central (SAB)	Mk10-A
j	31 Jul 2010	90	29 Oct 2010	Central (SAB)	KiwiSat 101
k	31 Jul 2008	795	16 Feb 2011	South (SE GoM)	KiwiSat 101
l	21 May 2009	932	9 Dec 2011	South (Bahamas)	Mk10-AFB
m	29 May 2009	478	19 Sept 2010	South (FL Keys)	Mk10-AFB
n	30 July 2009	378	12 Aug 2010	South (Bahamas)	KiwiSat 101

Abbreviations are as follow: platform terminal transmitter (PTT), day (d), Mid-Atlantic Bight (MAB), South-Atlantic Bight (SAB), South East Gulf of Mexico (SE GoM), Florida Keys (FL Keys).

Geographic Variability in Stable Isotope Ratios

The $\delta^{13}\text{C}$ values of RBCs from tracked female loggerheads ranged from -17.50 ‰ to -10.48 ‰, and $\delta^{15}\text{N}$ varied between 5.46 ‰ and 14.00 ‰. The multiple regression analysis and AIC model selection revealed that average latitude alone was the best predictor of $\delta^{13}\text{C}$ values in female tissues for both winter (Table 2.2) and summer (Table 2.3) feeding areas. $\delta^{13}\text{C}$ decreased significantly with increasing latitude for both winter feeding areas ($F_{1,12} = 75.04$, $r^2 = 0.862$, $p < 0.001$, Figure 2.3A) and summer feeding areas ($F_{1,12} = 46.13$, $r^2 = 0.794$, $p < 0.001$). Likewise, winter feeding area latitude was the best explanatory variable for $\delta^{15}\text{N}$ ($F_{1,12} = 23.01$, $r^2 = 0.657$, $p < 0.001$; Figure 2.3B), while the additive model of latitude and distance from shore explained the relationship better than latitude alone with regard to summer feeding areas ($F_{1,12} = 21.96$, Adjusted $r^2 = 0.763$, $p < 0.001$)

Table 2.2. Comparison of linear regression models describing the relationship between RBC $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and geographic location of winter non-breeding foraging areas for the 14 loggerheads fitted with satellite tags.

	Model variables	R^2	Adj. R^2	RSS	N	K	AIC_c	ΔAIC_c	AICc Weights	P
$\delta^{13}\text{C}$	lat	0.862	0.851	0.797	14	3	-31.7	0	0.885	<0.0001
	lat + dist shore	0.870	0.846	0.808	14	4	-27.5	4.2	0.106	
	lat * dist shore	0.877	0.840	0.825	14	5	-22.1	9.6	0.007	
	dist shore	0.121	0.048	2.013	14	3	-18.8	13.0	0.001	
$\delta^{15}\text{N}$	lat	0.657	0.629	1.617	14	3	-21.8	0.0	0.818	0.0004
	lat + dist shore	0.714	0.662	1.543	14	4	-18.4	3.4	0.150	
	dist shore	0.026	-0.055	2.726	14	3	-14.5	7.3	0.021	
	lat * dist shore	0.732	0.652	1.566	14	5	-13.2	8.7	0.011	

Model selection used Akaike's Information Criterion, corrected for small sample sizes (AIC_c). Abbreviations are as follow: RSS = residual sum of squares, N = number of observations, K = number of parameters, ΔAIC_c = difference between each model and the best model, AIC_c weight = relative information content, P = probability associated with the best model, lat = average latitude of foraging ground based on tracking data, dist shore = distance from shore (in km) calculated from the point having as coordinates average latitude and longitude of foraging ground, lat * dist shore = lat + dist shore + lat * dist shore.

Table 2.3. Comparison of linear regression models describing the relationship between RBC $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and geographic location of summer non-breeding foraging areas for the 14 loggerheads fitted with satellite tags.

	Model variables	R²	Adj.R²	RSS	N	K	AIC_c	Δ AIC_c	AIC_c Weights	P
δ¹³C	lat	0.794	0.776	0.976	14	3	-28.9	0	0.884	<0.0001
	lat + dist shore	0.804	0.768	0.994	14	4	-24.6	4.3	0.103	
	lat * dist shore	0.826	0.774	0.981	14	5	-19.7	9.2	0.009	
	dist shore	0.026	-0.055	2.119	14	3	-18.0	10.9	0.004	
δ¹⁵N	lat + dist shore	0.800	0.763	1.291	14	4	-20.9	0.0	0.551	0.0001
	lat	0.549	0.511	1.855	14	3	-19.9	1.0	0.329	
	dist shore	0.304	0.246	2.304	14	3	-16.9	4.1	0.072	
	lat * dist shore	0.823	0.769	1.275	14	5	-16.0	4.9	0.048	

Model selection used Akaike's Information Criterion, corrected for small sample sizes (AIC_c). Abbreviations are as follow: RSS = residual sum of squares, N = number of observations, K = number of parameters, ΔAIC_c = difference between each model and the best model, AIC_c weight = relative information content, P = probability associated with the best model, lat = average latitude of foraging ground based on tracking data, dist shore = distance from shore (in km) calculated from the point having as coordinates average latitude and longitude of foraging ground, lat * dist shore = lat + dist shore + lat * dist shore.

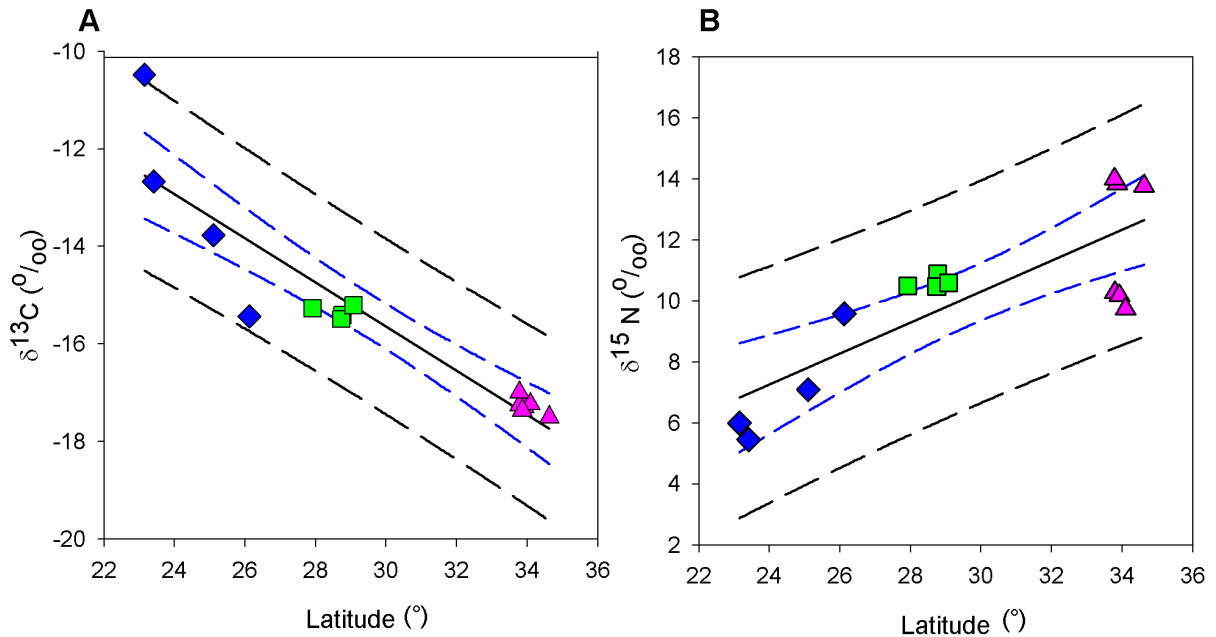


Figure 2.3. Relationship between RBC stable isotope ratios and post-nesting foraging ground location. RBC $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) values of satellite-tracked adult female loggerheads ($n = 14$) versus mean latitudes of winter foraging areas calculated based on satellite telemetry. Blue diamonds represent individuals migrating to southern foraging grounds (southern), green squares females residing in eastern central Florida (resident) and pink triangles females that migrated to northern foraging areas (northern). Only northern loggerheads undertook seasonal migration between winter and summer foraging ground. In the case of northern females, the latitude plotted represents the average latitude of the winter foraging area for each individual. The remaining eight females did not show seasonal movement; therefore, the latitude plotted represents the average latitude of the year-round foraging area. Dashed blue and black lines indicate 95% confidence and predictive interval (respectively) for the regression analysis.

Females from the three foraging areas segregated by their overall isotopic signatures

(MANOVA, Pillai's trace test, $F_{4, 22} = 4.147$, $p = 0.012$) and, in univariate analysis, both $\delta^{13}\text{C}$ (ANOVA, $F_{2, 11} = 17.695$, $p < 0.001$) and $\delta^{15}\text{N}$ values ($F_{2, 11} = 10.217$, $p = 0.003$) differed among foraging aggregations (Figure 2.4). Mean $\delta^{13}\text{C}$ values per group varied from $-17.27 \pm 0.17\%$ in females using northern foraging areas to $-13.09 \pm 2.08\%$ in southern individuals. $\delta^{15}\text{N}$ values ranged from $11.97 \pm 2.09\%$ (northern females) to $7.04 \pm 1.83\%$ (southern females). Individuals

residing in eastern central Florida exhibited intermediate values between northern and southern loggerheads in both $\delta^{13}\text{C}$ ($-15.35 \pm 0.13\text{‰}$) and $\delta^{15}\text{N}$ ($10.62 \pm 0.19\text{‰}$). Post hoc Games-Howell (GH) multiple comparison tests indicated that the northern aggregation $\delta^{13}\text{C}$ differed significantly from the resident aggregation ($p < 0.001$) and marginally from the southern ($p = 0.054$), while resident and southern aggregations did not differ from each other in $\delta^{13}\text{C}$ ($p = 0.222$). $\delta^{15}\text{N}$ signatures of loggerheads using southern foraging areas differed significantly from the northern aggregation ($p = 0.013$) and marginally from the resident ($p = 0.058$) group, while northern and resident aggregations did not differ from each other in $\delta^{15}\text{N}$ ($p = 0.336$).

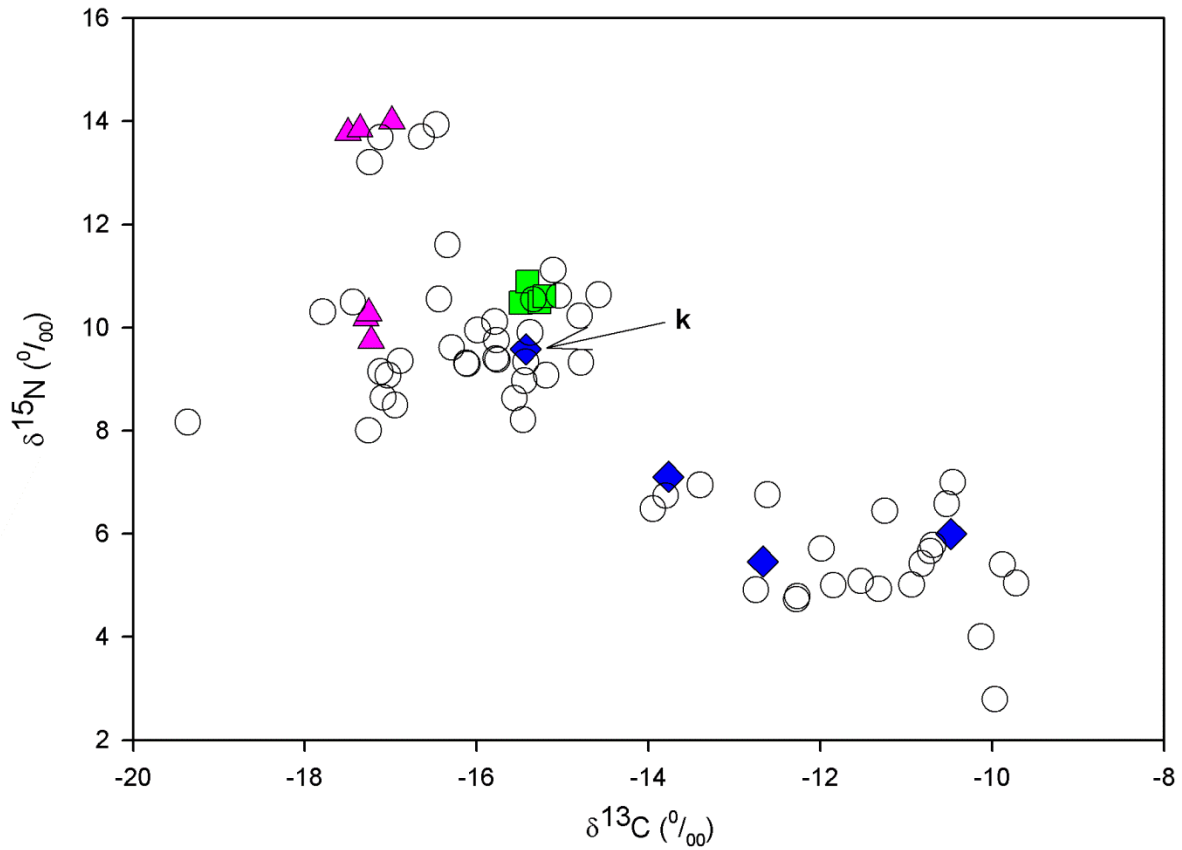


Figure 2.4. Scatterplot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the 71 nesting loggerhead turtles sampled at the Carr NWR, Florida (USA). Pink triangles represent females equipped with satellite tags that migrated to northern foraging areas, green squares those foraging in eastern central Florida, blue diamonds those foraging in the south, while empty circles represent untracked females. The arrow indicates turtle “k”, which foraged in the SE Gulf of Mexico. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of this individual were extremely similar to the ones found in eastern central Florida residents, while the average latitude of the foraging ground used by this female for almost two years was intermediate between residents and the other southern individuals. RBC stable isotope ratios of untracked females ($n = 57$) have a similar distribution pattern to the 14 satellite-tracked loggerheads.

Assignment of Untracked Females to Foraging Areas

The discriminant analysis of the training data set (14 loggerheads equipped with satellite tags) was significant ($P > \text{Wilks' Lambda} < 0.002$). Two discriminant functions were calculated, with a combined $X^2(4) = 16.785$, $p = 0.002$. After removal of the first function, the association between groups (foraging areas) and predictors ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) became not significant $X^2(1) = 0.867$, $p = 0.352$. The first discriminant function accounted for 97.6% of the between-group variability. Overall the discriminant analysis of the training data set was able to correctly classify the foraging ground used for all but one individual (92.9% of original grouped cases correctly classified). The only misclassified loggerhead was assigned to the resident aggregation, while satellite telemetry indicated this loggerhead belonged to the southern aggregation as it migrated to the SE Gulf of Mexico. The stability of the classification procedure was checked by a leave-one-out cross validation, which classified 92.9% of the test data set correctly. In the untracked females, RBC $\delta^{13}\text{C}$ ranged from -19.36 ‰ to -9.72 ‰ and $\delta^{15}\text{N}$ varied between 2.79 ‰ and 14.00 ‰. Putative foraging ground was predicted for 57 untracked turtles in the test data set and was based on the above classification functions. The discrimination analysis assigned 15 of the 57 untracked individuals (26.3%) to the northern aggregation, 20 females (35.1%) to the resident group and 22 females (38.6%) to the southern aggregation (Figure 2.5, Table 2.4). When we considered the entire dataset ($n = 71$), the relative importance of the three foraging areas remains similar with 21 females considered northern (29.6% of all females), 24 resident (33.8% of all females) and 26 southern (36.6% of all females).

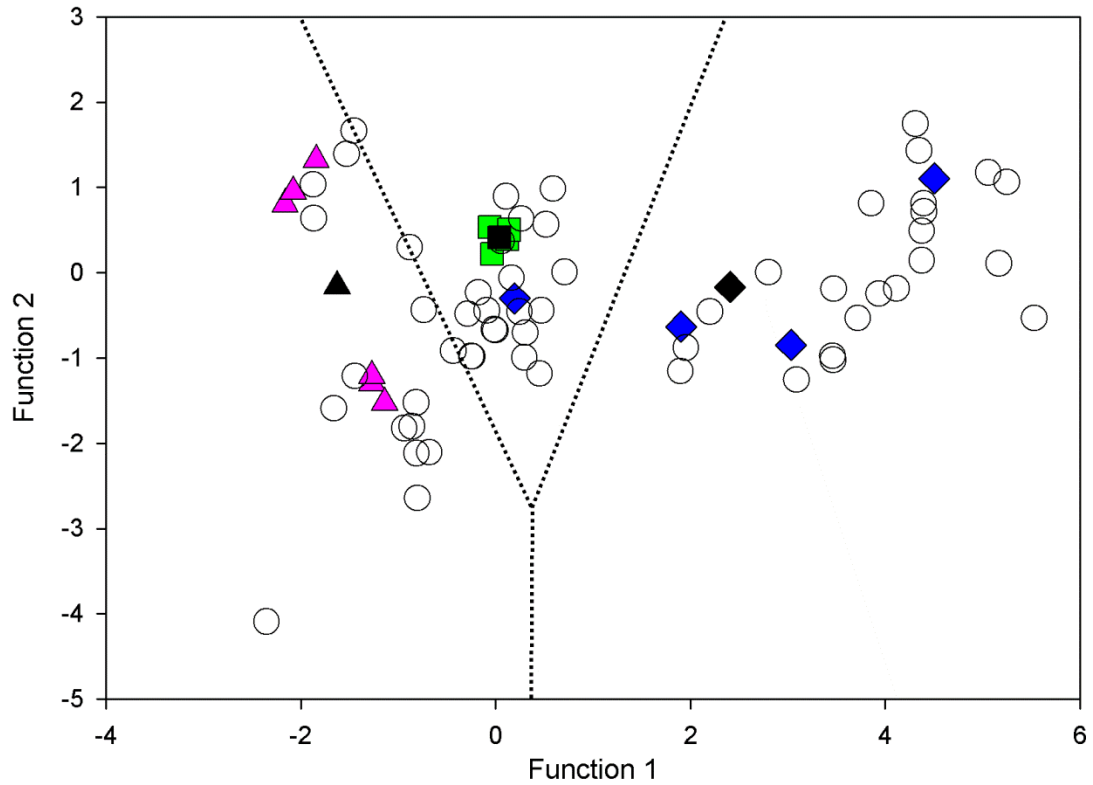


Figure 2.5. Discriminant function analysis (DFA) of foraging groups based on the stable isotope ratios. Function 1 accounted for 97.6% of the between-group variability. Pink triangles represent females equipped with satellite tags that migrated to northern foraging areas, green squares those foraging in eastern central Florida and blue diamonds those foraging in the south. Black markers represent the centroids for the respective foraging groups. Empty circles represent untracked females. Dotted lines define the three DFA territories.

Table 2.4. Foraging ground assignment (number and %) for the discriminant model based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of loggerhead RBCs.

Group		Predicted Group Membership			Total
		Northern	Central	Southern	
Training data (n=14)	Northern	6 (100%)	0 (0%)	0 (0%)	6
	Central	0 (0%)	4 (100%)	0 (0%)	4
	Southern	0 (0%)	1 (25%)	3 (75%)	4
Test data (n=57)	Untracked	15 (26.3%)	20 (35.1%)	22 (38.6%)	57
	Total	21	25	25	71

Number and % of loggerheads assigned to each foraging ground based on the classification results. Observed classes are in rows, predicted in columns. We used $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the females equipped with satellite tags (n=14) as training data set to develop the discriminant functions and the untracked turtles (n=57) as test data set for the discriminant classification. 92.9% of original and of cross-validated grouped cases were classified correctly. Only one southern individual (turtle ID k, Table 2.1) was misclassified and assigned to the central group.

Discussion

Satellite Telemetry

Our telemetry data identified new foraging areas used by female loggerheads of the NWA Florida Peninsular Recovery Unit. Six of the 14 individuals we tracked moved north and four resided in eastern central Florida, demonstrating for the first time that the Mid- and South-Atlantic Bights, respectively, provide important foraging grounds for adult females of this Recovery Unit. This result is a major difference from the results of prior satellite tracking studies. Overall there are published tracking data for 47 females of the NWA Florida Peninsular

Recovery Unit (27, 28, 44). Prior to this study, only 19 females were tracked (between 1988 and 2000) from eastern central Florida (27, 44) despite the fact that the Carr NWR alone accounts for ~25% of the 30-60,000 nests laid in Florida each year (29, 42). Only one of the 19 previously tracked individuals moved north to North Carolina and one stayed in eastern central Florida, while the remaining 17 females migrated south along the east coast of Florida to the Bahamas Archipelago, Cuba, west coast of Florida and Gulf of Mexico.

The Mid- and South- Atlantic Bights are known to be important foraging areas for adult females of the NWA North Recovery Unit, which comprises loggerheads nesting from the Florida/Georgia border to southern Virginia (39). Of the 73 females of the NWA North Recovery Unit equipped with satellite tags between 1997 and 2008 in North Carolina, South Carolina and Georgia, 51 used the north strategy, nine stayed year-round in the South Atlantic bight, four migrated to the Bahamas, Florida Keys and Gulf of Mexico, while the remaining ceased transmitting before reaching post-nesting migration destinations (47, 59, 60).

Prior to our study, the documentation that adult females of the NWA Florida Peninsular Recovery Unit used Mid- and South- Atlantic Bights was limited to a few flipper tag returns (61). In fact, the majority of tag returns for this Recovery Unit are from Cuba (62), Bahamas and Florida Keys (Ehrhart, unpublished). Interestingly, migratory patterns similar to the ones we identified have been shown recently in male loggerheads tracked from Cape Canaveral (FL, USA), a major breeding aggregation only 40 km north of our study site (63). Twenty of the 29 males tracked used the Mid- (n = 8) and South Atlantic (n = 12) Bights. Among the 12 males that used the South Atlantic Bight, two individuals migrated to South Carolina, while 10 remained in

eastern central Florida suggesting that eastern central Florida supports a year round aggregation of adult loggerheads.

We can think of three plausible explanations for the novelty of our tracking data: (1) the high use of Mid- and South- Atlantic Bights may be a new phenomenon, (2) sample size of telemetry studies is small and our results, as well as prior studies', may be due to chance, (3) Mid- and South- Atlantic Bights have always been important foraging grounds for the Florida Peninsular Recovery Unit but the importance was not detected with prior technology such as flipper tag return. Even though considerable progress has been made into understanding sea turtle migration using recovery of flipper-tagged individuals (61, 62, 64-68), the use of this technique to assess post-nesting migration destinations has some drawbacks. Flipper tag recapture distribution may be affected by small sample sizes, differential fishing pressure and/or oceanographic features such as currents that may push carcasses offshore. In recent years advances in satellite telemetry, genetic analysis and stable isotope analysis have provided additional tools to unravel migratory connectivity. While it is not possible to discriminate between hypothesis (1) and (3), it is possible to test whether the importance of Mid- and South- Atlantic Bights is due to random chance and small sample size. To do so we can either (a) significantly increase the number of females equipped with satellite tags or (b) investigate the reliability of stable isotope analysis as a tool to infer post-nesting migration of a large number of females to obtain a better representation at the population level.

Relationship Between Loggerhead RBC Isotopic Signatures and Post-nesting Migratory Destinations

The variability we found among individuals in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ allowed us to identify three distinct foraging aggregations. Four gradients from enriched to depleted $\delta^{13}\text{C}$ in marine habitats (18, 69-73) can explain the variability in $\delta^{13}\text{C}$ we observed: (1) nearshore/offshore, (2) benthic/pelagic, (3) enriched/depleted $\delta^{13}\text{C}$ food webs and (4) low/high latitudes.

We reject the hypothesis that differences in $\delta^{13}\text{C}$ are due to a neritic/oceanic gradient because all the loggerheads we tracked stayed on the continental shelf (within the 200 m isobaths), thus in neritic habitat. Our data did not allow testing the benthic/pelagic gradient because we only have dive profile data for four (of the 14) loggerheads we tracked. Bathymetry is not a good proxy to investigate the benthic/pelagic gradient because individuals may use the water column differently and these differences can only be detected if diving profiles are available. Adult loggerheads are known to feed mostly on benthic invertebrates such as crabs and mollusks (35, 74). Since all loggerheads resided on the continental shelf and remained within their diving limit (up to 233 m: (75)), we hypothesize the majority of their diet will be made of benthos and, thus, exclude a primary role of the benthic/pelagic gradient in driving the differences in $\delta^{13}\text{C}$ among loggerheads. The benthic/pelagic and the enriched/depleted food web gradients are tightly connected. Benthic organisms will most likely feed on seagrass or algae-based webs that are enriched in $\delta^{13}\text{C}$ compared to pelagic environment based on phytoplankton food webs (76). The last known gradient that could explain variation in $\delta^{13}\text{C}$ is the latitudinal gradient. Latitudinal differences in $\delta^{13}\text{C}$ are due to temperature, surface water CO_2 concentrations and differences in

plankton biosynthesis or metabolism (77). The loggerheads we tracked moved across a wide latitudinal range (23°N to 38.6°N) and, therefore, provide an opportunity to test the latitudinal gradient hypothesis. The North-South latitudinal gradient in $\delta^{13}\text{C}$ isotopic values of our satellite-tracked loggerheads, with northern individuals being more depleted in ^{13}C , supports the conclusion that a latitudinal gradient is the main driver of the variation in $\delta^{13}\text{C}$ we observed. This conclusion agrees with previous studies in several marine taxa (cephalopods (78), penguins (79), North Pacific humpback whales (7), Cory's shearwater (33), albatrosses (19)).

For nitrogen, northern females were the most enriched, and southern females the most depleted, in ^{15}N . The relationship between latitude and $\delta^{15}\text{N}$ was weaker than for $\delta^{13}\text{C}$, suggesting that other factors may affect loggerhead RBC $\delta^{15}\text{N}$ values. Variation in $\delta^{15}\text{N}$ can be explained in three ways: (1) loggerheads at different latitudes forage at different trophic levels, (2) the differences in RBC $\delta^{15}\text{N}$ are a consequence of primary producers' baseline shift in nitrogen values associated with prevailing N cycling regimes that are maintained and amplified higher up the food chain and (3) a combination of the two hypotheses. The nitrogen stable isotope ratios of primary producers define the $\delta^{15}\text{N}$ value at the base of the food web and are a function of the $\delta^{15}\text{N}$ values of their nutrient sources (e.g. nitrate, ammonium, N), subsequent biological transformation (e.g. nitrogen fixation, which lowers the $\delta^{15}\text{N}$ values of primary producers, and denitrification, a process that increases values of $\delta^{15}\text{N}$) and isotopic fractionation (13, 80, 81). Data available in the literature on plankton $\delta^{15}\text{N}$ support a gradient in the NWA, with $\delta^{15}\text{N}$ values becoming progressively more enriched from the subtropics as we move north along the U.S. coastline (McMahon et al. as cited by [13]) (82). Loggerheads that migrated south moved to areas dominated by N_2 fixation, where source nitrogen has a lower isotopic composition (81, 83),

while loggerheads moving into the MAB entered a region whose nitrogen budget is mostly driven by denitrification and, thus, it is characterized by high phytoplankton $\delta^{15}\text{N}$ value in surface waters (84).

There also may be some individual variability in foraging preference, as reflected in our data on females using northern feeding areas. Within the northern aggregation, our $\delta^{15}\text{N}$ data show two clusters that may reflect two alternative foraging strategies. One group of females ($n = 3$) has $\delta^{15}\text{N}$ values ranging from 9.74 to 10.28 ‰ ($10.07 \pm 0.29\text{‰}$), while the second group ($n = 3$) $\delta^{15}\text{N}$ values range from 13.77 to 14‰ ($13.87 \pm 0.12\text{‰}$). These values suggest that females of the two clusters forage at different trophic levels. Despite previous paradigms that all turtles are benthic foragers, we suspect that the depleted group has a diet based mostly on jellyfish, while the enriched group forages mostly on benthos (crustacean and mollusks). These conclusions are supported by video footage of loggerheads foraging on sea scallop beds in the Mid-Atlantic (Haas et al. unpublished). Intraspecific variability in foraging preference in adult female loggerheads has been demonstrated using series of scute samples (54). Alternatively, differences in $\delta^{15}\text{N}$ between the two groups may reflect an anthropogenic effect. Recently McKinney et al. (85) found a gradient in $\delta^{15}\text{N}$ of particulate matter available to primary producers from estuaries (more enriched) to nearshore (average 30 km offshore) to mid-shelf (average 90 km offshore) in six locations at the same latitude (in the Mid Atlantic Bight). Our two groups of northern females also followed this pattern, with the enriched group residing an average of 17 km from shore (range = 10-29 km) and depleted group 71 km (range = 67-76 km) from shore. Thus, both groups may forage at the same trophic level and the differences in $\delta^{15}\text{N}$ may be attributed to agriculture runoff and anthropogenic waste that increase $\delta^{15}\text{N}$ in nearshore compared to mid-shelf

ecosystems (85). We cannot discriminate between these alternative hypotheses (different trophic level vs. anthropogenic effect) with our data, but further investigation using additional elements (oxygen and sulfur), compound specific stable isotope analysis, trace minerals and contaminant levels could be informative.

Discrimination of Stable Isotope Ratios According to Foraging Areas and Assignment of Untracked Females

Our use of the isotopic patterns identified in the 14 loggerheads equipped with satellite tags to assign putative post-nesting migration destinations of the remaining 57 untracked females allowed us to scale up the information obtained with satellite telemetry, gain a better idea at the population level and begin to understand relative importance of foraging grounds. Telemetry and assignment results were similar and highlighted a similar relative importance of foraging grounds. However, it should be noted that while telemetry results were obtained over the course of several years (2008, n = 2; 2009, n = 6; 2010, n = 6), all the untracked turtles analyzed were sampled in 2010. Therefore, our analysis does not take into consideration remigration interval, which may affect the relative importance of each foraging area on a year-to-year basis.

Several authors (18, 26, 77) have called for studies that integrate satellite telemetry data to ground truth the use of isotopic data as proxies for habitat use and diet. Validation of stable isotope analysis with tracking has recently been done in other migratory species (several sea bird species (86), albatrosses (19), kittiwake (52), Procellariiform species (33), fin whales (87)). With regard to sea turtles, a combination of satellite tracking and stable isotope analysis has been used

in juvenile (23), adult male (25) and adult female loggerheads nesting in Japan (20) and Greece (24), and adult leatherbacks [34]. Our study, as well as previous studies in loggerheads, supports the use of stable isotope analysis to infer post-nesting foraging grounds. However, while Zbinden et al. (24) found only $\delta^{15}\text{N}$ to be informative in the Mediterranean, our study in the NW Atlantic, as well as Hatase et al. (20) in the NE Pacific, used both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to assign post-nesting migration destinations. Interestingly, Hatase et al. (20) found differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to be caused by a neritic/oceanic gradient, while we found them to be associated with a latitudinal gradient. Therefore, while we support the use of stable isotope analysis *in lieu* of more expensive satellite tags, we emphasize the need to validate the use of isotopic signatures with satellite telemetry on a subsample of individuals because oceanographic processes that affect baseline stable isotope ratios differ among ocean basins and geographical regions and, thus, data interpretations without validation can be misleading.

Conclusions

The Carr NWR hosts approximately 25% of all the nests laid by the NWA loggerhead Florida Peninsular Recovery Unit, which in turn makes up the greatest majority of the NWA female population. Therefore, to identify key foraging areas used by females nesting at Carr NWR is particularly important for the persistence of the species as a whole. Using a combination of satellite telemetry and stable isotope analysis we not only identified prime foraging areas -whose importance was previously unknown- but also validated the use of stable isotope analysis as a tool to derive post-nesting migration destinations for the most important breeding aggregation of

this Recovery Unit. We provided the first documentation that the continental shelf of the Mid- and South Atlantic Bights offer essential foraging areas for a large number (61%) of adult female loggerheads of the NWA Florida Peninsular Recovery Unit. These same areas have been found to be extremely important for loggerheads of the NWA Northern Recovery Unit (47, 59, 60). Our findings suggest that a large proportion of NWA Florida Peninsular Recovery Unit loggerheads are likely to be found within the USA Economic Exclusive Zone, potentially simplifying strategies for the conservation of the two most numerous Recovery Units of the NWA loggerhead populations. We agree with Hawkes' conclusion (47) that models integrating loggerhead spatial data (e.g. home range, niche models), anthropogenic threat data (e.g. from commercial fisheries and future plans for offshore oil drilling) and climate change are needed to identify hotspots to prioritize for conservation management.

After validating stable isotope analysis with satellite tracking, we suggest using isotopic signatures to assign turtles to foraging regions to scale up knowledge obtained from a limited number of individuals equipped with satellite tags to sample sizes that are more representative at the population level. Regular monitoring of foraging locations for nesting females will open new opportunities to investigate carry-over effects (*sensu* Norris (88): any event occurring in one season that influences individual performance in a non-lethal manner in subsequent season) and assess variation in relative importance of foraging grounds that, in turn, may reflect changes in environmental conditions (e.g. food availability) or anthropogenic stress (e.g. differential fishing pressure, pollution).

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Supporting Information

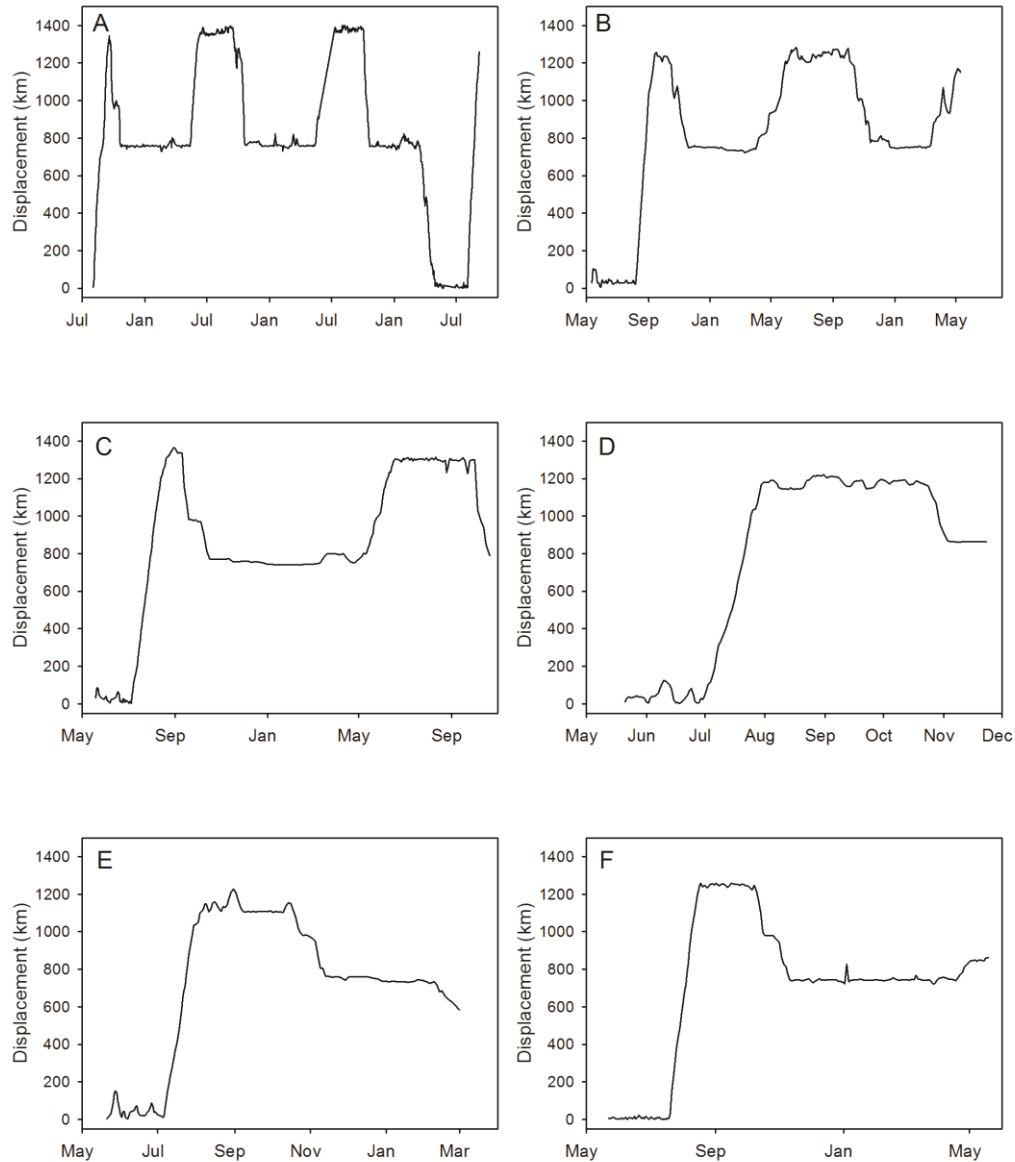


Figure S2.1. Displacement from release site plot of loggerheads equipped with satellite tags that followed the northern strategy and migrated between summer and winter foraging areas (turtle a-f). Phases of migration are represented by rapid changes in displacement distance; summer and

winter foraging areas can be seen where displacement values plateau. Note differences in y-axis scale among Figure S1, S2 and S3.

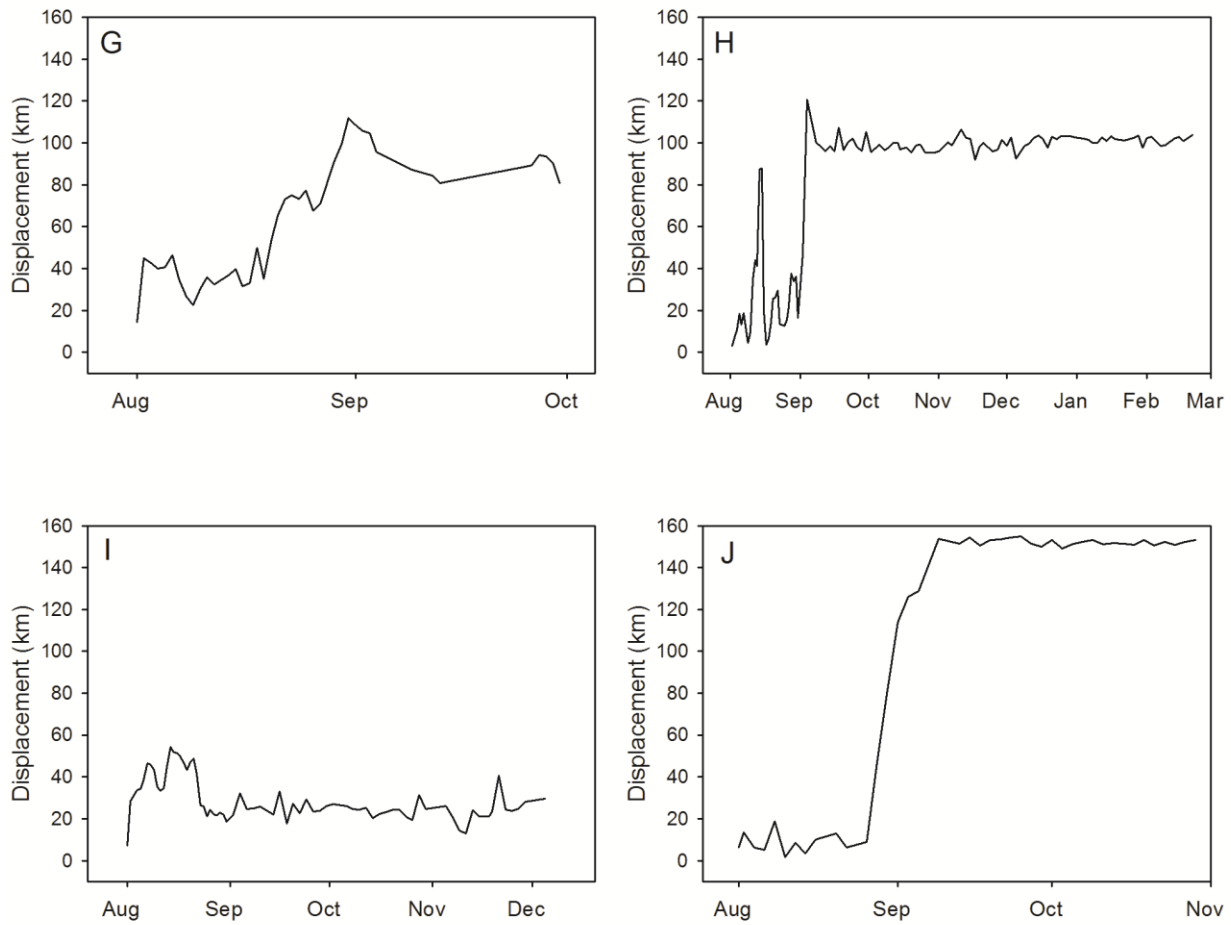


Figure S2.2. Displacement from release site plot of loggerheads equipped with satellite tags that resided in eastern central Florida (turtle g-j).

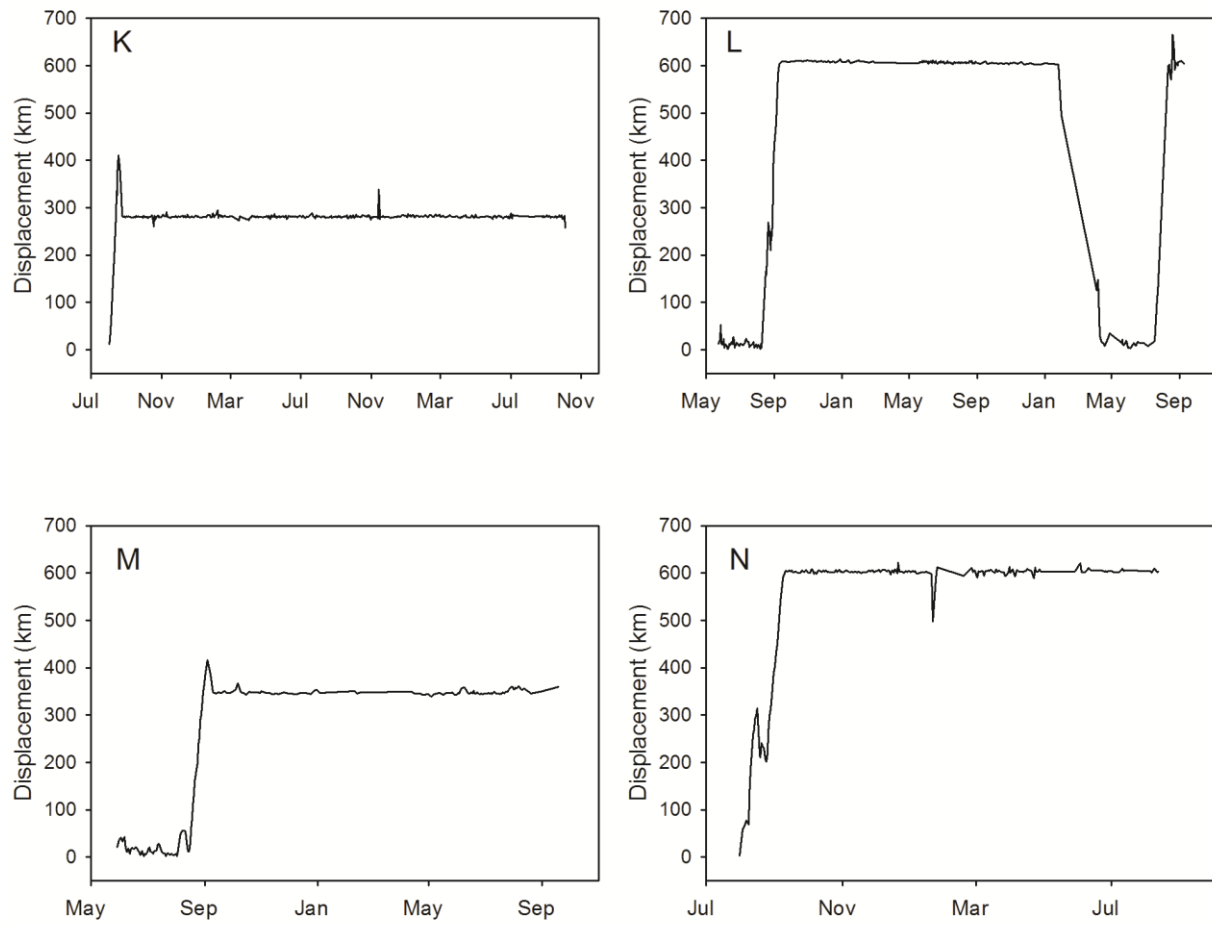


Figure S2.3. Displacement from release site plot of loggerheads equipped with satellite tags that followed the southern strategy and took up year-round residence in southern foraging grounds (turtle k-n). Phases of migration are represented by rapid changes in displacement distance. Year-round foraging areas can be seen where displacement values plateau.

CHAPTER 3: MODELING AND MAPPING ISOTOPIC PATTERNS IN THE NORTHWEST ATLANTIC DERIVED FROM THE LOGGERHEAD TURTLE²

Introduction

The study of animal migration has advanced in recent years thanks to a variety of techniques (e.g., satellite telemetry, stable isotope, genetic, trace element, and contaminant analyses). Among these techniques, stable isotope analysis of light elements (C, H, N, O and S) has emerged as a relatively cost-effective and rapid tool for studying migratory connectivity in a variety of taxa in both terrestrial and marine systems (Hobson 1999). The isotopic approach is effective because ratios of stable isotopes of naturally occurring elements change across the landscape, often in systematic ways and at the continental scale as a result of several biogeochemical processes (Hobson 1999). Patterns in stable isotope ratios at the base of food webs are amplified (depending on the element) at higher trophic levels. For stable isotopes to act as forensic tracers, individuals must move between isotopically distinct landscapes and maintain in one or more of their tissues measurable isotopic differences, either in metabolically inert tissues (e.g., feather, hair) or integrated in living tissues over some time duration (e.g., blood solutes, skin), that can be related to past locations (1). Hence, stable isotope ratios can function as intrinsic markers that reflect the isotopic composition of the ecological environment (geographic location and food web).

² **Prepared as:** Ceriani SA, Roth JD, Sasso CR, McClellan CM, James M, Haas HL, Smolowitz RJ, Evans DR, Addison DS, Bagley DA, Ehrhart LM, Weishampel JF(*in review*). Modeling and mapping isotopic patterns in the Northwest Atlantic derived from the loggerhead turtle. *Ecosphere*.

One application of the isotopic approach is the geographic assignment of origin of unknown individuals, which has both forensic and ecological applications (e.g., Ehleringer et al. 2008, Hobson et al. 2012a). To estimate the geographic origins of migratory animals, it is necessary to develop an assignment model based on stable isotope values of individual tissue of known geographic origin. The geographic assignment of unknown individuals uses nominal or continuous-surface assignment approaches (see Wunder 2012). Nominal assignment requires the predetermination of possible origins and includes methods such as regression trees and likelihood-based approaches (e.g., DFA). In contrast, the continuous-surface assignment approach depends on an underlying mechanistic geographic model of variation in stable isotope values, which is used to develop spatially explicit predictions of elemental stable isotope ratios (i.e., isoscapes) (Wunder 2010, 2012). Nominal assignment is spatially-implicit (i.e., it incorporates assumptions about spatial structure of biotic interactions but does not include geographical space) and requires smaller sampling coverage. The continuous-surface assignment approach has finer spatial resolution because it models the probability that the unknown individual comes from each specific geographic location across a region (Wunder 2010, 2012) and can assign individuals to a specific location rather than a coarser geographic bin.

Isoscapes that are used to track the movements of migratory animals (Hobson et al. 1999, Barnett-Johnson et al. 2008, Graham et al. 2010) are generated by spatially interpolating (typically using geostatistics) species-derived isotope values (e.g., the monarch butterfly; Hobson et al. 1999) or by calibrating an existing isoscape based on other sources (e.g., $\delta^2\text{H}$ in rainwater; Bowen et al. 2005, Wunder 2012). We refer to the former type as “species-specific isoscapes”

and to the latter as “environmental-based isoscapes” or “low trophic level-based isoscapes” depending on whether environmental (e.g., $\delta^2\text{H}$ rainwater) or low trophic level sources (e.g., particulate organic matter or phytoplankton in marine systems) are used to create the isotopic base maps. The key assumption of the latter type of isoscape is that stable isotope patterns are faithfully maintained or translated through food webs (Wunder and Norris 2008). In order to be applied to a specific animal tissue (e.g., bird feathers), this latter type of isotopic map must be first calibrated with samples of known origin to reflect the isotopic discrimination between the tissue (e.g., $\delta^2\text{H}$ feathers) and the environmental source ($\delta^2\text{H}$ rainwater). The main advantage of species-specific isoscapes is that the calibration of the base map is incorporated directly into the spatial interpolation, which removes an important source of error and underlying assumption of base maps derived from environmental sources (e.g., $\delta^2\text{H}$ feather isoscapes based on $\delta^2\text{H}$ rainfall precipitation patterns; Hobson and Wassenaar 1996, Bearhop et al. 2005, Hobson et al. 2012b) or low trophic-level sources (e.g., plankton $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in marine systems; McMahon et al. 2013). However, only a few species-specific isotopic base maps have been developed so far (e.g., monarch butterfly, Hobson et al. 1999; house sparrow, Hobson et al. 2009; albatross, Jaeger et al. 2010; tuna, Graham et al. 2010) because of inherent difficulties associated with sampling multiple individuals at multiple locations, across a broad spatial area and over an appropriate time period (Wunder and Norris 2008).

Many marine organisms move across broad geographic areas and are difficult to track with conventional methods (e.g., banding, surveys). Populations of apex marine predators and most commercially-exploited fish have declined significantly in the last century and the consequences

of these declines on marine ecosystems are not fully understood (Baum et al. 2003, Heithaus et al. 2008); thus, there is an urgency to better understand their spatial ecology and migratory connectivity in order to develop effective conservation strategies. Stable isotope analysis has contributed significantly to the unraveling of migratory connectivity of marine species (Killingley 1980, Hobson 1999), but despite this progress, isotopic patterns and their underlying drivers in marine systems are less understood compared to terrestrial systems. To validate the isotopic approach, satellite tracked individuals constitute the training data set for the development of assignment models of unknown origin individuals (e.g., Hatase et al. 2002, Zbinden et al 2011, Ceriani et al. 2012, Pajuelo et al. 2012, Seminoff et al. 2012). However, telemetry-based assignment models often rely on relatively small numbers of tracked individuals sampled over the course of several years due to high costs associated with sampling and tracking wildlife. To apply telemetry assignment models with confidence, it is critical to assess their performance by conducting external validation. This normally involves treating known origin samples as unknown for the purpose of the assignment and then calculating the percentage of correct assignments and is a common practice in food traceability studies (e.g., Alonso-Salces et al. 2010). However, in animal migration studies, external validation has been limited mostly to birds (Wunder et al. 2005, Hobson et al. 2012b) due to the difficulties of obtaining additional samples of known origin. The performance of telemetry-based assignment models has not been assessed for marine organisms. Although some of the first applications of stable isotopes to animal movements focused on marine animals (Killingley 1980, Killingley and Lutcavage 1983), few marine isoscapes have been developed (Graham et al. 2010, Jaeger et al. 2010, McMahon et al. 2013). In addition, while the long-term growing-season rainfall $\delta^2\text{H}$ has proven to be the best environmental predictor of $\delta^2\text{H}$ in terrestrial organisms (e.g., birds; Bowen et al. 2005), an

equivalent environmental parameter has not been clearly identified in the marine environment, although evidence suggests there is correspondence between sea surface temperature (SST) and $\delta^{13}\text{C}$ isotopic patterns in marine systems (MacKenzie et al. 2011).

The life history of sea turtles, and in particular, loggerheads, *Caretta caretta*, makes them an ideal taxon for stable isotope applications. Loggerheads are highly migratory organisms with a complex life cycle where different life stages occupy diverse ecological environments. In the Atlantic Ocean, loggerheads typically switch from an initial oceanic juvenile stage to a neritic stage, where maturity is reached (Bolten 2003). Females undertake breeding migrations every 1 to 4 years between spatially distinct foraging grounds and nesting areas. Each female from a nesting aggregation typically forages in one of several geographically distinct foraging grounds (Schroeder et al. 2003, Girard et al. 2009, Hawkes et al. 2011, Ceriani et al. 2012, Foley et al. 2013). Telemetry has revealed that loggerheads nesting in east central Florida, the largest nesting aggregation in the Atlantic, follow distinct migratory routes associated with three foraging grounds (Ceriani et al. 2012): (1) a seasonal shelf-constrained North-South migratory pattern along the northeast USA coastline, (2) a year-round residency in the South Atlantic Bight (SAB), mainly in waters adjacent to the breeding area and (3) a year-round residency in southern foraging grounds such as the Bahamas and southeast Gulf of Mexico. Individual females appear to show fidelity to both nesting and feeding areas throughout their adult life (Miller 2003, Broderick et al. 2007). Loggerheads in the Northwest Atlantic Ocean (NWA) are well studied at nesting beaches (Ehrhart et al. 2003, Witherington et al. 2009), and on some neritic foraging grounds used by adults and juveniles (e.g., Ehrhart et al. 2007, Epperly et al. 2007, Braun-McNeill et al. 2008, Eaton et al. 2008). Juveniles in the NWA generally mimic adult female

migratory behavior, encompass the same geographic areas (i.e., McClellan and Read 2007, Mansfield et al. 2009) and exhibit similar fidelity to foraging grounds (Avens et al. 2003, McClellan and Read 2007). While still incomplete, the spatial ecology of loggerheads that have recruited in neritic habitats is better understood than many other marine species making NWA loggerheads good candidate organisms to tackle problems of geographic assignment.

Ceriani et al (2012) examined the use of stable isotope analysis to infer foraging areas used by adult female loggerheads during the non-breeding season. Here, we include a larger number of loggerheads equipped with satellite tags as well as mostly juveniles sampled at foraging grounds across a broader geographic area. We then used a combination of satellite telemetry and stable isotope analysis to (1) evaluate the efficacy of stable isotopes to infer loggerhead migratory strategies and to (2) create loggerhead specific isotopic base maps to visualize isotopic geographic patterns and explore whether a spatially explicit approach could be used to gain further insight on the ecology of this highly migratory species.

Methods

Study Sites and Tissue Collection

We collected tissue samples (blood and/or a skin biopsy) for stable carbon and nitrogen isotope analysis from a total of 214 loggerheads in the NWA Ocean (Figure 3.1). Our data set is comprised of two subsets: (1) 58 loggerheads equipped with satellite devices either at the nesting

beach (n = 32 adult females) or foraging areas (n = 26) (*training subset*) and (2) 156 individuals captured at their foraging grounds (*test subset*). Although 48 individuals were equipped with satellite transmitters at foraging areas, only 26 transmitted long enough to determine their foraging ground and were included in the training subset.

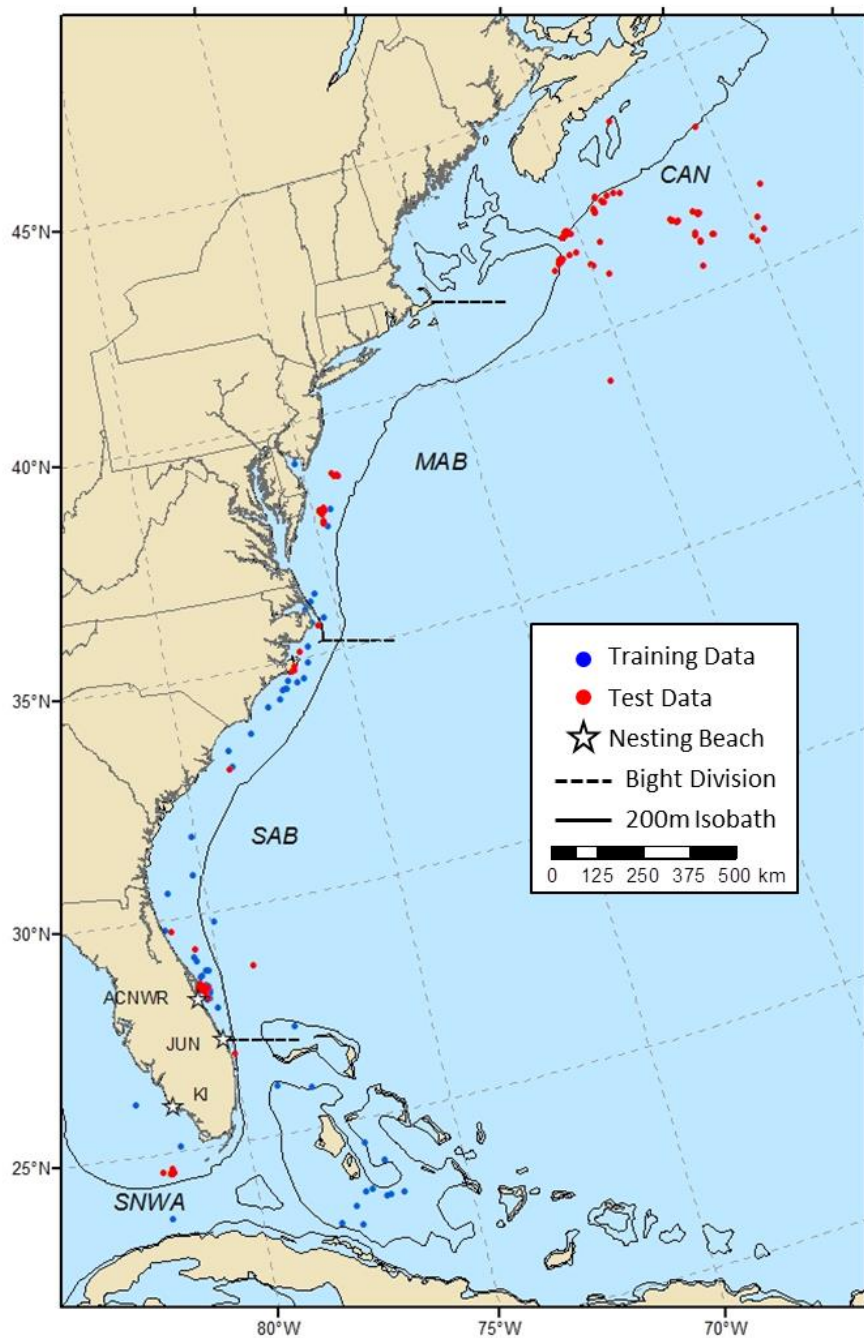


Figure 3.1. Sampling locations of the 214 loggerheads (32 nesting females and 156 individuals captured at foraging grounds) included in this study. We sampled four geographic areas: the waters off Nova Scotia, Canada (CAN), the Mid-Atlantic Bight (MAB), the South Atlantic Bight (SAB) and the Subtropical Northwest Atlantic (SNWA). CAN and MAB constitute the northern group. Dotted lines separate the geographic areas sampled: CAN, MAB, SAB and SNWA. Stars indicate the three nesting beaches where 32 females were equipped with satellite tags: the Archie Carr National Wildlife Refuge (ACNWR), Juno Beach (JUN) and Keewaydin Island (KI).

We collected a skin biopsy for stable carbon and nitrogen isotope analysis from the 32 nesting loggerheads between 2008 and 2012. Females included in this study were sampled from three locations: the Archie Carr National Wildlife Refuge (east central Florida; n = 21, 14 were included in Ceriani et al. (2012)), Juno Beach (south Florida; n = 6) and Keewaydin Island (southwest Florida; n = 5). For the in-water loggerhead sampling, we collected tissues from four foraging areas in the NWA (Figure 3.1): (1) the waters off Nova Scotia, Canada (CAN), in particular on the Scotian Shelf, Slope and the abyssal plain itself within Canada's Exclusive Economic Zone, (2) the Mid-Atlantic Bight (MAB), defined as the region enclosed by the coastline from Cape Cod (Massachusetts) to Cape Hatteras (North Carolina), (3) the South Atlantic Bight (SAB), which extends from Cape Hatteras to West Palm Beach (Florida), and (4) the Subtropical Northwest Atlantic (SNWA), defined as the area south of West Palm Beach and encompassing the waters around the Florida Keys, Bahamas and Cuba. The CAN data set consisted of skin samples collected from 68 loggerheads caught on and beyond the continental shelf break in the summer of 2011 (n = 1) and 2012 (n = 67). The MAB data set consisted of (1) skin and red blood cells (hereafter RBC) samples collected from 25 loggerheads captured using dip-nets in summer 2011 as part of a study conducted on the continental shelf by the Northeast Sea Turtle Collaborative (primarily Coonamessett Farm Foundation and National Marine Fisheries Service (NMFS) Northeast Fisheries Science Center) and (2) RBC samples obtained from 18 loggerheads caught in pound nets in North Carolina estuaries and equipped with satellite tags during the 2002-2004 summers and autumns (McClellan and Read 2007). The SAB data set consisted of skin sampled collected from 30 loggerheads that were trawled off Cape Canaveral (FL) during the 2013 winter and that were equipped with satellite tags as a part of a study conducted by the NMFS Southeast Fisheries Science Center. Lastly, the SNWA data set was

made of RBC and skin samples collected from 41 loggerheads that were caught using the rodeo capture technique (Limpus and Walter 1980) in 2010 (n = 23) and 2011 (n = 18) within the Key West National Wildlife Refuge (Florida), hereafter abbreviated as Key West NWR, by the Inwater Research Group, a Florida non-profit corporation. Our sampling encompassed several class sizes representing different life stages.

Live sea turtles cannot be aged; thus, body size is commonly used as a proxy of age and life stage though the relationship between age and length is quite variable. In addition to tissue samples, we collected standard size morphometrics, i.e., curved carapace length (CCL) and straight carapace length (SCL). We used the size classification (Stage I to Stage V) proposed by the Turtle Expert Working Group (2009). Little is known about loggerheads found off Nova Scotia (CAN), but Stage III juveniles ($41 < \text{SCL} < 82$ cm), and possibly some Stage II juveniles ($15 < \text{SCL} < 63$ cm) use this area in the summer (Brazner and McMillan 2008). Both MAB and North Carolina estuaries are known to be important summer foraging grounds (Epperly et al. 1995, Musick and Limpus 1997, McClellan and Read 2007, Epperly et al. 2007), and aerial surveys (Shoop and Kenney 1992) have documented that large numbers of loggerheads aggregate in the MAB from May to October and undertake seasonal north-south migrations along the US coastline between MAB (May to October) and SAB (November to April) (Mansfield et al. 2009). The loggerhead population off Canaveral consists of a mix of year-round residents and seasonal (winter) residents and in spring and summer hosts a major breeding aggregation (Henwood 1987). Loggerheads are year-round residents in the Key West NWR as suggested by the high recapture rates (22% of the 454 total captures since the beginning of the project in 2002, Jeff Guertin *personal communication*). All sites but CAN have been extensively

studied and host long-term in-water projects focusing on loggerhead population dynamics and contain mainly large juveniles (Stage III and IV) and adults (Stage V, SCL > 82 cm).

Tissue Processing and Stable Isotope Analysis

We measured the stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios of red blood cells (RBC) and epidermis. Tissue turnover rates in sea turtles are unknown (except for hatchlings and small juveniles – Stage II, Reich et al. 2008) but RBC and epidermis are estimated to reflect foraging habits at least 4 months prior to sampling (Brace and Altland 1955, Seminoff et al. 2007, Reich et al. 2008, 2010; Ceriani et al. *unpublished data*). Thus, RBC and skin samples represent the foraging area used by females during the non-breeding season (Caut et al. 2008, Reich et al. 2010, Ceriani et al. 2012, Pajuelo et al. 2012, Seminoff et al. 2012) and by juveniles and sub-adults that migrate between summer foraging grounds and overwintering areas (Wallace et al. 2009, McClellan et al. 2010).

Blood samples (4 ml) were collected from the cervical sinus with a 20-gauge needle and syringe (48), transferred to a non-heparanized container and placed in ice. Blood was separated into serum and cellular components by centrifugation (5000 rpm x 10 min) and frozen at -20°C until analysis. Skin samples were collected in two anatomical positions depending on the researcher permit: the right shoulder area (nesting females and Key West NWR loggerheads) and the soft skin from the trailing edge of the rear flipper (CAN, MAB and SAB loggerheads) using 4-6 mm biopsy punches. Skin samples were either stored in a non-frost-free freezer at -20°C or preserved

in saturated sodium chloride solution. Both preservation methods have no effect on tissue isotopic composition (Barrow et al. 2008).

Samples were prepared for stable isotope analysis following standard procedures. All samples with the exception of the 18 RBC from loggerheads captured in North Carolina estuaries (McClellan et al. 2010) were prepared at the University of Central Florida. RBC samples were either dried at 60°C (McClellan et al. 2010) or freeze-dried for 48 h before being homogenized with mortar and pestle. Skin samples were rinsed with distilled water and cleaned with 70% ethanol. We used a scalpel blade to separate and finely dice epidermis (stratum corneum) from the underlying tissue (stratum germinativum). Epidermis samples were then dried at 60°C for 48 h. Lipids were removed from all the samples (except those from North Carolina estuaries) using a Soxhlet apparatus with petroleum ether as solvent for 12 and 24 h (RBC and epidermis, respectively). A post hoc lipid correction factor (Post et al. 2007) was applied to carbon isotope ratios of the RBC samples collected in North Carolina (see McClellan et al. 2010). Sub-samples of prepared tissues (0.4-0.7 mg) were weighed with a microbalance and sealed in tin capsules. Most of the prepared samples were sent to the Paleoclimatology, Paleoceanography and Biogeochemistry Laboratory at the University of South Florida, College of Marine Science (St. Petersburg, FL, USA), where they were converted to N₂ and CO₂ using a Carlo-Erba NA2500 Series 2 Elemental Analyzer (Thermoquest Italia, S.p.A., Rodano, Italy) and analyzed with a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen). Stable isotope ratios were expressed in conventional notation as parts per thousand (‰) according to the following equation: $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where X is ¹⁵N or ¹³C, and R is the corresponding ratio ¹⁵N:¹⁴N or ¹³C:¹²C. The standards used were atmospheric

nitrogen and Pee Dee Belemnite for ^{15}N and ^{13}C , respectively. Estimates of analytical precision were obtained by replicate measurements of internal lab reference materials (1577b Bovine liver) and yield a precision (reflecting ± 1 SD) of $\pm 0.14\text{‰}$ for $\delta^{13}\text{C}$ and 0.12‰ for $\delta^{15}\text{N}$. Samples collected in North Carolina estuaries were analyzed at the Duke University Environmental Stable Isotope Laboratory (Durham, NC; see McClellan et al. 2010 and Wallace et al. 2009 for analytical details). RBC from the 25 loggerheads captured by Coonamessett Farm and the NEFSC were prepared at the University of Central Florida but the spectrometry was conducted at the MBL Stable Isotope Laboratory (Woods Hole, MA). Though there may be potential differences among the accredited laboratories, we do not expect them to have a significant effect on the analyses because potential measurement differences among labs are orders of magnitude smaller than the range of isotopic values sampled.

Tracking Analysis

We attached satellite transmitters (Wildlife Computers MK10-A, MK10 AFB and Mk10-PAT Pop-up Archival Transmitting Tag, Redmond, Washington, USA; SIRTRACK KiwiSat 101 K1G 291A, New Zealand) to 32 nesting loggerheads and tracked their post-nesting migrations. Transmitters were affixed to the turtle carapace using epoxy or direct attachment for PAT tags (Sasso et al. 2011, Ceriani et al. 2012). In addition, 48 juveniles were equipped with satellite tags after being captured in the estuaries of North Carolina ($n = 18$; McClellan and Read 2007) and off Cape Canaveral, FL ($n = 30$; Sasso *unpublished data*). Only 26 of the 48 juveniles ($n = 13$ from North Carolina and $n = 13$ from Cape Canaveral, FL) exhibited a defined migratory behavior and transmitted long enough to determine their summer and overwintering areas, and

thus, were included in the training subset. Loggerheads sampled off Cape Canaveral were included in the training subset if they transmitted for at least 80 days and remained within the SAB. We chose the 80-day cut-off because loggerheads were sampled in early March 2013 and individuals undergoing seasonal migration between the SAB and the MAB usually leave the SAB by the end of April/early May (i.e., within 60 days from capture date) (Epperly et al. 1995, Mansfield et al. 2009, Ceriani et al. 2012).

Tracking data were filtered as described in McClellan and Read (2007) and Ceriani et al. (2012). Service Argos, Inc provided position estimates and associated location accuracy. We employed a customized script in the R package software (R Development Core Team 2009) that was based on a two-stage filtering algorithm (land/sea and Freitas' speed-distance-angle filters) to reject implausible locations (Freitas et al. 2008). Loggerhead movements were reconstructed by plotting the best location estimate per day of the filtered location data using ArcGIS 10.1. Post-nesting foraging ground used by each adult female was calculated following the procedures described in Ceriani et al. (2012). Briefly, foraging areas were determined by plotting displacement from deployment site (see Ceriani et al. 2012; Figure 3.1). Migration was considered to have ceased when displacement began to plateau. We averaged the locations of all filtered data (best estimate/day) from the plateau to derive foraging ground location of females that used the same area year-round. If an individual undertook seasonal migration, summer and winter foraging phases were considered to have ended when displacement values started to change. To calculate mean latitudes and longitudes of summer and winter foraging areas, we averaged the locations of all filtered data (best estimate/day) from each plateau. Foraging

locations were classified as ‘oceanic’ if off the continental shelf, as defined by the 200 m isobath, or ‘neritic’ if on the shelf.

Statistical Analysis

We converted RBC stable isotope values of the juvenile loggerheads equipped with satellite tags in North Carolina estuaries into equivalent epidermis values using a linear regression equation derived from 66 of the juvenile loggerheads sampled at the foraging grounds for which we analyzed both epidermis and RBC stable isotope values (Appendix A).

We used multivariate analysis of variance (MANOVA) with the Pillai’s trace test to test for significant differences in isotopic signatures among foraging areas used by the 58 juveniles and adult females equipped with satellite tags (training subset). Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnov and Levene’s test, respectively. Data were normal but did not meet the equal variance assumption even after transformation. We selected the Pillai’s trace test because it is the most robust of the tests when the assumption of similar-covariance matrix is not met (Johnson and Field 1993). Post hoc Games-Howell (GH) multiple comparison tests for unequal variance was used to determine groups responsible for statistical differences (Day and Quinn 1989).

Loggerheads of different sizes may consume different foods, which in turn could affect their stable isotope ratios. Thus, we used analysis of variance (ANOVA) to test for differences in body size (a proxy of age in sea turtles) among the foraging areas used by the 58 loggerheads in the

training subset (CCL measurements were unavailable for two nesting turtles). Post hoc GH multiple comparison tests for unequal variance was used to determine groups responsible for statistical differences (data were normal but did not meet the equal variance assumption even after transformation). We, then, performed analysis of covariance (ANCOVA) to test for the effect of foraging area location on isotopic values after controlling for turtle class size.

DFA was used to investigate how well $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ predict the general location of loggerhead foraging grounds (SPSS v. 19). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the 58 loggerheads equipped with satellite tags represented the training data set to develop the discriminant functions and the remaining 156 loggerheads sampled at foraging grounds were the test data set for the classification. We chose to compute from group sizes for prior probabilities because our test data did not have an equal chance of being in either group (i.e., we did not sample the same number of individuals at each foraging site). Loggerheads sampled at foraging grounds were treated as “unknown” for the purpose of the DFA and used as external validation to assess how well the classification model performed. We evaluated the model performance under a variety of assignment scenarios based on different probabilities of membership.

Development of Isoscapes

Of the 214 samples, we used only 205 that had specific geocoordinate locations (i.e., latitudes and longitudes) associated with foraging areas to generate $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes. Since loggerhead body size differed among foraging areas, we generated two sets of isoscapes: (1) isoscapes based on all the geolocated data and (2) isoscapes based on turtles with $\text{CCL} \geq 64.0$ cm

(n = 168) to exclude smaller and presumably oceanic loggerheads (Stage II), which are characterized by different habitat use and diet compared to the other individuals we sampled (exclusively oceanic vs. mostly neritic). We chose a cut-off of 64.0 cm, which corresponds roughly to SCL = 59.5 cm, because that is the size at which almost all Atlantic loggerheads are presumed to have recruited out of the oceanic stage according to the length frequency analysis conducted by Bjorndal et al. (2000). The two sets of isoscapes fundamentally generated the same isotopic patterns; thus, we present and discuss only the isoscapes that were generated using the larger data set (n = 205).

We developed isoscape models using the empirical Bayesian kriging (EBK; Pilz and Spöck 2007) routine available in ArcGIS 10.1 to interpolate between data points. This kriging method differs from more traditional methods as it automatically calculates semivariogram parameters using restricted maximum likelihood by running numerous simulations based on sample subsets. By generating and evaluating many semivariogram models, this approach produces more accurate standard error estimates and interpolations based on small data sets.

To adjust for non-normality in the data, which was more apparent with the $\delta^{13}\text{C}$ data, we applied a multiplicative skewing normal score transformation using an empirical base distribution. This transformation forces EBK to use a simple kriging model fitted with an exponential semivariogram. We evaluated interpolation models, resulting from differences in subset size, overlap factor, and neighborhood search parameters, based on cross validation statistics (e.g., root mean square and average standard error values).

Results

Satellite Telemetry: Post-nesting Migrations and Juvenile Foraging Areas

As found by Ceriani et al. (2012), post-nesting loggerheads moved across a wide range of latitudes spanning from the Great Bahamas Bank (23°N) to the MAB (38.6°N) following three migratory strategies. Migratory destinations of each of the 32 females were classified into one of the following geographic bins: northern (with seasonal migration between summer foraging areas in the MAB and wintering areas in the SAB; n = 11), central (year-round residence within the SAB, n = 5) and southern foraging area (year-round residence within the SNWA, n = 16), respectively.

Twenty-six juveniles equipped with satellite tags in North Carolina (n = 13) and Cape Canaveral, FL (n = 13) were assigned to one of the three foraging areas and included in the training subset. Movements of North Carolina juveniles have been described elsewhere (McClellan and Read 2007). For the purpose of this paper, these individuals belonged to the northern group since North Carolina represented their foraging area (McClellan and Read 2007, McClellan et al. 2010), and thus, shared the same geographic bin used by the adult females following the northern strategy. The 13 loggerheads sampled off Cape Canaveral that were included in the training subset belong to the central group as they either remained off the east central Florida coast or moved within the limits of the SAB. All 58 tracked loggerheads were considered “neritic” since all individuals took up residency within the limits of the continental shelf (water depth < 200 m).

Geographic Variability in Loggerhead Class Size and Stable Isotope Ratios

Foraging areas used by the 58 tracked loggerheads (32 nesting females and 26 juveniles) segregated by their combined bivariate ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) isotopic signatures (MANOVA, Pillai's trace test, $F_{4, 110} = 21.128$, $p < 0.001$), and in univariate analyses where both $\delta^{13}\text{C}$ (ANOVA, $F_{2, 55} = 130.286$, $p < 0.001$) and $\delta^{15}\text{N}$ values ($F_{2, 55} = 26.305$, $p < 0.001$) differed among foraging aggregations (Figure 3.2A). Post hoc GH multiple comparison tests indicated that all aggregations differed significantly in $\delta^{13}\text{C}$ among each other ($p < 0.001$ in all comparisons). The $\delta^{15}\text{N}$ signatures of loggerheads using southern foraging areas differed significantly from both northern ($p < 0.001$) and central ($p < 0.001$) aggregations, while northern and central aggregations did not differ from each other in $\delta^{15}\text{N}$ ($p = 0.623$). The “unknown” test subset seemed to exhibit similar isotopic patterns (Figure. 3.2B) as the training subset.

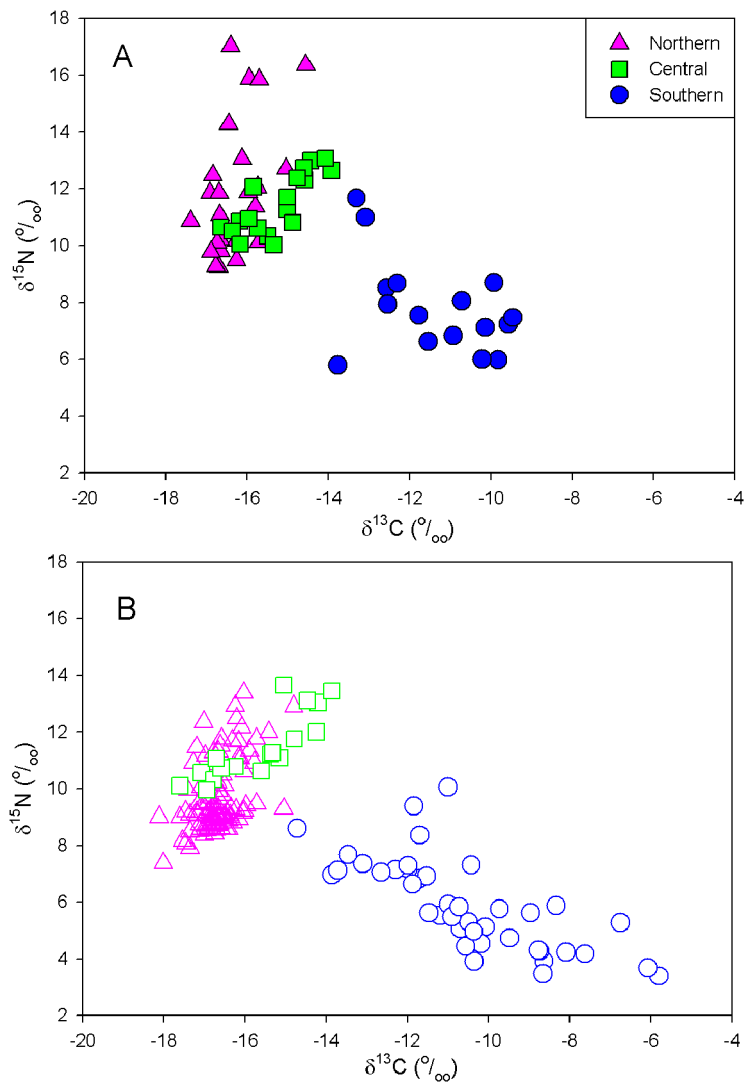


Figure 3.2. Stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) of (A) the 58 loggerheads equipped with satellite tags (training subset) and (B) the 156 untracked loggerheads (test subset) sampled at foraging areas (test subset) in the Northwest Atlantic. The northern area in (B) includes CAN and MAB loggerhead samples.

The MANOVA showed that stable isotope ratios differed among foraging areas (suggesting DFA could be used to assign unknown turtles), but our ability to apply DFA could be confounded if size varies among foraging areas. Thus, we tested for differences in body size among foraging grounds. We found significant differences in body size ($F_{2, 55} = 9.310$, $p < 0.001$) among

loggerheads using the three isotopically distinct foraging areas. Post hoc GH multiple comparison tests indicated that loggerheads in the southern foraging areas (SNWA) were significantly larger than the ones in the northern ($p < 0.001$) and central ($p < 0.001$) foraging grounds. This result was not surprising because the northern and central groups in the training data set included both tracked adult females and juveniles, while the southern group included only adult females as none of the juveniles equipped with satellite tags used the southern foraging area. Since body size differed among foraging areas, we used ANCOVA to determine whether the effect of foraging area was significant. After controlling for size, both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ differed significantly among foraging grounds ($\delta^{13}\text{C}$: $F_{2,52} = 94.85$, $p < 0.0001$; $\delta^{15}\text{N}$: $F_{2,50} = 4.50$, $p = 0.0160$). The interaction of loggerhead size and foraging location was significant only for $\delta^{15}\text{N}$ ($F_{2,50} = 13.56$, $p < 0.0001$). Appendix B provides a summary of body size and stable isotope values for the entire data set. Appendix C shows differences in body size and the effect of foraging area after accounting for size in the testing subset ($n = 156$).

Evaluation of the Stable Isotope Approach to Assign Foraging Grounds

The discriminant analysis of the training data set (58 loggerheads equipped with satellite tags) was significant ($P > \text{Wilks' Lambda} < 0.001$). Two discriminant functions were calculated, with a combined $X^2(4) = 108.8$, $p < 0.001$. After removal of the first function, the association between groups (foraging areas) and predictors ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) became not significant $X^2(1) = 0.301$, $p = 0.583$. The $\delta^{13}\text{C}$ skin values contributed the most to separation among groups ($\delta^{13}\text{C}$ $r = 0.817$, $\delta^{15}\text{N}$ $r = -0.673$). The first discriminant function accounted for 99.9% of the between-group variability (Figure 3.3). Overall the discriminant analysis of the training data set was able

to correctly classify the foraging ground used for 47 of the 58 loggerheads (81.0% of original grouped cases correctly classified). Two adults and one juvenile from the northern aggregation were incorrectly assigned to the central group, one adult and five juveniles from the central group were incorrectly assigned to the northern bin and two adults from the southern aggregation were incorrectly assigned to the central one. The stability of the classification procedure was checked by a leave-one-out cross validation, which classified 79.3% of the test data set correctly. The 156 loggerheads in the training subset were treated as “unknown” in the classification analysis and their putative foraging ground was predicted in the test data set and was based on the above classification functions (Table 3.1). Foraging areas used by those 156 loggerheads were known and, thus, provided the data set to conduct an external validation and assess how well the assignment model based on the 58 satellite tracked loggerheads performed under a variety of assignment scenarios based on different probabilities of membership (Figure 3.4). When we allowed the highest probability to determine assignment, the model correctly identified the foraging ground of 143 (of 156) “unknown” individuals (91.7%). When we considered only loggerheads that were assigned to one of the three groups with $\geq 66.66\%$ probability of membership (2:1 odds ratios), only 73.1% of the test turtles (114 of 156) exceeded that threshold, but of those, 93.0% were classified correctly. When we considered higher probabilities of membership, the number of turtles that could be assigned decreased rapidly but the percentage of correct assignment did not improve.

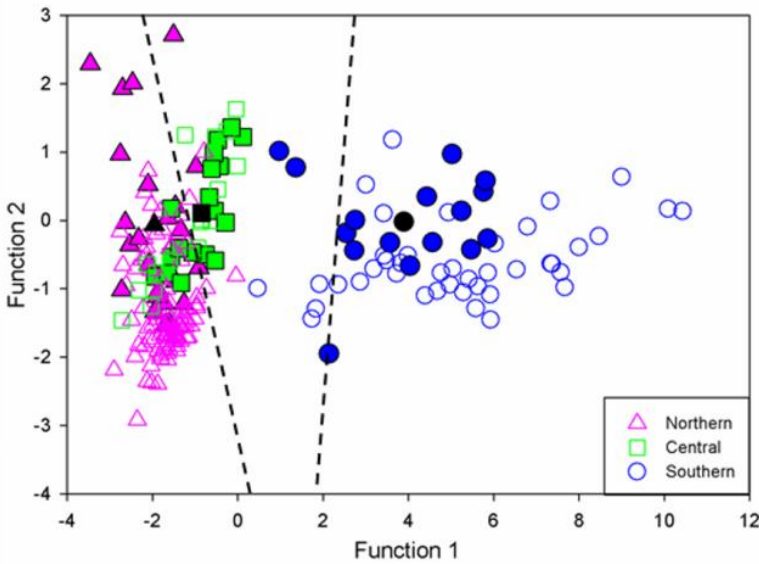


Figure 3.3. Discriminant Function Analysis (DFA) of foraging groups based on the stable carbon and nitrogen isotope ratios. The filled markers correspond to the training subset. The empty markers correspond to the test subset. The black symbols correspond to the group centroid. Dashed lines define the DFA territories.

Table 3.1. Foraging ground assignment, number and percent (in parentheses) for the discriminant model based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of loggerhead epidermis.

		Data Source		Predicted Group Membership			Total
		Nesting ¹	Foraging ²	North	Central	South	
Training data (n = 58)	North	11	13	21 (87.5)	3 (12.5)	0 (0)	24
	Central	5	13	6 (33.3)	12 (66.7)	0 (0)	18
	South	16	0	0 (0)	2 (12.5)	14 (87.5)	16
Test data (n = 156)	“Unknown”	0	156	100 (64.1)	16 (10.3)	40 (25.6)	156
Total		32	182	127	33	54	214

Note: Loggerheads were treated as unknown in the classification although their origin was actually known.

¹Loggerheads that were sampled and equipped with satellite tags at the nesting beach

²Loggerheads that were sampled at their foraging grounds; the ones used for training were equipped with satellite tags

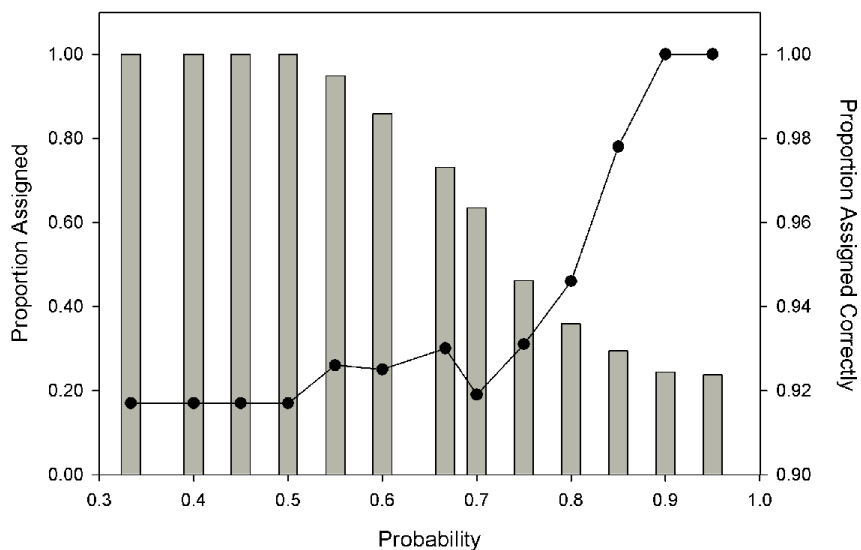


Figure 3.4. External validation and evaluation of assignment model performance under different probabilities of membership scenarios. Histogram represents the proportion of the 156 “unknowns” that could be assigned for a given cut-off probability or odds ratio (e.g., 2:3 = 66.66%). The black line indicates what proportion of the “unknown” that met the probability criterion was assigned correctly.

Isoscapes

Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ varied considerably for loggerheads across the sampled geographic area. Loggerhead $\delta^{13}\text{C}$ values followed the latitudinal gradient as shown previously by Ceriani et al. (2012) of more enriched values at low latitudes (SNWA) to more depleted values at higher latitudes (CAN) and ranged from -5.80 ‰ to -18.12 ‰. Loggerhead $\delta^{15}\text{N}$ ranged from 3.39 ‰ to 17.02 ‰ and exhibited a more complex pattern with depleted values at the lowest latitudes we sampled, intermediate $\delta^{15}\text{N}$ values at the higher offshore latitudes and most enriched values at nearshore intermediate latitudes in proximity of large river/estuary systems, i.e., Pamlico and Albemarle Sound, Chesapeake and Delaware Bays. The isoscapes based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ levels

in loggerhead epidermal tissue are presented in Figure 3.5A and Figure 3.6A, respectively. Both were derived from 100 simulations using a subset size of 100 samples with an overlap factor of 2. We used a smooth circular searching neighborhood with a radius of 1000 km. The interpolated surfaces (i.e. predicted) explained 86% of the variance in the measured values (i.e., observed) for $\delta^{13}\text{C}$ (observed $\delta^{13}\text{C} = 1.03 \cdot \text{predicted } \delta^{13}\text{C} + 0.42\text{‰}$) and 83% for $\delta^{15}\text{N}$ (observed $\delta^{15}\text{N} = 1.07 \cdot \text{predicted } \delta^{15}\text{N} - 0.66 \text{‰}$). All sample points were included in the cross-validation which yielded root mean square standardized values of 0.96 and 0.93 for the interpolations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Though we observed strong spatial structure for both carbon and nitrogen isotopes in the heavily sampled areas, there was uncertainty and the standard error of the predictions varied from 0.12 to 3.33 ‰ (Figure 3.5B) for $\delta^{13}\text{C}$ and from 0.18 to 3.15 ‰ (Figure 3.6B) for $\delta^{15}\text{N}$. As expected, the greatest uncertainty was associated with geographic areas in the central and south-eastern portions (lower right quadrant) of the modeled region for which we did not have loggerhead samples. Hence, the patterns in those areas should be interpreted with caution.

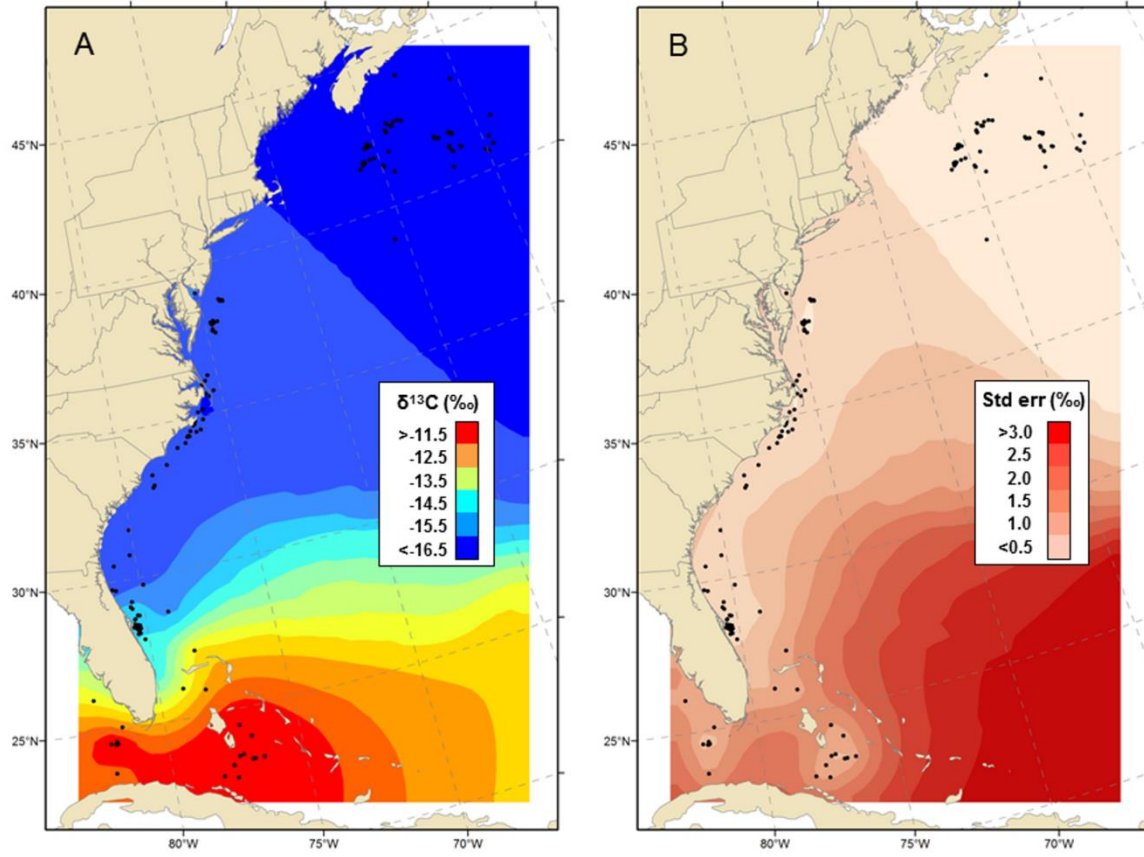


Figure 3.5. Isoscape of $\delta^{13}\text{C}$ (A) derived from loggerhead epidermal tissue and associated standard error surface (B) based on cross validation of observed and predicted values.

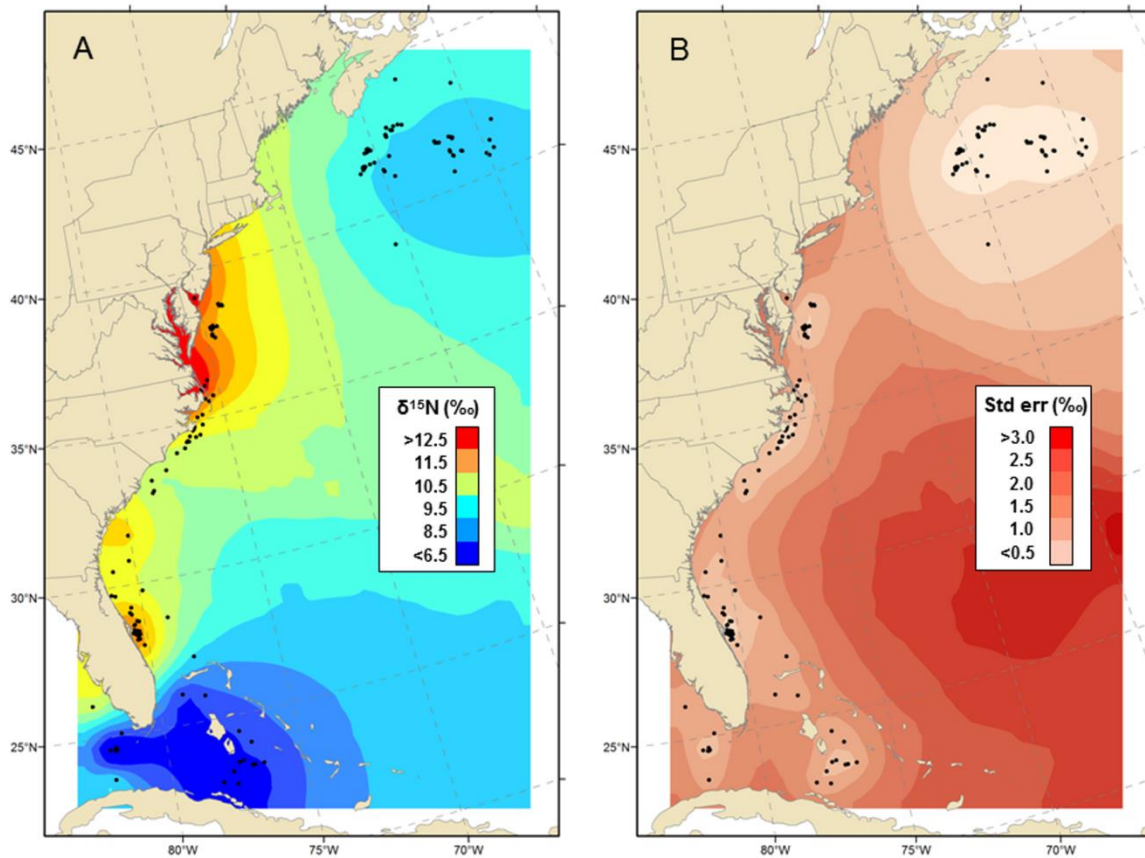


Figure 3.6. Isoscape of $\delta^{15}\text{N}$ (A) derived from loggerhead epidermal tissue and associated standard error surface (B) based on cross validation of observed and predicted values.

Discussion

Identifying Loggerhead Foraging Grounds with Stable Isotope Signatures

The east coast of North America constitutes essential habitat for both juvenile and adult loggerheads providing both foraging and nesting grounds for the world's second largest population of endangered loggerhead turtles (Ehrhart et al. 2003). We evaluated the use of

carbon and nitrogen stable isotopes to infer foraging grounds for juvenile (CCL > 51.0 cm) and adult loggerheads in the NWA by using a two-fold approach. First, we used a combination of satellite telemetry and stable isotope analysis of tissue with a slow turnover rate (months) from nesting females and juveniles equipped with satellite tags to develop a spatially implicit model to assign migratory strategies used by loggerheads at a relatively broad (100-1000 km) spatial scale. The DFA model correctly assigned 81% of original group and 79.3% of cross-validated cases, respectively. Then we treated 156 epidermis values of loggerheads whose foraging areas were known as “unknown” to evaluate the assignment model. This external validation confirmed that DFA models based on a relatively few tracked loggerheads in the NWA are robust and provide independent evidence supporting this spatially implicit approach for migratory marine organisms.

Isoscape Patterns

We produced the first species-specific isoscapes for a marine predator (the loggerhead turtle) in the Atlantic Ocean. Other species-specific isoscapes on marine predators have been developed for albatrosses equipped with tracking devices (n = 45) in the Southern Ocean (Jaeger et al. 2010) and for untracked bigeye (n = 196) and yellowfin (n = 387) tuna that were sampled in conjunction with fishery operations in the Pacific Ocean (Graham et al. 2010). However, with tuna the isotopic values were assumed to reflect the signature of the capture location, although they may have been in transit (i.e. sampled during migration). We found clear spatial patterns in loggerhead $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the NWA. The isotopic ranges were considerable ($-18.12\text{‰} \leq \delta^{13}\text{C} \leq -5.80\text{‰}$, $3.39\text{‰} \leq \delta^{15}\text{N} \leq 17.02\text{‰}$) and greater than expected if loggerheads were feeding at

the same trophic level with the same baseline suggesting the influence of phenomena (e.g., trophic differences) beyond geographic variability in the primary productivity isotopic baseline. Latitudinal differences in $\delta^{13}\text{C}$ have been found in previous studies in several marine predators (cephalopods, Takai et al. 2000; penguins, Cherel and Hobson 2007; North Pacific humpback whales, Witteveen et al. 2009; Cory's shearwater, Roscales et al. 2011; albatrosses Jaeger et al. 2010). Latitudinal differences in $\delta^{13}\text{C}$ are due to temperature, surface water CO_2 concentrations and differences in plankton biosynthesis or metabolism (Rubenstein and Hobson 2004). Recently, Mackenzie et al. (2011) showed that differences in marine organism $\delta^{13}\text{C}$ values correlate with SST because water temperature affects both cell growth rates and dissolved carbonate concentrations, and thus have a direct effect on the $\delta^{13}\text{C}$ values of primary producers. Therefore, an environmental parameter (SST) appears to be a good proxy for phytoplankton $\delta^{13}\text{C}$, which, in turn, is reflected in the $\delta^{13}\text{C}$ values of marine organisms at higher trophic levels. In addition, the south to north $\delta^{13}\text{C}$ gradient, to a certain extent, matches seagrass distribution along the eastern U.S. coastline and the Caribbean (Short et al. 2007). Seagrasses are the dominant primary producer for low-latitude neritic systems (e.g., SNWA). Compared to phytoplankton, seagrasses are enriched in $\delta^{13}\text{C}$ values falling within the range associated with C_4 metabolism (McMillan et al. 1980, Hemminga and Mateo 1996). Hence benthic seagrass- or macro-algae-based food webs are more $\delta^{13}\text{C}$ enriched than pelagic phytoplankton-based systems (e.g., the Scotian Shelf Slope) (Rubenstein and Hobson 2004). Loggerheads are generalist carnivores feeding mainly on benthos when on the continental shelf (Hopkins-Murphy et al. 2003 but see McClellan et al. 2010); therefore, variations in $\delta^{13}\text{C}$ loggerhead tissues are due to a

combination of low/high latitudes, nearshore/offshore, benthic/pelagic and seagrass/phytoplankton-based food webs gradients.

While $\delta^{13}\text{C}$ isopleths exhibited a clear latitudinal trend, $\delta^{15}\text{N}$ patterns were less linear. We attribute these patterns to a combination of three factors: (1) a baseline shift in primary producer $\delta^{15}\text{N}$, (2) differences in foraging strategies among the aggregations we sampled and, in particular, between CAN loggerheads off the Scotian Shelf Slope and the other areas sampled and (3) an anthropogenic effect. Ceriani et al. (2012) found that a combination of latitude and distance from shore was the best predictor of loggerhead $\delta^{15}\text{N}$ values in the NWA but their northernmost sampling location was at 38.6°N , while our sampling extended as far north as 44°N and farther offshore (beyond the continental shelf). Differences in loggerhead $\delta^{15}\text{N}$ have been attributed to primary producers' baseline shift in nitrogen values (Ceriani et al. 2012, Pajuelo et al. 2012) related to prevailing N cycling regimes that are retained at higher trophic levels and oceanic/neritic foraging strategies (McClellan et al. 2010). Nitrogen stable isotope ratios of primary producers are a function of $\delta^{15}\text{N}$ values of their nutrient pools (e.g., nitrate, ammonium, N_2), biological transformations (e.g., denitrification increases $\delta^{15}\text{N}$ and nitrogen fixation lowers $\delta^{15}\text{N}$) and isotopic fractionation (Sigman and Casciotti 2001, Montoya et al. 2007, Graham et al. 2010). Loggerheads in the SNWA reside in areas with higher rates of N_2 fixation, with a more depleted isotopic composition (Montoya et al. 2002, 2007), while turtles at higher latitudes are in a region with higher rates of denitrification, leading to enriched phytoplankton $\delta^{15}\text{N}$ (Fennel et al. 2006).

We believe the observed nitrogen patterns are also partially driven by differences in foraging strategies among the aggregations we sampled. Loggerheads are generalists that feed opportunistically on a wide range of food items, from benthic bivalves to crustaceans to jellyfish (Hopkins-Murphy et al. 2003 but see McClellan et al. 2010). Our northernmost sampling location (CAN; the Scotian Shelf, Slope and the abyssal plain) occurred farther from shore, on the continental shelf break and in deeper waters (depth > 200 m) and consisted mostly of Stage III juveniles and possibly some Stage II juveniles, which are exclusively oceanic (TEWG 2009). This difference in age class and habitat may explain why $\delta^{15}\text{N}$ values of turtles from this location were intermediate (higher than the SNWA but lower than the MAB and SAB). Loggerheads sampled off the Scotian Shelf Slope most likely feed in the epipelagic zone at a lower trophic level compared to those on the continental shelf that feed mostly on benthos. As ^{15}N becomes enriched at higher trophic levels (Peterson and Fry 1987), turtles feeding lower on the food web are less enriched as confirmed by McClellan et al. (2010), who found that loggerheads that moved into the oceanic environment had significantly lower $\delta^{15}\text{N}$ than those remaining on the continental shelf. In addition, loggerheads on the continental shelf may forage on a variety of benthic prey; thus, variation in $\delta^{15}\text{N}$ values may be due also to differences in diet (trophic differences) among individuals within and among sites. Despite being generalist consumers, we found low within site isotopic variation (Appendix B) suggesting that individual loggerheads feed on a similar mixture of diet within an area. Therefore, the isoscapes we produced appear to be a good representation of the overall isotopic values of loggerheads at each site.

Lastly, we found that loggerheads that were sampled from or took up residence off large river/estuary systems (e.g., Savannah River, Chesapeake Bay, Delaware Bay) had the most $\delta^{15}\text{N}$ -

enriched values even though they most likely share the same foraging strategy of loggerheads in the SNWA (feeding upon benthos in the neritic habitat). We expected turtles at intermediate latitudes to be more $\delta^{15}\text{N}$ -enriched than individuals sampled in the SNWA due to the shift in nitrogen fixation/denitrification rates, but we suspect that anthropogenic factors such as agricultural runoff and anthropogenic waste, which are known to increase $\delta^{15}\text{N}$ in nearshore compared to mid-shelf ecosystems (McKinney et al. 2010), are responsible for the higher values observed. Sampling prey items from these areas, the use of additional elements (in particular contaminants associated with anthropogenic activities) and examining the spatial and temporal (seasonal and annual) variation in isotopic signatures could provide further insights.

The stable isotope patterns in loggerhead tissues are only partially in agreement with the recently published zooplankton $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes for the Atlantic Ocean (McMahon et al. 2013). Contrary to the patterns we observed, McMahon et al.'s $\delta^{13}\text{C}$ isoscape shows little spatial structure within the geographic area we sampled, while their $\delta^{15}\text{N}$ isoscape indicates a progressive northward enrichment in $\delta^{15}\text{N}$ values between the SNWA and the Grand Banks. These discrepancies are likely due to the differences in scale (ocean basin vs. continental shelf) and resolution (sample locations) of study as well as species (zooplankton-primary consumer vs. loggerhead-apex predator).

Isoscape Model Assumptions

The isoscapes we developed based on epidermis have some implicit assumptions and considerations. First, tissue turnover rates and discrimination factors are unknown for most taxa

and several authors have called for more captive studies (e.g., Seminoff et al. 2007, Martinez del Rio et al. 2009) to address this critical knowledge gap and related assumptions commonly used in stable isotope studies. We, like others (e.g., McClellan et al. 2010, Reich et al. 2010, Pajuelo et al. 2012, Seminoff et al. 2012), assumed epidermis and RBC turnover rates were on the order of months; thus, results could slightly differ between samples representing summer foraging grounds versus overwintering areas. Migratory differences may also affect tissue turnover rates in loggerheads sampled in different geographic areas. Telemetry and long-term studies at feeding grounds have shown that juvenile and adult loggerheads reside year-round in southern foraging areas (e.g., the Florida Keys, the Bahamas, south west Florida) with the exception of breeding migrations (Eaton et al. 2008, Girard et al. 2009, Ceriani et al. 2012). Thus, even though skin turnover rate for large loggerhead class sizes can only be estimated, we can assume that skin represents the isotopic signature of the foraging area for loggerheads in the SNWA. Similarly, SAB loggerheads are either year-round or seasonal residents (Henwood 1987, Hawkes et al. 2011, Arendt et al. 2012a, Ceriani et al. 2012); therefore, their skin represents the isotopic signature of the SAB foraging area. On the other hand, satellite telemetry, fishery interaction and aerial survey data have shown that loggerheads form seasonal aggregations and forage at high latitudes (MAB and off the Scotian Shelf) from May to October every year (Shoop and Kenney 1992, Epperly et al. 1995, Witzell 1999, Brazner and McMillan 2008, Mansfield et al. 2009). MAB loggerheads as well as many from North Carolina estuaries overwinter south of Cape Hatteras (NC) or move as far south as North Florida (McClellan and Read 2007, Mansfield et al. 2009, Hawkes et al. 2011). We suspect that metabolic rate and, thus, tissue turnover rates, increase during summer months as with other ectotherms (Gillooly et al. 2001, Wallace and Jones 2008). Slow-turnover rate tissues (skin and RBC) collected at northern, summer foraging

grounds reflect an integration of the food and the habitat experienced at both summer foraging grounds and overwintering areas (McClellan et al. 2010), but the relative contribution of each is unclear. This could be further investigated by modeling the effect of differential metabolic rates on tissue turnover rates.

One goal of generating isoscapes is to examine the movement patterns and habitat use of migratory animals with unknown behaviors. Although these isoscapes represent a promising starting point, much can be done to constrain the maps before using them to track loggerhead movements and identify habitat use on ecologically relevant spatial scale. To develop meaningful predictive models, future studies need to examine temporal isotopic variability and improve the sampling across the geographic area of interest. As with other marine isoscapes (Graham et al. 2010, Jaeger et al. 2010, McMahon et al. 2013), our maps are necessarily constrained over time and space scales by our sampling ability. The isoscapes we generated are based on tissues sampled over a five-year period (2009-2013). Aside from 18 individuals (McClellan and Read 2007), our data set prevented us from investigating isotopic temporal variability. However, a previous study found that adult NWA loggerheads exhibit high consistency in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ over an estimated 4 to 12-years time span (Vander Zanden et al. 2010) suggesting temporal isotopic stability, which is also supported by our analysis of scute samples of satellite-tracked loggerheads (Ceriani *unpublished data*). Spatially, our data set consisted of clumped samples and lacked isotopic values for the coastal areas between southern New Jersey and New England, while the coastal area off Georgia and South Carolina were based on only a few samples. Moreover, the majority of our sampling took place on the continental

shelf (with the exception of the waters off Nova Scotia); thus, our isoscapes for the northern MAB, SAB and the oceanic environment should be interpreted with caution as suggested by the standard error distribution maps (Figures 3.5B, 3.6B). Little is known about loggerheads found during summer months off the Scotian Shelf. This smaller class of loggerheads will mostly leave the area after the water reaches a threshold temperature and move either south or to deeper waters near the warmer Gulf Stream (as seen by McClellan and Read 2007 and Mansfield et al. 2009); thus, satellite telemetry could help elucidate their movements and associated foraging behavior and inform future isoscapes. Lastly, our isoscapes were based on juvenile and adult loggerhead samples, whose body sizes ranged from 51.0 to 111.2 cm (CCL); therefore, the isoscapes we produced should not be used to generate hypotheses on isotopic values of smaller and exclusively oceanic loggerheads (Stage II). Future studies should investigate the full extent of juvenile and adult loggerhead geographic range in the NWA (e.g., the Gulf of Mexico) and model the contribution of environmental factors (e.g., SST, bathymetry) that affect the geographic distribution of isotope signatures and how they could be included to improve the isoscapes (Bowen et al. 2005). Ideally, continuous surface assignment models should incorporate and model several levels of isotopic variation such as (1) within site variation (namely isotopic variation found in individuals sampled at a given site), (2) isotopic differences between age groups and (3) inter-annual isotopic variation (Wunder and Norris 2008, Wunder 2010, 2012).

Conclusions

Recently, Ramos and Gonzalez-Solis (2012) suggested that marine top predators are ideal candidates to assess ocean health and sustainability. Along with sea birds, marine mammals and

sharks, sea turtles are caught in large numbers as a result of fishery by-catch (Hall et al. 2000, Baum et al. 2003, Lewison et al. 2004); thus, a better understanding of their spatial ecology has become a conservation and management priority (Hamann et al. 2010). In addition to conserving *Sargassum* and nesting habitats, essential for oceanic and breeding adult loggerheads, respectively, critical foraging grounds for larger class sizes with high reproductive value (Crouse et al. 1987) must be identified and protected in order to develop a holistic management approach for this imperiled species. Our exploratory isoscapes demonstrate that it may be possible to develop predictive foraging habitat models tailored to sea turtles; thus, the spatially explicit isotopic approach may be used as a conservation tool to identify loggerhead foraging areas with a spatial resolution greater than the one currently provided by the nominal approach (e.g. DFA).

Hundreds of sea turtles (and loggerheads, in particular) have been equipped with satellite tags in the last decade in the NWA and the Gulf of Mexico alone (e.g., McClellan and Read 2007, Girard et al. 2009, Mansfield et al. 2009, Hawkes et al. 2011, Sasso et al. 2011, Arendt et al. 2012a,b,c, Ceriani et al. 2012) and tissue samples have been collected for genetics and/or stable isotope analysis. Extensive spatial and temporal tracking data sets are becoming available that could be integrated to develop refined isoscapes based on isotopic values of satellite tracked individuals. Once refined, these species-specific isoscapes could be integrated to develop a dual-element isoscape, overlaid with different geographic features (e.g. SST, sea grass distribution) and used to develop continuous-probability surfaces for the assignment of unknown origin individuals that are commonly sampled both on the nesting beaches (e.g., Hatase et al. 2002, Zbinden et al. 2011, Ceriani et al. 2012, Van der Zanden et al. 2013) and at sea (e.g., the U.S. NMFS fishery observer program). This, in turn, may provide resource managers the ability to

identify higher probability areas of interaction with anthropogenic activities (e.g., fishery operations, military activities, oil exploration) and where to apply finer scale resolution tools (e.g. aerial surveys, satellite telemetry) in order to pin point conservation priority areas.

Thus, this study provides further evidence supporting the use of the isotopic approach to unravel migratory connectivity in marine systems. We provided independent evidence supporting the use of nominal assignment models based on a relatively small number of tracked individuals in the NWA and developed the first species-specific isoscapes for this region. Our isoscapes even though basic suggest that a spatial explicit approach may provide an additional tool to explore migratory connectivity in this endangered species and visualize geographic isotopic patterns at a finer spatial resolution than previous studies in the Atlantic Ocean (McMahon et al. 2013).

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CHAPTER 4: DEVELOPING A COMMON CURRENCY FOR STABLE ISOTOPE ANALYSES OF NESTING MARINE TURTLES³

Introduction

Studying animal migration is challenging as migratory species often traverse vast distances and are often elusive. Nonetheless, identifying linkages between habitats used by migratory species during their lifecycle is necessary to understand their ecology, demography and evolutionary biology. There is an urgency to understand migratory connectivity because it is unknown how imperiled migratory species will respond to threats posed by climate change and habitat loss and degradation (Hobson and Norris 2008), which typically differ between foraging and breeding areas. Despite difficulties, our understanding of animal migration has seen tremendous improvements over the last two decades thanks to advances in genetics, stable isotope applications and tracking device technology (e.g., miniaturization, light geo-locators and solar-powered satellite tags). Multi-technique approaches have proven to be the most powerful at unraveling linkages between breeding, wintering and intermediate stopover sites used by migrants (Clegg et al. 2003; Roscales et al. 2011; Chabot et al. 2012).

Stable isotope analysis of light elements (C, H, N, O and S) is commonly used to identify migratory linkages. This approach is based on the idea that the ratios of stable isotopes of naturally occurring elements vary across the landscape, often in systematic ways and at the

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continental scale due to a variety of biogeochemical processes. Patterns in stable isotope ratios at the base of food webs are amplified to various degrees (depending on the element) at higher trophic levels. Stable isotopes act as forensic recorders of animal migratory and foraging behaviors if organisms move between isotopically distinct landscapes and maintain quantifiable isotopic differences in one or more tissues, either permanently (e.g., feathers, hairs, whiskers, nails) or integrated over some time duration (e.g., blood solutes, skin), that can be linked to past locations (Hobson and Norris 2008). If these prerequisites are met, stable isotopes function as intrinsic markers that reflect the isotopic composition of the environment (location and food web) where the tissue under consideration was synthesized.

A growing body of literature has used stable isotope analysis of slow-turnover-rate (e.g., skin) and metabolically inert (e.g., feathers) tissues to investigate migratory connectivity (e.g., Hobson and Wassenaar 1997; Witteveen et al. 2009). In the majority of cases, migrants are intercepted either at foraging (e.g., whale sharks, Wilson et al. 2006) or breeding areas (e.g., song birds, Norris et al. 2004) or stopover sites (e.g., song birds, Wilson et al. 2008) because only one of these locations is known or is logistically feasible to sample. Many organisms tend to aggregate during the breeding season (e.g., geese, Cooper 1978; humpback whales, Witteveen et al. 2009; salmon, Gross 1991). Sampling breeding aggregations are ideal because these aggregations (i) are often spatially and temporally predictable and (ii) usually represent a mix of individuals coming from several isotopically distinct foraging areas. In addition, a variety of marine migratory organisms across taxa are tied to land for reproduction (e.g., penguins, sea lions, marine turtles, pelagic sea birds), which facilitates sampling. Having access to marine organisms during their reproductive stage on land has enabled a variety of questions to be addressed related

to their reproductive physiology (Cherel et al. 2005), feeding ecology (Cherel 2008; Paez-Rosas et al. 2012) and migration routes (Roscales et al., 2011; Ceriani et al. 2012).

Stable isotopes have been increasingly used to study marine turtle migratory connectivity (Hatase et al. 2002), foraging ecology (Wallace et al. 2009; McClellan et al. 2010) and ontogenetic habitat shifts (Arthur et al. 2008). The technique has proven to be particularly effective to study migratory linkages for the adult life stage. The life history and reproductive biology of sea turtles makes them ideal for stable isotope applications. Sea turtles are highly migratory and tend to move across broad geographic scales. Females embark on breeding migrations every 1 to 4 years between spatially distinct foraging grounds and breeding areas. Each female from a nesting aggregation typically forages in one of several geographically distinct foraging grounds (Schroeder et al. 2003; Girard et al. 2009; Hawkes et al. 2011; Ceriani et al. 2012; Foley et al. 2013). Individual females appear to show fidelity to both nesting and feeding areas throughout adult life (Miller 2003; Broderick et al. 2007). Lastly, sea turtles are capital breeders, using energy stored at the non-breeding ground for reproduction (Stearns 1992), for whom maternal investment ends with egg deposition.

Researchers have collected a variety of tissue samples from nesting females for stable isotope analysis to assign the putative foraging area used during the non-breeding season. Most studies have used to some extent satellite telemetry to validate the use of stable isotopes to assign foraging areas. These studies have employed a variety of tissues and all but one (Caut et al. 2008) has used a single tissue approach: skin samples (loggerheads, Reich et al. 2010; Pajuelo et al. 2012; leatherbacks, Seminoff et al. 2012; green turtles, Vander Zanden et al. 2013), red blood

cells -hereafter RBC- (leatherbacks, Caut et al. 2008; loggerheads, Ceriani et al. 2012), freshly laid eggs (loggerheads, Hatase et al. 2002; leatherbacks, Caut et al. 2008) and a combination of freshly laid and unhatched eggs (loggerheads, Zbinden et al. 2011). Despite the variety of tissues used and the fact that tissue turnover rates in adult sea turtles are unknown, all of the tissues examined so far have been shown to represent the foraging area used by the female, most likely because sea turtles are large bodied ectotherms and, as such, have slow metabolic and tissue turnover rates.

Given the success of this relatively inexpensive technique, studying migratory connectivity with stable isotope analysis has become widespread. However, protocols, such as the choice of tissue to sample, are not standardized, which makes comparisons and meta-analysis problematic. We initiated this study to (1) define the relationship among tissues that are commonly used to infer sea turtle non-breeding areas, (2) test whether freshly laid egg yolks and unhatched non-viable eggs are isotopically equivalent and (3) test whether a single, non-intrusive sampling event (collecting unhatched eggs from a single nest) over the course of the 3-4 month nesting season adequately represents the isotopic signature of the foraging area.

Materials and Methods

Study Site and Sampling

We collected multiple tissues (blood, skin biopsy and at least one unhatched whole egg retrieved during post-hatch clutch excavation) for stable carbon and nitrogen isotope analysis from $n = 80$ loggerheads nesting along the Atlantic coast of Florida between 2009 and 2012. Females included in this study were sampled in two locations: the Archie Carr National Wildlife Refuge (ACNWR) in east central Florida and Juno Beach in South Florida. These two beaches (total length = 28 km) are loggerhead hotspots in the western hemisphere and account for approximately 22% (i.e., 27,000 nests in 2012) of all the nests laid in Florida each year (Florida Fish and Wildlife Conservation Commission, unpub data). Nesting activity is monitored at both sites and a subsample of females is encountered and tagged using both Inconel flipper tags and passive integrated transponders during night surveys. Blood samples (4 ml) were collected from the cervical sinus with a 20-gauge needle and syringe (Owens and Ruiz 1980) as soon as the turtle began to cover her nest. Blood was transferred to a non-heparanized container and placed in ice. Skin samples were collected from the right shoulder (the area between the neck and the front flipper) using sterile 4 mm biopsy punches. Thirty six (of the 80) females included in this study were sampled in conjunction with telemetry projects investigating movement patterns during the inter-nesting period, post-nesting migration destinations or estimating demographic parameters such as annual survival (Ceriani et al. 2012; Ceriani et al. in review; Ceriani et al. unpub data; Sasso et al. unpub data). We obtained a freshly laid egg at deposition only from individuals equipped with tracking devices as it is sometimes impossible to retrieve unhatched

eggs from a given clutch at post-hatching excavation due to beach erosion or predation. We followed the fate of the nest laid by the 80 individual females and excavated nests after hatching emergence (3 to 14 days post-emergence) to retrieve at least one unhatched egg for stable isotope analysis.

Loggerheads lay several clutches (mean = 4, range 2-8; Miller 2003; Tucker 2010) over the course of the 4-month nesting season, at approximately 2-week intervals, and each clutch contains an average of 112 eggs (Miller 2003). To examine inter-clutch variability in egg isotopic values, we collected a single freshly laid egg per nest from 11 females equipped with a tracking device that were encountered repeatedly over the course of the nesting season. To examine intra-clutch variability in egg isotopic values, we retrieved 5-10 unhatched eggs at time of post-hatching excavation from 19 (of the 80) nests. We used unhatched eggs to minimize the amount of destructive sampling because sea turtles are species of conservation concern and unhatched eggs are readily available in most nests, varying in number from one egg per clutch (or none in some rare cases) to the entire clutch (Ehrhart unpub data). If several unhatched eggs were available, we retrieved whole undeveloped eggs; we did not collect broken eggs or those containing late stage development fetuses. Samples were stored in ice until returning to the field station. Blood was separated into serum and cellular components by centrifugation (5000 rpm x 10 min). Tissue samples (RBC, serum, skin, freshly laid egg and unhatched undeveloped eggs) were frozen at -20°C until analysis.

Sample Preparation and Stable Isotope Analysis

To address our objectives, we measured the stable carbon and nitrogen isotope ratios of RBC, epidermis, fresh egg yolk, unhatched eggs and serum. Other than serum, these tissues are assumed to have slow turnover rates that should reflect an integration of diet and habitat at the foraging ground prior to breeding migration. Tissue turnover rates in adult sea turtles are unknown, but it has been estimated that RBC, epidermis and egg yolk reflect the foraging habits at least four months prior to sampling (Brace and Altland 1955; Ceriani et al. unpub data; Hamann et al. 2003; Reich et al. 2008, 2010; Seminoff et al. 2007).

Samples were prepared following standard procedures. Skin samples were rinsed with distilled water and cleaned with 70% ethanol. Epidermis (*stratum corneum*) was separated from the underlying tissue (*stratum germinativum*) and finely diced using a scalpel blade. Epidermal samples were then dried at -60°C for 48 hours. Fresh eggs were separated into egg components (albumen and yolk) using an egg separator. In the case of unhatched eggs, it is often not possible to distinguish between egg components so the entire egg content was used for analysis after removing the eggshell.

RBC, egg (egg yolk and unhatched egg) and serum samples were freeze-dried for 24 to 72 h before being homogenized with mortar and pestle. Lipids were removed from all tissues using a Soxhlet apparatus with petroleum ether as solvent for 12 h (RBC and serum) and 24 h (epidermis, egg yolk and unhatched egg). Subsamples of prepared tissues (0.4-0.7 mg) were weighed with a microbalance and sealed in tin capsules. Prepared samples were sent to the

Paleoclimatology, Paleoceanography and Biogeochemistry Laboratory at the University of South Florida College of Marine Science (St. Petersburg, FL, USA), where they were converted to N₂ and CO₂ using a Carlo-Erba NA2500 Series 2 Elemental Analyzer (Thermoquest Italia, S.p.A., Rodano, Italy) and analyzed with a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen). Stable isotope ratios were expressed in conventional notation as parts per thousand (‰) according to the following equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where X is ¹⁵N or ¹³C, and R is the corresponding ratio ¹⁵N:¹⁴N or ¹³C:¹²C. The standards used for ¹⁵N and ¹³C were atmospheric nitrogen and Peedee Belemnite, respectively. Estimates of analytical precision were obtained by replicate measurements of internal lab reference materials (1577b Bovine liver) and yielded a precision (reflecting ± 1 SD) of 0.13‰ for δ¹³C and 0.16‰ for δ¹⁵N.

Statistical Analyses

Principal Component Analysis (PCA) and simple linear regressions were used to illustrate the relationships among tissues sampled: RBC, epidermis, fresh egg yolk, unhatched egg, and serum. Since we collected a fresh egg only from females equipped with tracking units, we performed two sets of analyses. PCA was used to define the overall pattern in the 36 tracked loggerheads for which we had δ¹³C and δ¹⁵N values for the 4 different tissues (RBC, epidermis, fresh egg yolk and unhatched egg) that have been used in previous studies to assign females to non-breeding foraging areas. We did not include serum in the PCA. Even though tissue turnover rates are not known for adult loggerheads, it is commonly assumed that serum has a much faster

turnover rate compared to the other tissues (Hobson 1999), and thus, it is not the tissue of choice for foraging area assignment.

We used PC-ORD v5 (2006) for the PCA and the R Statistical Package (R Development Core Team 2011) for the remainder of the analyses with an alpha level set to 0.05 for all statistical analyses. We were interested in developing predictive equations to derive isotopic values of unhatched egg from other tissues because only this tissue can be collected widely and non-invasively. Therefore, unhatched egg isotopic values were treated as the dependent variables in all comparisons. Although we collected up to ten unhatched eggs from each individual, when results for multiple unhatched eggs were available for a single clutch, we randomly chose one unhatched egg to include in the analysis. We conducted four simple linear regressions for each element, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($n = 36$ for independent variables fresh egg and serum, which we had only from the tracked females; $n = 80$ for RBC and epidermis, which were sampled from all females); we applied the Bonferroni correction to account for the risk of inflating Type I error and set an adjusted α -level (α') to 0.006 ($\alpha = 0.05/8$). Lastly, we used a paired t-test to compare stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) between unhatched egg and fresh egg yolk from each female.

To determine whether a single sampling event occurring at any time during the nesting season provides an adequate isotopic representation of the foraging area used in the non-breeding season, we examined inter-clutch variation in fresh egg-yolk isotopic values for females ($n = 11$ individuals) that were repeatedly sampled throughout the nesting season. We developed general

linear models (GLM) in which the dependent variable was each stable isotope ratio ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and the independent variables were the individual turtle (random effect) and the time in days since the female's first clutch was observed (time 0 corresponded to the day we observed the first clutch for each female). Normality of the dependent variables was evaluated prior to the analyses.

To examine intra-clutch variation, we used clutches for which we collected at least five unhatched eggs ($n = 19$ individual clutches from different females). We followed a Monte Carlo approach to calculate the mean isotopic values for each element ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) as a function of number of eggs sampled from randomly drawn combinations of eggs for each of the 19 clutches. We used the overall standard deviation of the reference material (lab precision) to determine how many eggs per clutch should be sampled to maintain the representative level of isotopic variation for the clutch, keeping within the measurement precision of the lab.

Results

Isotopic Relationships Among Tissues

Isotopic signatures of the five tissues examined (RBC, epidermis, fresh egg yolk, unhatched egg and serum) are summarized in Figure 4.1. We found marginal differences and no significant

difference in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between fresh egg yolk and unhatched egg from each female ($\delta^{13}\text{C}$: Paired t-test, $t_{35} = 1.97$, $P = 0.06$; $\delta^{15}\text{N}$: Paired t-test, $t_{35} = -1.01$, $P = 0.32$), respectively.

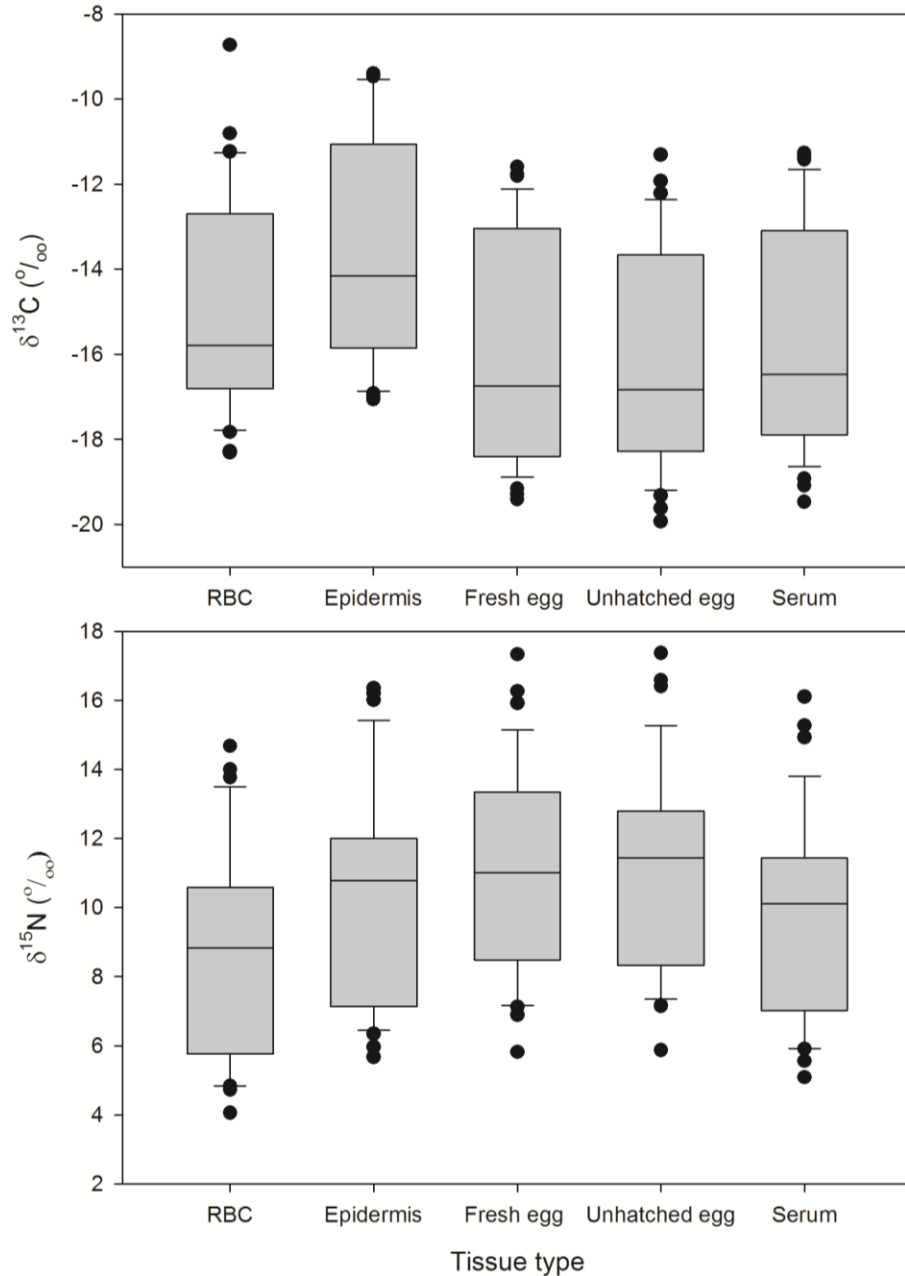


Figure 4.1. Box plot summarizing the distribution of $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) ratios found in five tissues (RBC, skin, fresh egg yolk, unhatched egg and serum) from 36 nesting loggerhead turtles. The box extends from the 25th to the 75th percentile; the central line indicates the median. The whiskers extend from the 10th to the 90th percentile. Black circles represent outliers.

We used PCA to reduce the number of inter-correlated variables, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of several turtle tissues, into one or a few variables. The single significant PCA-derived variable (PC1), based on Jackson's "heuristic" approach to determine the number of significant axes (Jackson 1993 cited in McCune and Grace 2002), explained 83.5% of the variance of the isotopic values. The first two principal components (PC1 and PC2) accounted for 97.5% of the variance. PC1 was highly positively correlated to $\delta^{15}\text{N}$ and highly negatively correlated to $\delta^{13}\text{C}$ (Table 4.1). Though all tissues should work well, the large differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ correlation values with both freshly laid and unhatched eggs in axis 1, suggest that these tissues should discriminate sufficiently among isotopic signatures. Foraging areas used by 19 of the 36 tracked loggerheads included in the PCA were known, but for the remaining 17 individuals the tag failed prematurely and did not transmit all the data at once one year after deployment as programmed. PCA results showed distinct clustering of the PC scores for females using the three different foraging areas described in Ceriani et al. (2012) with only partial overlap between clusters (Figure 4.2). The distribution of isotopic signatures from the combined tissues show a similar grouping pattern to the latitudinal gradient found in Ceriani et al. (2012). As shown by the correlation matrix (Table 4.2), levels of a particular element were highly correlated among tissues. However, $\delta^{13}\text{C}$ was negatively associated with $\delta^{15}\text{N}$.

Table 4.1. Pearson and Kendall correlations (r) and relative weights (tau) of carbon and nitrogen stable isotope ratios by tissue on the first two principal component axes.

Axis	1		2	
	r	tau	r	tau
$\delta^{13}\text{C}_{\text{RBC}}$	-0.905	-0.726	-0.386	-0.291
$\delta^{15}\text{N}_{\text{RBC}}$	0.924	0.737	-0.349	-0.187
$\delta^{13}\text{C}_{\text{epidermis}}$	-0.908	-0.719	-0.382	-0.270
$\delta^{15}\text{N}_{\text{epidermis}}$	0.924	0.732	-0.353	-0.199
$\delta^{13}\text{C}_{\text{fresh egg}}$	-0.915	-0.724	-0.384	-0.295
$\delta^{15}\text{N}_{\text{fresh egg}}$	0.915	0.781	-0.388	-0.168
$\delta^{13}\text{C}_{\text{unhatched}}$	-0.921	-0.748	-0.354	-0.253
$\delta^{15}\text{N}_{\text{unhatched}}$	0.900	0.748	-0.411	-0.195

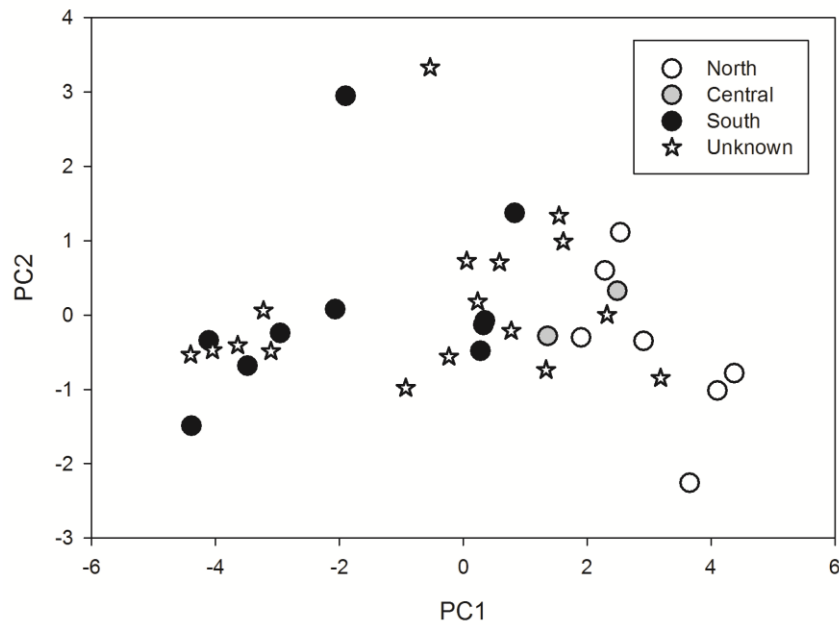


Figure 4.2. Principal component analysis (PCA) ordination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of loggerhead tissues. Foraging areas used by 19 of the tracked loggerheads were known to be north of the nesting area (white circles), near the nesting area in eastern central Florida (grey circles), or south of the nesting area (black circles; see Ceriani et al. 2012). Star symbols represent loggerheads whose foraging ground destination was unknown.

Table 4.2. Cross-products matrix with correlation coefficients among isotopes measured in the four tissue types included in the Principal Component Analysis. Abbreviations are as follow: RBC = Red Blood Cells, epi = epidermis, fresh = fresh egg, unhatched = unhatched egg.

	$\delta^{13}\text{C}_{\text{RBC}}$	$\delta^{13}\text{C}_{\text{epi}}$	$\delta^{13}\text{C}_{\text{fresh}}$	$\delta^{13}\text{C}_{\text{unhatched}}$	$\delta^{15}\text{N}_{\text{RBC}}$	$\delta^{15}\text{N}_{\text{epi}}$	$\delta^{15}\text{N}_{\text{fresh}}$	$\delta^{15}\text{N}_{\text{unhatched}}$
$\delta^{13}\text{C}_{\text{RBC}}$	1.000							
$\delta^{13}\text{C}_{\text{epi}}$	0.954	1.000						
$\delta^{13}\text{C}_{\text{fresh}}$	0.969	0.970	1.000					
$\delta^{13}\text{C}_{\text{unhatched}}$	0.959	0.961	0.975	1.000				
$\delta^{15}\text{N}_{\text{RBC}}$	-0.695	-0.712	-0.715	-0.721	1.000			
$\delta^{15}\text{N}_{\text{epi}}$	-0.698	-0.699	-0.714	-0.725	0.971	1.000		
$\delta^{15}\text{N}_{\text{fresh}}$	-0.683	-0.684	-0.687	-0.699	0.973	0.977	1.000	
$\delta^{15}\text{N}_{\text{unhatched}}$	-0.658	-0.656	-0.659	-0.695	0.964	0.966	0.982	1.000

We found highly significant positive relationship between unhatched egg $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the corresponding RBC ($\delta^{13}\text{C}$: $r^2 = 0.91$, $F_{(1,78)} = 820.39$, $P < 0.001$; $\delta^{15}\text{N}$: $r^2 = 0.86$, $F_{(1,78)} = 494.81$, $P < 0.001$), epidermis ($\delta^{13}\text{C}$: $r^2 = 0.83$, $F_{(1,78)} = 451.91$, $P < 0.001$; $\delta^{15}\text{N}$: $r^2 = 0.86$, $F_{(1,78)} = 468.61$, $P < 0.001$), fresh egg-yolk ($\delta^{13}\text{C}$: $r^2 = 0.95$, $F_{(1,34)} = 736.75$, $P < 0.001$; $\delta^{15}\text{N}$: $r^2 = 0.96$, $F_{(1,34)} = 861.69$, $P < 0.001$) and serum ($\delta^{13}\text{C}$: $r^2 = 0.78$, $F_{(1,34)} = 130.14$, $P < 0.001$; $\delta^{15}\text{N}$: $r^2 = 0.83$, $F_{(1,34)} = 147.59$, $P < 0.001$) values (Figure 4.3).

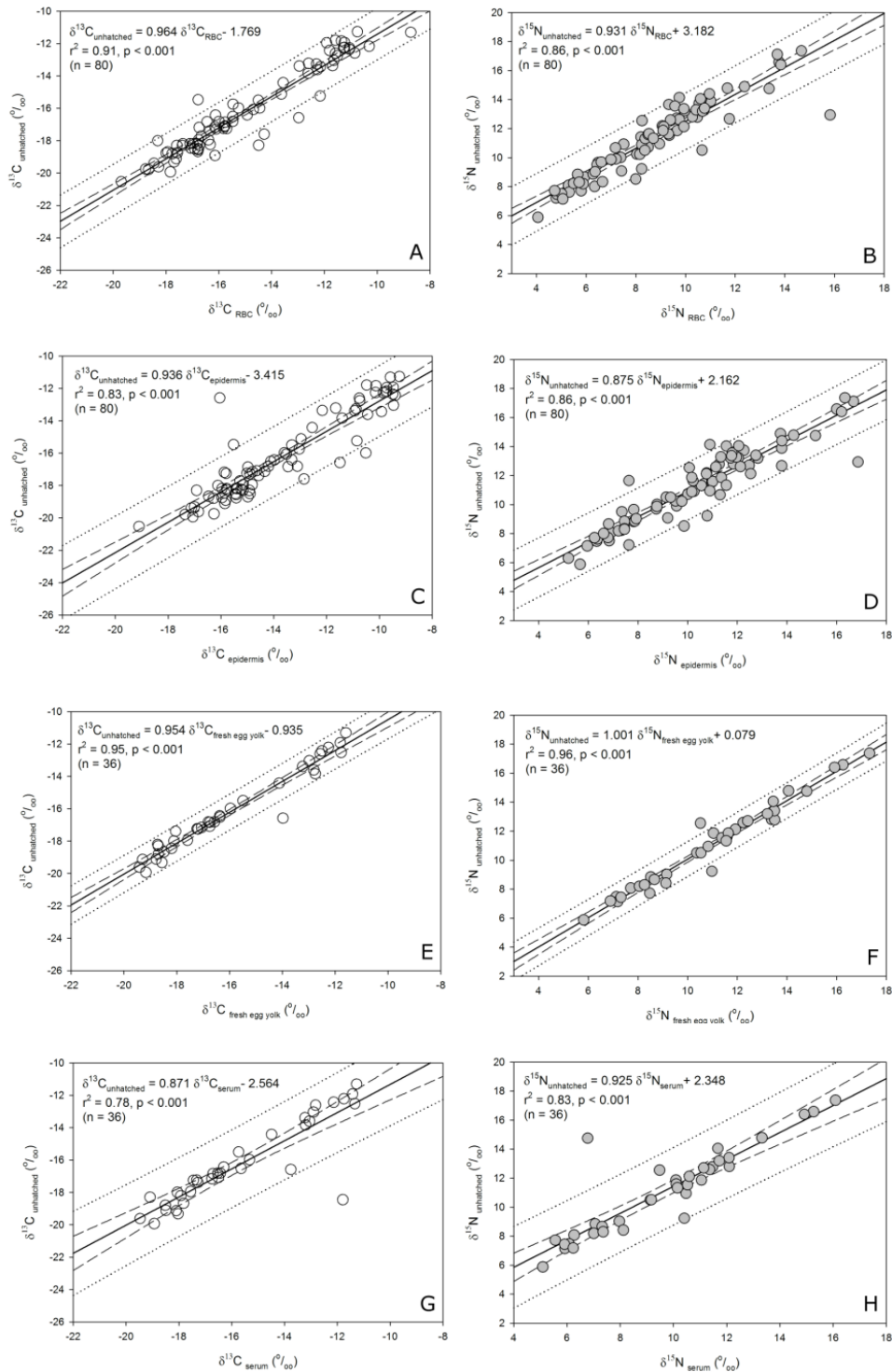


Figure 4.3. Relationships between unhatched egg isotopic values and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for RBC (A & B), epidermis (C & D), fresh egg yolk (E & F) and serum (G & H).

Inter-clutch Egg Isotopic Variability

We had fresh eggs from multiple clutches for 11 loggerheads equipped with tracking devices and used these females to investigate among-clutch variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ egg values within a given nesting season. We were unable to retrieve unhatched eggs at post-hatching excavation from each clutch for all 11 turtles as some nests were lost due to beach erosion or raccoon depredation. Therefore, we used fresh eggs to examine inter-clutch variability in stable isotope ratios. Eggs from one female were sampled five times, eggs from another female were sampled four times, eggs from five females were sampled three times and eggs from four females were sampled twice. The time of each laying event marginally affected $\delta^{13}\text{C}$ in fresh egg yolk, becoming progressively more depleted from one clutch to the next (rate of $\delta^{13}\text{C}$ change = -0.066 ‰, $F_{1,10} = 3.97$, $P = 0.074$, Figure 4.4A), while $\delta^{15}\text{N}$ did not change significantly over time ($F_{1,10} = 0.0006$, $P = 0.98$, Figure 4.4B).

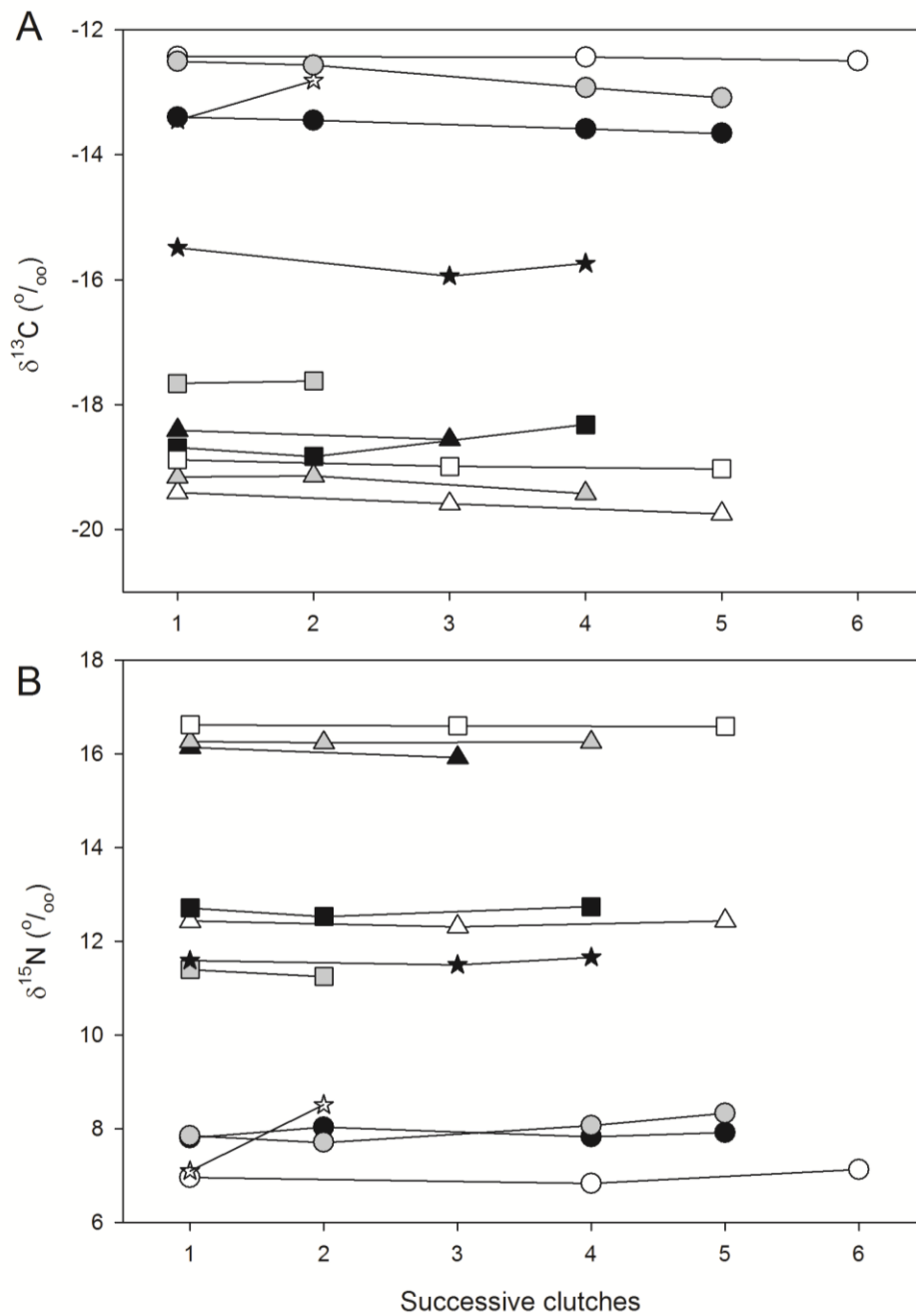


Figure 4.4. Inter-clutch isotopic variability: trends in isotopic values for $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) in fresh egg yolk of 11 nesting loggerhead turtles. Each point represents a sampled clutch; each line connects successive clutches laid by one female. Markers correspond to specific females.

Intra-clutch Egg Isotopic Variability

Five or more unhatched eggs (range = 5 to 10 eggs/clutch) were analyzed from a single clutch from 19 different females. The overall lab analytical uncertainty standard deviation was 0.13 ‰ for $\delta^{13}\text{C}$ (range 0.08 to 0.23 ‰) and 0.16 ‰ for $\delta^{15}\text{N}$ (range 0.10 to 0.23 ‰). The overall unhatched egg mean standard deviation within each clutch was 0.21 ‰ for $\delta^{13}\text{C}$ (range 0.04 to 0.53‰) and 0.20 ‰ for $\delta^{15}\text{N}$ (range 0.04 to 0.47 ‰). The Monte Carlo analyses showed (Figure 4.5) the level of variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ associated with the number of unhatched eggs with respect to the analytical precision of the lab. The probability that the clutch sample mean fell within the range of lab precision improved as the number of unhatched eggs analyzed per clutch increased up to three eggs. This sampling exercise suggests that three unhatched eggs sufficiently provide an accurate isotopic representation of the clutch as a whole.

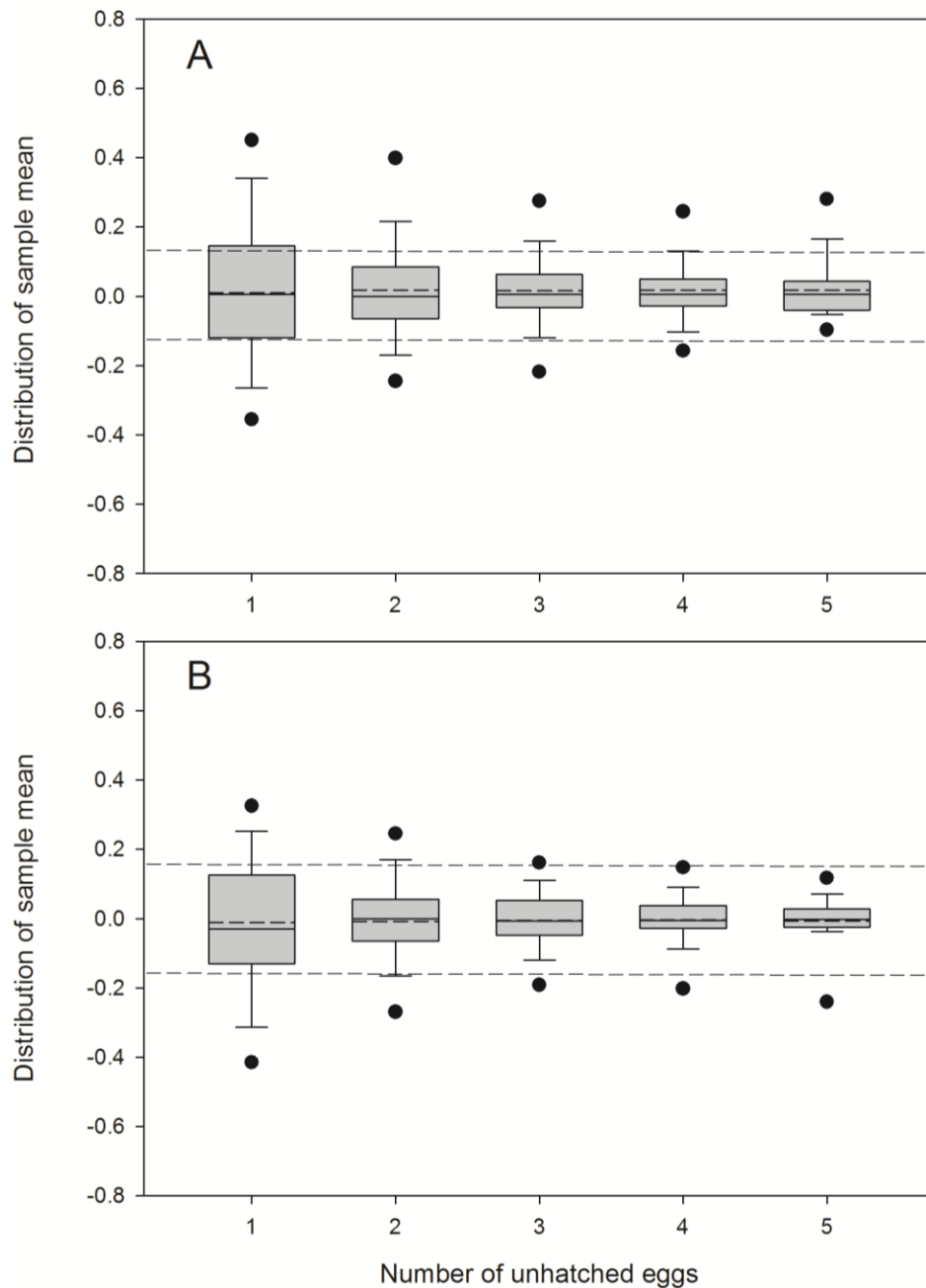


Figure 4.5. Intra-clutch isotopic variability in $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) for the 19 clutches for which we collected more than 5 unhatched eggs. The boxplots summarize 100 Monte Carlo simulations and represent the distribution of the isotopic mean as a response of the number of unhatched eggs sampled per clutch. The solid line indicates the 50th percentile (median) and the dashed line the mean. The box encompasses the 25th to the 75th percentile. The whiskers extend from the 10th to the 90th percentile. Black circles represent the 5th and 95th percentiles. The black horizontal dashed lines represent the lab analytical precision of $\pm 0.13\text{‰}$ for $\delta^{13}\text{C}$ and 0.16‰ for $\delta^{15}\text{N}$.

Discussion

Isotopic Relationship Between Tissues

We designed our study to investigate whether it is possible to (i) develop predictive equations to convert stable isotope results from one tissue into another one and (ii) implement the use of a common tissue among researchers undertaking stable isotope studies on nesting marine turtles.

In the last decade, several researchers have used stable isotope analysis to investigate migratory connectivity in adult sea turtles (Hatase et al. 2002; Caut et al. 2008; Reich et al. 2010; Zbinden et al. 2011; Ceriani et al. 2012; Pajuelo et al. 2012; Seminoff et al. 2012). Researchers have used a variety of tissues and, in most cases, a single tissue approach. We were interested in developing predictive equations that can be used to integrate results from different studies. Isotopic relationships between a few tissues have been investigated to some extent in previous studies but did not constitute their main scope. Caut et al. (2008) used fresh egg-yolk and RBC stable isotope values to identify post-nesting migration destination of leatherbacks nesting in French Guyana. In doing so, they examined the relationship between fresh egg-yolk and RBC values and found a positive and significant relationship between signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of the blood and their fresh egg yolk. However, the relationship between $\delta^{15}\text{N}$ of fresh egg yolk and RBC explained only 64% of the variation in the data (Caut et al. 2008, Table 1). Zbinden et al. (2011) used similar methods to assign putative non-breeding areas used by loggerheads nesting in Greece. Although they found a significant relationship between female carapace keratin and unhatched egg yolk $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{unhatched egg yolk}} = 0.73 \times \delta^{15}\text{N}_{\text{carapace}} + 4.69$, $r = 0.92$, $P < 0.001$),

they did not find a good fit in $\delta^{13}\text{C}$ between the two tissues (due to unequal distribution of data points across the range), and, thus, did not propose a predictive equation for $\delta^{13}\text{C}$.

We found that the relationships between unhatched egg isotopic values and RBC, epidermis, fresh egg yolk and serum were all highly significant and characterized by narrow confidence and predictive intervals. In all cases, the slope of the relationship was close to 1, indicating that the five tissues are isotopically equivalent and all represent the isotopic signature of the foraging area used by the female during the non-breeding season. Intercepts differed significantly from zero in all but one comparison (fresh yolk vs. unhatched egg) for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. These results suggest diet-tissue discrimination (i.e., the difference between isotopic values of turtle tissues and its diet) differs between unhatched eggs and the other three tissues (RBC, epidermis, and serum). Eggs were the most ^{13}C -depleted of all the tissues and the most enriched in ^{15}N .

We expected to find highly significant relationships between unhatched egg, RBC, epidermis and fresh egg-yolk isotopic values. In fact, previous studies have used satellite telemetry to validate the use of RBC (Ceriani et al. 2012; Pajuelo et al. 2012), epidermis (Seminoff et al. 2012), fresh egg yolk and unhatched eggs (Hatase et al. 2002; Caut et al. 2008; Zbinden et al. 2011) in both loggerhead and leatherback turtles and were able to assign post-nesting migration destinations based on values of $\delta^{15}\text{N}$ alone (Zbinden et al. 2011; Seminoff et al. 2012) or using a combination of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Hatase et al. 2002; Caut et al. 2008; Ceriani et al. 2012; Pajuelo et al. 2012). However, we were surprised by the strength of the relationship between unhatched egg and serum isotopic values. In endotherms, serum is a fast-turnover tissue (Hobson 1999) that

is used to investigate short-term diet and habitat use. Even though there is increasing evidence suggesting that serum has a slow turnover rate in reptiles (summarized by Rosenblatt and Heithaus 2013, Table 4.7), there are no data available on tissue turnover rate in adult sea turtles. The significant positive relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of serum and unhatched egg suggests that all the tissues we analyzed are in equilibrium with the diet of the female at the foraging ground.

Methodological Validation: Inter- and Intra-clutch Isotopic Variation

The use of egg components (i.e., fresh egg yolk, unhatched egg) or egg products (i.e., hatchling) *in lieu* of other maternal tissues (i.e., RBC, epidermis, carapace) to study sea turtle trophic ecology and infer foraging grounds has been previously investigated to some extent (Caut et al. 2008; Zbinden et al. 2011; Frankel et al. 2012). Zbinden et al. (2011) examined the relationship between stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in fresh egg yolk and unhatched eggs and found what appeared to be a systematic enrichment of yolk at clutch excavation compared to fresh egg yolk, but the conclusions were obtained using a small sample size ($n = 5$ pairs). In contrast, we found no consistent differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between unhatched egg and fresh egg yolk ($n = 36$ comparisons) and found a positive and highly significant relationship for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the two tissues, supporting the conclusion that fresh egg yolk and unhatched eggs are isotopically equivalent.

Marine turtles lay several clutches, each of which contains an average of 50-130 eggs (depending on the species, Miller 2003); thus, we examined both inter- and intra- clutch isotopic variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. We found that $\delta^{13}\text{C}$ values in egg yolk decreased slightly from one clutch to the next, becoming progressively more depleted, while $\delta^{15}\text{N}$ did not change significantly over time. Previous studies addressing inter-clutch isotopic variation found mixed results. Hatase et al. (2002) found no significant difference in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of fresh egg yolk among four serial clutches of the one loggerhead they examined but found a significant enrichment in $\delta^{15}\text{N}$ with the progression of the nesting season in one green turtle for which they sampled five serial clutches (Hatase et al. 2006). Caut et al. (2008) observed a significant decrease of $\delta^{13}\text{C}$ in fresh egg yolk in consecutive clutches of leatherbacks ($n = 23$ females) but no differences in $\delta^{15}\text{N}$, while Zbinden et al. (2011) found a small but significant decrease of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with clutch order in unhatched eggs, with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ on average becoming depleted by 0.14 ‰ and 0.13 ‰ with each successive clutch ($n = 14$ females). Despite a lack of agreement in results among studies, we agree with Zbinden's conclusion (2011) that the low magnitude of isotopic inter-clutch variability is not a concern if the purpose of the study is to use stable isotopes as intrinsic markers to infer origin of female foraging ground.

Caut et al. (2008) examined intra-clutch variability in fresh egg yolk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($n = 2$ fresh eggs from 3 clutches) and found that within-clutch variability was similar to lab measurement error. Thus, despite the small sample size, they concluded that a single egg yolk reflected the whole clutch. We found some level of isotopic variability in unhatched eggs, which may represent natural isotopic variation or may be affected by decomposition processes. Nests are

excavated and unhatched eggs retrieved anywhere between 72 hours (if hatching emergence is observed) to 2 weeks after expected emergence date (if no sign of emergence is noticed) depending on the protocol implemented by different research groups and permit guidelines. Thus, the effect of biological decomposition on unhatched eggs may vary and affect stable isotope values to different degrees and should be further investigated. Overall unhatched egg mean standard deviation within each clutch was 0.21 ‰ for $\delta^{13}\text{C}$ (range 0.04 to 0.53‰) and 0.20 ‰ for $\delta^{15}\text{N}$ (range 0.04 to 0.47 ‰). These values are comparable to the within-clutch unhatched egg isotopic variability reported by Zbinden et al. (2011). We found that analyzing three unhatched eggs is sufficient to obtain isotopic values that are representative of the whole clutch. However, such level of precision is perhaps unnecessary when the purpose of the study is to assign foraging ground used by the females because previous studies have shown that isotopic differences among distinct foraging areas are greater than 1 ‰ for at least one of the elements considered (Ceriani et al. 2012; Pajuelo et al. 2012; Seminoff et al. 2012). Even though we advise collecting three unhatched eggs during post hatching excavation, we suggest analyzing two eggs per clutch to limit analysis costs. If intra-clutch variability in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ is greater than 0.5 ‰, we recommend analyzing the remaining unhatched egg to obtain an isotopic signature that represents the female adequately to avoid mis-assignments. This precaution is particularly relevant when female isotopic signature does not belong clearly to a single foraging ground but it falls on the edge of two foraging areas.

Recently, Frankel et al. (2012) proposed to use nest content (hatchling epidermis tissue) retrieved at clutch excavation to derive the isotopic values of female foraging ground. They found that fresh dead hatchling $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were significantly correlated to the values of their

mothers, but the relationship between hatchling and female epidermis $\delta^{13}\text{C}$ should be interpreted with caution due to the small range in $\delta^{13}\text{C}$ values and low r^2 . Hatchlings that were sampled after being discovered dead in the nests (three days after hatching emergence) had significantly different discrimination values from those of live hatchlings, suggesting decomposition affects the reliability of stable isotope ratios. In addition, they found little isotopic variation in live hatchling collected from the same clutch ($n = 5$ hatchlings from three distinct nests) and suggested collecting a skin biopsy from a single live hatching to obtain an acceptable estimate of the whole nest and derive an isotopic value for the mother. We recognize the potential of sampling live hatchlings; however, we believe that unhatched eggs are the preferred tissue to use to investigate female sea turtle migratory connectivity. Even though repeated sampling of skin did not affect growth rates and health status of hatching loggerheads raised in captivity (Bjorndal et al. 2010), collecting a skin biopsy from live hatchling is invasive, logistically more difficult (hatchlings must be intercepted when leaving the nest) and requires appropriate training and permits.

Conclusions and Conservation Implications

Our results support the conclusions that (i) unhatched eggs are isotopically equivalent to RBC, epidermis, fresh egg yolk and serum, (ii) inter-clutch variability in egg isotopic values is minimal and, therefore, a single sampling event during the nesting season adequately represents the foraging ground used by the female during the non-breeding season and (iii) three unhatched eggs should suffice to account for intra-clutch isotopic variability.

The strength of the predictive equations we derived to convert tissues used in earlier studies (RBC, epidermis, fresh egg yolk) into unhatched egg stable isotope ratios provides an opportunity to combine the results of previous and future studies. Stable isotope analysis is being increasingly used to unravel sea turtle migratory connectivity and large datasets are becoming available. Being able to convert results obtained by different studies into a common tissue provides an opportunity to explore these datasets to undertake meta-analysis of stable isotope results to derive more general isotopic patterns at broader geographic scales. Moreover, our results provide empirical data supporting the choice of unhatched eggs as a common currency in stable isotope studies of nesting sea turtles. The use of unhatched eggs has profound practical and management implications. Unhatched eggs are an ideal tissue to sample because collecting unhatched eggs does not require seeing the nesting female. Few research groups intercept and work with nesting females, while morning nest monitoring programs are extremely common and in place worldwide. For example, Florida accounts for approximately 90% of all the loggerhead nests laid in the Southeast USA (2008-2012 nest number average = 62,867; Florida Index Nesting Beach Survey program) and it is debated whether it is the first or the second largest loggerhead aggregation in the world (Ehrhart et al., 2003). Despite its importance and the fact that Florida beaches are generally easy to access, only a handful of research groups encounter nesting females at night. On the other hand, the State of Florida - as well as the entire Southeast USA and many other nesting beaches around the world - have very well established and comprehensive morning monitoring programs where sea turtle nests are counted daily or on a regular basis. Thus, retrieving unhatched eggs can be done much more simply and provide an opportunity to sample at a much larger scale (both numerically and geographically). Using

unhatched eggs in turn, provides an opportunity to (i) reduce sampling biases, (ii) obtain information that is more representative at the population level, (iii) investigate relative importance of foraging grounds on a yearly basis, (iv) investigate how contribution from different foraging grounds varies over time and (v) elucidate remigration intervals and environmental parameters that may affect nesting patterns. Moreover, collecting unhatched eggs is a non-invasive and non-destructive sampling method, which is preferred in general and especially when dealing with threatened and endangered species. We recognize it is not always possible to retrieve unhatched eggs at inventory because nests may be lost due to stochastic events such as storms, hurricanes and, consequent, beach erosion, or predation (e.g., raccoons, Barton and Roth 2008). Thus, while we advocate sampling unhatched eggs, we recommend collecting a fresh laid egg at time of deposition or a skin sample if an individual is particularly important for a specific study (i.e., the female is equipped with a tracking device).

Sampling for stable isotope analysis has become a standard procedure to study animal migration across taxa and large datasets using various tissues are becoming available; thus, there is a need to develop common currency for stable isotope studies at the family or species level. While we have not compared isotopic values among tissues in other sea turtle species, we expect the strong relationships we found between tissues will be maintained across marine turtle species. This hypothesis is supported by the work Caut et al. (2008) conducted on leatherback turtles. Sea turtles are widely distributed species of conservation concerns but our understanding of their life at sea is still relatively limited. The use of unhatched eggs as a common currency can be applied to loggerhead breeding aggregations worldwide (assuming that females forage in areas that are isotopically different) and opens new opportunities to conduct large-scale studies that can

improve our comprehension of this taxon's ecology and migratory connectivity. A better understanding of sea turtle migratory connectivity is particularly important given that many of the recently compiled research priorities for marine turtle conservation and management have a significant spatial component (Hamann et al. 2010; Wallace et al. 2011).

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CHAPTER 5: CARRY-OVER EFFECTS AND FORAGING GROUND DYNAMICS OF THE LOGGERHEAD BREEDING AGGREGATION IN FLORIDA⁴

Introduction

Resource availability influences animal life history characteristics: organisms have a limited energy budget available to partition among survival, growth and reproduction so that their interaction optimizes individual fitness (Smith & Fretwell 1974, Brockelman 1975, Krebs and Davies 2007). A female's reproductive output is constrained by the amount of resources she can allocate to reproduction. As food availability is the main factor limiting the energy budget, the foraging strategy (i.e., the combination of diet and habitat use) adopted by an individual female will have a direct effect on her reproductive output. Intra-population variations in foraging strategies and their effects on annual and long-term reproductive output have been documented in many species across taxa (Marra et al. 1998, Bolnick et al. 2003, Norris et al. 2004, 2005, Zbinden et al. 2011, Hatase et al. 2013).

Migrants often spend different periods of their lives in widely separated and ecologically different locations. Advances in telemetry, genetics and biogeochemical analyses have contributed to the elucidation of migratory connectivity and to the investigation of intra-population variation in foraging strategies. There is increasing evidence that arrival time to breeding areas, reproductive success and annual breeding population size are linked to non-

⁴ Prepared as: Ceriani SA, Roth JD, Quintana-Ascencio PF, Tucker AD, Evans DR, Addison DS, Ehrhart LM, Weishampel JF (*in prep*). Carry-over effects and foraging ground dynamics of the loggerhead breeding aggregation in Florida.

breeding habitat quality (Marra et al. 1998, Bearhop et al. 2005, Norris 2005, Vander Zanden et al. 2013), a phenomenon that is described under the umbrella term of “carry-over effects.” What we observe at one location is the result of a complex set of interactions occurring at the prior foraging location and migratory route; hence, connectivity among populations influences their demographics, genetic structure and response to environmental change.

Sea turtles are long living, highly migratory and late maturing species of conservation concern (IUCN 2013) that are primarily studied at nesting beaches where they are more easily accessible. Nesting females exhibit large variation in body size and reproductive parameters such as clutch size, clutch frequency (the number of clutches laid within a season) and remigration interval (the number of years between consecutive nesting seasons) (Miller 1997, Broderick et al. 2003, Hatase et al. 2008). Each female from a nesting aggregation typically forages in one of several geographically distinct foraging grounds (Schroeder et al. 2003, Caut et al. 2008). Individual females appear to show fidelity to both nesting and feeding areas throughout adult life (Broderick et al. 2007, Vander Zanden et al. 2010, Hawkes et al. 2011). Sea turtles are capital breeders (using energy stored at the nonbreeding feeding area for reproduction; Stearn 1992) that reproduce every few years but lay several clutches of eggs (Miller 1997). Moreover, as most oviparous ectothermic vertebrates, sea turtles lack parental care; hence, the maternal investment in reproduction is limited to pre-ovipositional allocation of resources to the number and size of eggs and the number of clutches per reproductive season (Wallace et al. 2007). Though body size may set the upper limit to the number of eggs per clutch that a female can lay, this limit may not be reached if conditions are sub-optimal (Shine 1992). Thus, eggs are the link between nesting female (body conditions and foraging ecology) and reproductive output. These characteristics

suggest that carry-over effects may play an important role in the ecology of sea turtles since the resources required for reproduction are acquired months before the nesting season while at the feeding area.

Carbon and nitrogen stable isotopes have been increasingly used as intrinsic markers to identify migratory linkages and determine geographic origin of migrants. Stable isotope ratios of these elements vary across land- and sea-scapes, often in systematic ways due to a variety of biogeochemical processes (Bowen et al. 2005, Graham et al. 2010). Patterns in stable isotope ratios at the base of food webs are assimilated through diet and retained at higher trophic levels. Isotopic signatures may be influenced by diet, habitat type and geographic location (Hobson and Norris 2008). Individuals moving between isotopically distinct regions may maintain quantifiable isotopic differences in one or more tissues that can be linked to past locations. Thus, stable isotopes function as intrinsic markers that reflect the isotopic composition of the environment (location and food web) where the tissues were synthesized (Hobson and Norris 2008). Since geographic variation in stable isotope ratios in marine systems have been described only at very coarse scales (McMahon et al. 2013), the isotopic approach has been validated with telemetry of several marine species (sea birds: Jaeger et al. 2010, González-Solis et al. 2011; sea turtles: Hatase et al. 2002, Caut et al. 2008, McClellan et al. 2010, Ceriani et al. 2012, Pajuelo et al. 2012, Seminoff et al. 2012).

Foraging strategies of adult female sea turtles have been studied using a combination of satellite telemetry and stable isotope analysis (Hatase et al. 2002, Caut et al. 2008, Zbinden et al. 2011, Ceriani et al. 2012, Pajuelo et al. 2012, Seminoff et al. 2012, Tucker et al. *in press*). Carry-over

effects have been documented in some sea turtle populations. Loggerheads nesting in Greece and foraging in two distinct regions within the Mediterranean differed in body size and clutch size (Zbinden et al. 2011). Similarly, loggerheads nesting in Georgia (Southeast USA) and feeding in three different regions in the Northwest Atlantic (NWA) differed in body size, clutch size and remigration interval (Vander Zanden et al. 2013), while differences in cumulative reproductive output have been found in loggerheads nesting in Japan depending on the foraging strategy of choice (neritic vs. oceanic) (Hatase et al. 2013). Moreover, foraging strategy has been shown to affect remigration interval in leatherbacks nesting in the Atlantic (Caut et al. 2008) and the Pacific (Lontoh et al. 2013).

The NWA loggerhead population is made of five recovery units identified based on genetic differences and a combination of geographic distribution of nesting densities and geographic separation, whose trends in abundance have been significantly fluctuating over the past two decades (NMFS and USFWS 2008). We focus on the NWA Peninsular Florida Recovery Unit, the largest loggerhead nesting population in the western hemisphere and one of the two largest in the world (Ehrhart et al. 2003). A detailed analysis of Florida's long-term loggerhead nesting data (1989-2013) revealed three distinct annual trends in nest numbers: (1) they increased by 23% between 1989 and 1998, (2) declined by 43% between 1999 and 2007 and (3) increased by 16% over the last six years (2008-2013) (FWC 2013a). Examining only the period between the high-count nesting season in 1998 and the most recent (2013) nesting season, researchers found no demonstrable trend in nest numbers, indicating a reversal of the post-1998 decline (FWC 2013b). Reasons for this oscillating trend in annual nest numbers (a proxy to estimate female population size) are unclear, but loggerhead fishery-related mortality has been suggested as the

main driver of the trend (Witherington et al. 2009, Bolten et al. 2010). However, changes in nest numbers might also be a consequence of female foraging strategy or of a variation in resource availability that could lead ultimately to a change in reproductive output (e.g., affecting clutch frequency, clutch size, egg quality and remigration intervals). For example Seney and Musick (2007) reported that loggerheads stranded in Virginia had incurred a double shift in diet during the past two decades from horseshoe crabs to blue crabs to finfish discarded by fisheries.

Intra-population variation in foraging strategies has been identified previously in the NWA loggerhead population using telemetry (Girard et al. 2009, Hawkes et al. 2011, Foley et al. 2013, Griffin et al. 2013), stable isotope analyses (Reich et al. 2010) or a combination of the two methods (e.g., Ceriani et al. 2012, Pajuelo et al. 2012); thus, there may be differential carry-over effects in this loggerhead population. Recently Vander Zanden et al. (2013) explored possible reproductive consequences of differential use of foraging areas in loggerheads of the Northern Recovery Unit, which represents ~10% of the overall NWA loggerhead nesting population (NMFS and USFWS 2008) and found differences in body size, clutch size (when not accounting for body size) and remigration intervals among the foraging areas. Carry-over effects have not been investigated in the Florida Peninsular Recovery unit despite the disproportionate importance of this management unit (~90% of the NWA nesting population, NMFS and USFWS 2008) to the overall NWA loggerhead population.

The overall goal of this study was to investigate carry-over effects on Florida loggerheads, which exhibit variation in foraging strategies. To do so we used a combination of telemetry and stable isotope analysis, and incorporated reproductive parameters. The specific objectives were to: (1)

identify the foraging regions used by loggerheads nesting at the Archie Carr National Wildlife Refuge (ACNWR), their relative contribution on an annual basis and over the six-year period of the study; (2) assess whether differences in foraging sites are associated with female phenotypic variability and (3) evaluate whether foraging site affects female reproductive output measured as a suite of five metrics (body size, clutch size, hatching success, emerging success and remigration interval). Individuals from different non-breeding regions in the NWA experience disparate environmental conditions and resource availability (e.g., temperature regimes and primary productivity, Wilkinson et al. 2009). Thus, resources acquired at the foraging ground and associated environmental conditions have implications for estimating overall number of breeding females in the population.

Material and methods

Sample Collection and Stable Isotope Analysis

A total of 330 loggerhead females were sampled at the Archie Carr National Wildlife Refuge (ACNWR) and vicinity during the 2007 – 2012 nesting seasons (May – August). This 21+ km stretch of beach in east-central Florida hosts the most important loggerhead rookery in the western hemisphere and accounts for approximately 22.5% (8,000-15,000 nests/year) of all the loggerhead nests in the NWA (Ehrhart et al. 2003). Here, all nesting activity is monitored, and a subsample of females is encountered and tagged using both Inconel flipper tags and passive integrated transponders during night surveys. Tissues were primarily collected for stable isotope

analysis or as a part of a genetic study. Blood and/or skin samples were collected for stable isotope analysis. Skin samples were collected if the female was originally part of an associated genetics study.

Skin samples were obtained using a sterile 4 mm biopsy punch from either the “shoulder” area of each female (between the neck and the front flipper) or from the soft skin on the trailing edge of the rear flipper. Stable isotope values of loggerhead epidermis sampled at these two anatomical locations were not significantly different (Ceriani *unpublished data*, $\delta^{13}\text{C}$: $t_{31,2}=0.992$, $p = 0.663$; $\delta^{15}\text{N}$: $t_{31,2}=0.165$, $p = 0.870$). Blood samples (4 ml) were collected from the cervical sinus with a 20-gauge needle and syringe (Owens and Ruiz 1980), transferred to a non-heparanized container and placed in ice. Blood was separated into serum and cellular components by centrifugation (5000 rpm x 10 min) and frozen at -20°C until analysis. Skin samples were either stored in a non-frost-free freezer at -20°C or preserved in 70% ethanol. Both preservation methods have no effect on tissue isotopic composition (Barrow et al. 2008).

Samples were prepared for stable isotope analysis following standard procedures at the University of Central Florida. RBC samples were freeze-dried for 48 h before being homogenized with mortar and pestle. Skin samples were rinsed with distilled water and cleaned with 70% ethanol. After the epidermis was separated from the underlying dermal tissue with a scalpel, the samples were dried at 60°C for 48 h. Lipids were removed from all the samples using a Soxhlet apparatus with petroleum ether as solvent for 12 and 24 h (RBC and epidermis, respectively). Sub-samples of prepared tissues (0.4-0.7 mg) were weighed with a microbalance

and sealed in tin capsules. Isotopic composition of weighed samples was determined at the Paleoclimatology, Paleoceanography and Biogeochemistry Laboratory at the University of South Florida, College of Marine Science (St. Petersburg, FL, USA). Samples were converted to N₂ and CO₂ using a Carlo-Erba NA2500 Series 2 Elemental Analyzer (Thermoquest Italia, S.p.A., Rodano, Italy) and analyzed with a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen). Stable isotope ratios were expressed in conventional notation as parts per thousand (‰) according to the following equation: $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where X is ¹⁵N or ¹³C, and R is the corresponding ratio ¹⁵N:¹⁴N or ¹³C:¹²C. The standards used were atmospheric nitrogen and Pee Dee Belemnite for ¹⁵N and ¹³C, respectively. Estimates of analytical precision were obtained by replicate measurements of internal lab reference materials (1577b Bovine liver) and yield a precision (reflecting ± 1 SD) of ± 0.14‰ for δ¹³C and 0.16‰ for δ¹⁵N.

Development of Assignment Model to Foraging Areas

Tissue turnover rates in adult sea turtles are not known. Previous studies on cheloniids and juvenile loggerheads estimated RBC and skin tissue to reflect foraging habits at least 4 months prior to sampling (Brace and Altland 1955, Seminoff et al. 2007, Reich et al. 2008, 2010; Ceriani et al. *unpublished data*). Thus, RBC and skin samples represent the isotopic signature of foraging areas used by females during the non-breeding season prior to migration and nesting activity (Caut et al. 2008, Reich et al. 2010, Ceriani et al. 2012, Pajuelo et al. 2012, Seminoff et al. 2012).

Several studies have used successfully RBC, epidermis, fresh and unhatched eggs combined with satellite telemetry to infer foraging areas of sea turtles (Caut et al. 2008, Zbinden et al. 2011, Ceriani et al. 2012, Pajuelo et al. 2012, Seminoff et al. 2012, Vander Zanden et al. 2013). Ceriani et al. (in review, Chapter 4) investigated the relationship among these same four tissues commonly used for stable isotope assignment and concluded that they are all isotopic equivalents for inferring foraging areas and developed equations to convert one tissue into another.

Skin samples were available from 279 (of the 330) individual females sampled at the ACNWR. Skin isotopic values were derived from red blood cell values for the remaining 51 females for which only a blood sample was available using an equation based on females for which both RBC and skin have been analyzed ($n = 165$; $\delta^{13}\text{C}_{\text{skin}} = 0.953 \delta^{13}\text{C}_{\text{RBC}} + 0.734$, $r^2 = 0.92$; $\delta^{15}\text{N}_{\text{skin}} = 1.031 \delta^{15}\text{N}_{\text{RBC}} + 1.576$, $r^2 = 0.93$).

Twenty-three of the 330 loggerheads sampled at the ACNWR and vicinity were equipped with satellite tags between 2008 and 2012. Telemetry revealed that loggerheads nesting at the ACNWR use four main foraging areas (Dodd and Byles 2003, Ceriani et al. 2012, Foley et al. 2013) (Figure 5.1): (1) a seasonal shelf-constrained movement between summer foraging areas located in the Mid-Atlantic Bight (off the coast of the Delmarva Peninsula) and wintering areas south of Cape Hatteras NC, (2) a year-round residence in the South Atlantic Bight (SAB), (3) a year-round residence in the Subtropical Northwest Atlantic (SNWA), which we define as the water encompassing the waters around the Florida Keys, Bahamas and Cuba, and (4) a year-round residence on the Southwest Florida continental shelf (SW FL), here defined as the waters between Tampa (FL) and the Florida Keys.

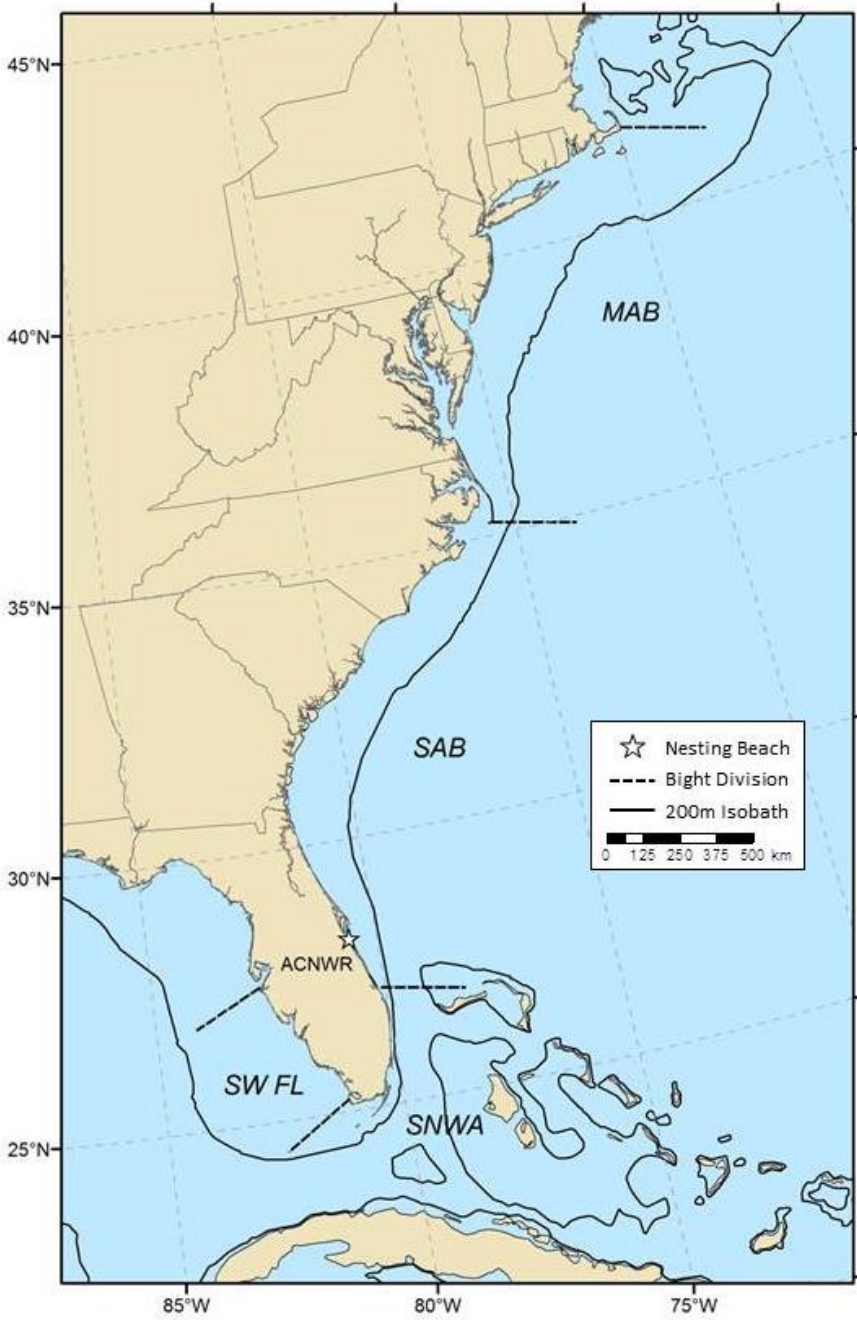


Figure 5.1. Adult loggerhead foraging areas identified by telemetry studies in the Northwest Atlantic and study site location (Archie Carr National Wildlife Refuge, Florida). The 200 m isobath is delineated (black line). Dashed lines separates Mid-Atlantic Bight (MAB), South-Atlantic Bight (SAB), Subtropical NWA (SNWA) and Southwest Florida continental shelf (SW FL).

Stable isotope analysis has proven to be a valid complementary tool to infer foraging areas in loggerheads in the NWA (Ceriani et al. 2012, in review, Pajuelo et al. 2012). The 23 adult females equipped with satellite tags in this study, 14 of which were used in Ceriani et al. (2012), are the only nesting ACNWR loggerheads that have also been sampled for stable isotope analysis. We included an additional 33 loggerheads equipped with satellite tags from other nesting locations in Florida and sampled for stable isotope analysis to increase the number of females with known foraging destinations to develop the assignment model for the remaining 307 untracked individuals. Epidermis isotopic values were available for 8 of the 33 females, but other tissues were available for the remaining 25 females (RBC: n = 7; fresh egg: n = 3; unhatched egg: n = 15). We converted all the isotopic values into epidermis values to unify the dataset. If epidermis values were not available, the tissue sampled (RBC, fresh or unhatched egg) was converted in epidermis isotopic values using regression equations derived from the dataset compiled by Ceriani et al. (in review, Chapter 4). The 33 loggerheads equipped with satellite tags outside the ACNWR vicinity were included in the development of the assignment model but were removed from subsequent analyses because our goal was to describe the female aggregation nesting at the ACNWR. All 56 loggerheads transmitted long enough to determine their foraging region. For details on tracking data processing and identification of post-nesting foraging areas used by each adult female see Ceriani et al. (2012) and Tucker et al. (*in press*).

We used discriminant function analysis (DFA – SPSS v. 19) of epidermis stable isotope values to assign females to one of the four foraging areas identified by telemetry: MAB, SAB, SNWA and SW FL. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the 56 loggerheads equipped with satellite tags represented the training data set to develop the discriminant functions and the remaining 307 females

sampled at the ACNWR were the test data set for the classification. We chose to compute from group sizes for prior probabilities because previous telemetry data (Dodd and Byles 2003, Ceriani et al. 2012, Foley et al. 2013) have shown unequal contribution of foraging areas to this breeding aggregation: 23 (65.7%) of the 35 females previously satellite tagged at the ACNWR migrated to SNWA and SW FL. Thus, the 56 loggerheads in the training dataset reflected the relative contribution of each foraging area identified by prior telemetry-based studies (Dodd and Byles 2003, Ceriani et al. 2012, Foley et al. 2013). Next, we used DFA to assign the 307 untracked loggerheads sampled at the ACNWR into one of the four foraging areas. Only assignments with posterior probabilities $\geq 50\%$ were retained for the following analyses on phenotypic variability and reproductive output differences attributed to foraging area. We used a cut off of 50% probability of membership, which translates to a 2:1 odds ratio, because an external validation of the isotopic approach for NWA loggerheads showed that this odds ratio was adequate to assign loggerheads to foraging grounds correctly (Ceriani et al. in review, Chapter 3). Two hundred and sixty four of the 307 untracked nesting females (86%) had posterior probabilities of assignment $\geq 50\%$.

Previous telemetry studies (Dodd and Byles 2003, Ceriani et al. 2012, Foley et al. 2013) found unequal contributions of foraging areas to the loggerhead aggregation nesting at the ACNWR but each study was based on a small number of individuals ($4 < n < 16$) tracked over multiple years ($2 < \text{years} < 4$); thus, this conclusion might have been biased by the small sample size. We used the larger dataset of 287 loggerheads (23 females equipped with satellite tags and 264 females assigned to a foraging area with a probability $> 50\%$ to evaluate the annual contribution of the foraging areas to the nesting assemblage at the ACNWR over the 2007 to 2012 nesting seasons.

We used Chi-square tests to assess the independence of frequencies of arriving turtles among foraging areas by year and combined across years, and among years. We assumed a null equal arrival frequency among sources or years.

Reproductive Parameters and Analyses

Tissues from 287 females were sampled at the ACNWR and used to evaluate whether foraging site preference affects phenotypic variability and reproductive output. Three days after an emergence or 70 days after deposition (if hatchlings were not observed), the nest content was inventoried to determine reproductive success rates. When the situation allowed, we analyzed five parameters for each female: female body size, clutch size, hatching and emerging success, and remigration interval.

- *Body size (CCL)* – Female body size was measured as standard curved carapace length (CCL notch-to-tip) for the year in which the female was sampled. Body size measurements were available for 274 (95.5%) of the 287 females.
- *Clutch size (CS)* – Clutch size (the number of eggs deposited in a single nesting event) was determined either within 12 hours of deposition (2008 – 2011) or at time of post-hatching excavation (2007 and 2012) whenever possible. Clutch size measurements were available for 203 (70.7%) of the 287 females. Eighty-four females were sampled for stable isotope analysis after a non-nesting emergence (n = 58) or their nest was lost due to erosion or predation prior to post-hatching excavation (n = 26), and therefore, no clutch size data are available. If a female

was encountered repeatedly during the nesting season ($n = 15$), clutch size was calculated as the mean number of eggs per clutch within the year.

- *Hatching success (HS)* – Hatching success is the percentage of eggs that hatch in an individual nest, calculated as:

$$(\# \text{ of hatched eggs} \times 100 / \text{CS}).$$

- *Emerging success (ES)* – Emerging success is the percentage of hatchlings that emerge from an individual nest, calculated as:

$$[\# \text{ of hatched eggs} - (\text{dead hatchlings} + \text{live hatchlings})] \times 100 / \text{CS}.$$

Sixty-five (32.0%) of the 203 clutches marked were either depredated by raccoons or lost due to beach erosion; thus, data on hatching success and emerging success were available for only 138 nests. If more than one nest was marked and hatched for an individual female within a nesting season ($n = 15$ females), we calculated mean HS and ES for the year of sampling.

- *Remigration interval (RI)* – Remigration interval is defined as the number of years between successive nesting seasons. RI measurements were available for 105 (36.6%) of the 287 females. If a female was seen in more than one year ($n = 105$), the remigration interval was determined as the mean of all the RI available for that individual. Most loggerheads in the Southeast USA nest every two or three years (Schroeder et al. 2003). Sixty-two of the 105 loggerheads were seen over assumed consecutive nesting events and had remigration intervals of two ($n = 28$) and three ($n = 20$) years. If a female had a remigration interval of 4 years ($n = 14$), we assumed the turtle was on a two-year cycle.

Pearson's correlation was used to explore relationships among the five reproductive parameters examined. To test for significant differences in phenotypic and reproductive parameters (CCL, CS, HS, ES) among foraging areas and years, we used two-way analyses of variance (ANOVA). We examined differences in remigration interval among foraging areas using a one-way ANOVA. In addition, we used a Pearson's Chi-squared test on the subset of females that were seen over assumed consecutive nesting seasons ($n = 62$) to evaluate whether individuals originating from the three foraging areas were more likely to be on a two or three year remigration interval. Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnov and Levene's test, respectively. Body size and clutch size data were normal; HS and ES required arcsin square root transformation, while RI required squared root transformation to meet the normality assumption. All data met the equal variance assumption; thus, we used post-hoc Tukey's HSD multiple comparison tests to identify groups responsible for statistical differences. Previous studies have demonstrated that clutch size positively correlates with body size (Bjorndal and Carr 1989, Van Buskirk and Crowder 1994). Thus, when comparing clutch size among foraging areas, we used an ANCOVA to control for the effect of body size (covariate) on clutch size (dependent variable). Data were analyzed using SPSS (vs. 19) and the R Statistical Package (R Development Core Team 2011) with an alpha level set to 0.05 for all statistical analyses.

Results

Assignment to Foraging Grounds

The post-nesting migratory destination of the 56 satellite tagged loggerheads used as the training dataset to develop the assignment model was as follows: ten females migrated to the MAB, seven took up year-round residence in the SAB, 21 females resided year-round in the SNWA and 18 individuals migrated to year-round foraging areas on the SW FL continental shelf.

The discriminant analysis of the training data set was significant ($P > \text{Wilks' Lambda} < 0.001$). Two discriminant functions were calculated, with a combined $X^2(6) = 79.7$, $p < 0.001$. After removal of the first function, the association between groups (foraging areas) and predictors ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) remained significant $X^2(2) = 10.454$, $p = 0.05$. The first discriminant function accounted for 92.6% of the between-group variability. Overall the discriminant analysis of the training data set was able to correctly classify the foraging ground used for 45 of the 56 loggerheads (80.4% of original grouped cases correctly classified). Three females (30%) from the northern aggregation (MAB), two females (28.6%) from the central group (SAB) and three individuals (14.3%) from SNWA group were incorrectly assigned to the SW FL bin, while three (16.7%) of the females that migrated to SW FL were assigned incorrectly to the SNWA area. The stability of the classification procedure was checked by a leave-one-out cross validation, which classified 75.0% of the test data set correctly. The putative foraging ground of the 307 untracked loggerheads in the test data set was based on the above classification functions (Table 5.1). Not all females sampled could be assigned to a foraging area (unassigned females, Table

5.1). Two hundred and sixty-four of the 307 untracked nesting females (86.0%) had posterior probabilities of assignment $\geq 50\%$ (Table 5.1, Figure 5.2) and were retained for further analyses. Only four (of 23) loggerheads equipped with satellite tags at the ACNWR and vicinity during this study took up residence in the SAB. None of the untracked loggerheads was assigned with posterior probability $\geq 50\%$ (2:1 odds ratio) to the SAB; therefore, we excluded the SAB from any comparison of reproductive parameters among foraging areas.

Table 5.1. Foraging ground assignment, number and proportion (in parentheses) for the discriminant model based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of 330 loggerheads sampled at the ACNWR and vicinity over a six-year period (2007 – 2012).

Year	MAB	SAB	SNWA	SW FL	Females assigned (prob ≥ 0.50)	Females unassigned (prob < 0.50)	Females sampled/year
2007	3 (0.14)	0 (0.00)	11 (0.5)	8 (0.36)	22	1	23
2008	9 (0.25)	0 (0.00)	15 (0.42)	12 (0.33)	36	6	42
2009	7 (0.14)	1 (0.02)	18 (0.35)	25 (0.49)	51	7	58
2010	10 (0.17)	3 (0.05)	29 (0.49)	17 (0.29)	59	13	72
2011	13 (0.20)	0 (0.00)	31 (0.48)	21 (0.32)	65	9	74
2012	11 (0.20)	0 (0.00)	32 (0.60)	11 (0.20)	54	7	61
Total	53 ^a (0.18)	4 ^b (0.01)	136 ^c (0.48)	94 ^d (0.33)	287	43	330

^a Eight of the 53 females assigned to the MAB were equipped with satellite tags

^b The only four females assigned to the SAB were equipped with a satellite tag

^c Nine of the 136 individuals assigned to the SNWA were equipped with satellite tag

^d Only one of the 94 females assigned to the SW FL foraging ground was satellite tagged

MAB – Mid-Atlantic Bight; SAB – South-Atlantic Bight; SNWA – Subtropical Northwest Atlantic; SW FL – Southwest Florida continental shelf

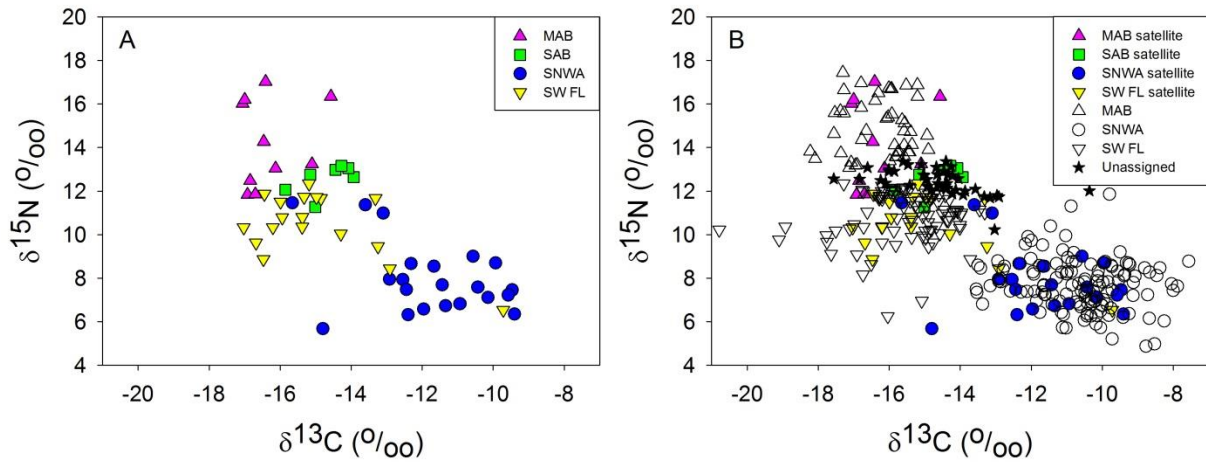


Figure 5.2. Stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) of (A) the 56 nesting loggerheads equipped with satellite tags (training subset) and (B) the entire dataset (56 satellite tagged and 307 untracked females sampled at the ACNWR). Colored markers (up triangle, down triangle, circle and squares) represent the 56 satellite turtles, while unfilled markers represent the 264 females that were assigned to a foraging area with probability $> 50\%$. Stars indicate the 43 females with posterior probability of assignment $< 50\%$ that could not be assigned definitively to a foraging area. MAB - Mid Atlantic Bight, SAB - South Atlantic Bight, SNWA – Subtropical Northwest Atlantic, SW FL – Southwest Florida continental shelf.

Reproductive Parameters

A Pearson product-moment correlation coefficient matrix was computed to assess the relationship between five reproductive variables: body size, clutch size, hatching success, emerging success and mean remigration interval. We found significant correlations between body size and clutch size ($r = 0.655$, $n = 200$, $p < 0.001$), between hatching success and emerging success ($r = 0.996$, $n = 138$, $p < 0.001$) and between emerging success and mean remigration interval ($r = -0.285$, $p = 0.049$, $n = 105$). Since hatching and emerging success were very highly correlated, we removed the latter from further analyses (Table 5.2).

Table 5.2. Pearson product-moment correlation coefficient matrix with two-tails.

	Body Size	Clutch Size	Hatching Success	Emerging Success	Mean Remigration Interval
Body Size	1 (n = 274)				
Clutch Size	0.655*** (n = 200)	1			
Hatching Success	0.044 (n = 136)	0.038 (n = 138)	1 (n = 138)		
Emerging Success	0.037 (n = 136)	0.028 (n = 138)	0.996*** (n = 138)	1 (n = 138)	
Mean Remigration Interval	-0.036 (n = 100)	-0.164 (n = 57)	-0.271 (n = 48)	-0.285 (n = 48)	1 (n = 105)

(*<.05, **<.01 and ***<.001)

We examined differences in reproductive parameters (body size, clutch size and hatching success) among years and foraging areas. Foraging area was the only significant predictor in all analyses; year and the interaction of year and foraging area were not significant in any of the analyses. Females using the three distinct foraging areas (MAB, SNWA, SW FL) differed significantly or marginally significantly in three of the parameters examined (CCL, CS, CI; Table 5.3). The two-way ANOVA indicated that body size was significant different among foraging areas ($F_{2,257} = 19.029$, $p < 0.001$). Post-Hoc Tukey's HSD multiple comparison tests revealed significant differences among all three foraging areas. Female body size decreased significantly as we move south from MAB to SW FL (Fig. 5.19A). Females residing year-round in SW FL were significantly smaller than those foraging in the MAB ($p < 0.001$) and in the SNWA ($p < 0.001$) and individuals foraging in the MAB were significantly larger than those residing year-round in the SNWA ($p = 0.038$). Clutch size differed significantly among the three

foraging areas ($F_{2,181} = 7.641$, $p = 0.001$, Fig. 5.19B). Females from SW FL laid significantly fewer eggs than those from the MAB ($p < 0.001$) and the SNWA ($p < 0.001$), but there was no difference in clutch size between MAB and SNWA females ($p = 0.882$). Since body size and clutch size were significantly correlated ($r = 0.655$, $p < 0.001$), we used an ANCOVA to investigate the effect of female body size on clutch size. Clutch size differed among females using the three foraging areas even after accounting for body size ($F_{2,195} = 3.576$, $p = 0.03$). Hatching success did not differ among females using the three foraging grounds ($F_{2,119} = 0.859$, $p = 0.427$, Figure 3C). Mean remigration interval was marginally different among foraging areas ($F_{2,102} = 2.885$, $p = 0.06$, Fig. 5.3D). Females originating from the MAB had longer mean remigration interval than females from the SNWA ($p = 0.05$) but there was no difference in mean remigration interval between MAB and SW FL ($p = 0.442$) and between SNWA and SW FL ($p = 0.609$). When we analyzed the subset of individuals that were seen over assumed consecutive nesting seasons ($n = 62$), we found that females foraging in the SNWA were more likely to be on a two-year remigration interval ($n = 39$, $\chi^2_1 = 7.4$, $p = 0.006$), while females foraging in the MAB and SW FL had the same probability of being on a two- or three-year remigration interval (MAB: $n = 9$, $\chi^2_1 = 0.1$, $p = 0.739$; SW FL: $n = 14$, $\chi^2_1 = 1.1$, $p = 0.285$).

Table 5.3. Size and reproductive parameters of female loggerheads nesting at the ACNWR and foraging in three different foraging regions.

	MAB			SNWA			SW FL			<i>p</i> -value
	Mean	SD	n	Mean	SD	n	Mean	SD	n	
CCL (cm)	102.3 ^a	6.2	51	99.3 ^b	5.4	131	95.5 ^c	6.4	88	<0.001
CS (# eggs)	123 ^a	19	35	121 ^a	24	93	102 ^b	21	31	<0.001
HS (%)	46.2	17.9	27	50.4	20.0	64	51.3	20.6	46	0.546
Mean RI (year)	5.5 ^a	3.1	21	4.1 ^b	2.2	61	4.6 ^{a,b}	2.4	23	0.06

Mean, standard deviation (SD) and sample size (n) are indicated for each of the three foraging areas compared. Bolded *p* values indicate parameters differed significantly among regions, while superscripts indicate foraging areas responsible for statistical differences. Abbreviations are as follow: body size (CCL), clutch size (CS), hatching success (HS), remigration interval (RI). The body size (CCL) of the four satellite tagged females that foraged in the SAB was 91.8 ± 1.2 cm and their average clutch size was 93 ± 13 eggs.

MAB – Mid-Atlantic Bight; SAB – South-Atlantic Bight; SNWA – Subtropical Northwest Atlantic; SW FL – Southwest Florida continental shelf.

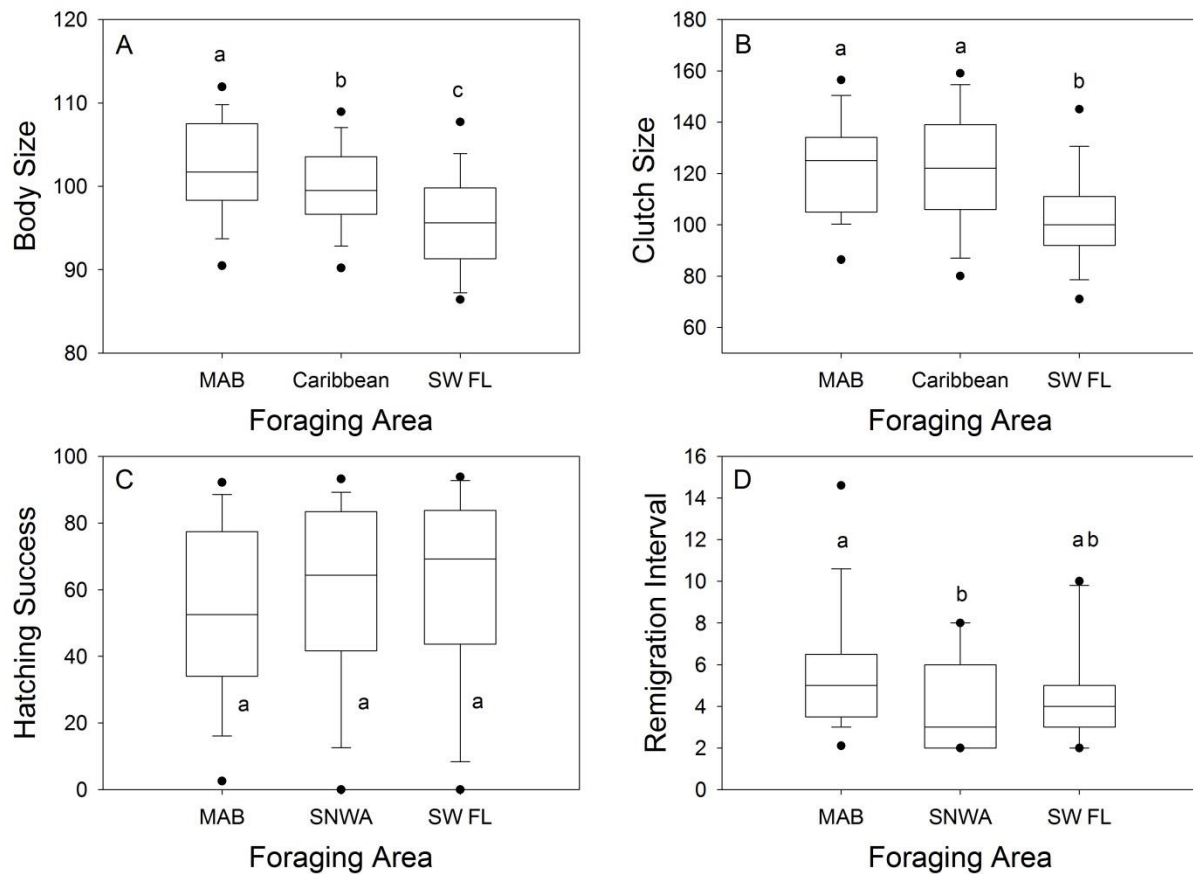


Figure 5.3. (A) Body size (CCL) differed significantly among females using three different foraging areas. (B) Clutch size (CS) differed significantly among female loggerheads originating from three different feeding regions. (C) Hatching success (HS) did not differ among foraging areas. (D) Remigration interval (RI) differed marginally among females using three distinct foraging areas. Letters indicate foraging areas responsible for statistical differences. MAB - Mid Atlantic Bight, SAB - SNWA – Subtropical Northwest Atlantic, SW FL – Southwest Florida continental shelf.

Dynamics of Foraging Ground Contributions

Our dataset allowed us to examine (i) the annual frequency contribution of the three foraging grounds to the breeding aggregation as well as (ii) trends in the relative frequency contribution of the three areas over a six-year consecutive period (2007 – 2012). There was an association

between presence and foraging ground in the last four years (2009: $\chi^2_2=9.88$, $P=0.007$; 2010: $\chi^2_2=9.89$, $P=0.007$; 2011: $\chi^2_2=7.51$, $P=0.02$; 2012: $\chi^2_2=16.33$, $P=0.003$), but these variables were independent during the first two years (2007: $\chi^2_2=4.45$, $P=0.11$; 2008: $\chi^2_2=1.50$, $P=0.47$). For all but one (2009) of the six years, the SNWA foraging area contributed the largest proportion of females to the nesting assemblage followed by SW FL (Fig. 5.4A). The MAB contributed consistently the fewest females in the years sampled with the exception of the 2012 nesting season when MAB and SW FL contribution was equal (20%). On average, 47% ($\pm 8\%$, range 35% – 59%) of the females sampled each year foraged in the SNWA, 33% ($\pm 9\%$, range 20% - 49%) foraged in SW FL and 18% ($\pm 4\%$, range 14% - 25%) foraged in the MAB. Across year frequency contributions were independent of year for MAB ($\chi^2_5=6.89$, $P=0.23$) but were associated to year for SNWA ($\chi^2_5=18.24$, $P=0.003$) and SWFL ($\chi^2_5=13.5$, $P=0.02$). Overall, egg contribution by foraging areas exhibited a similar pattern (Fig. 5.4B).

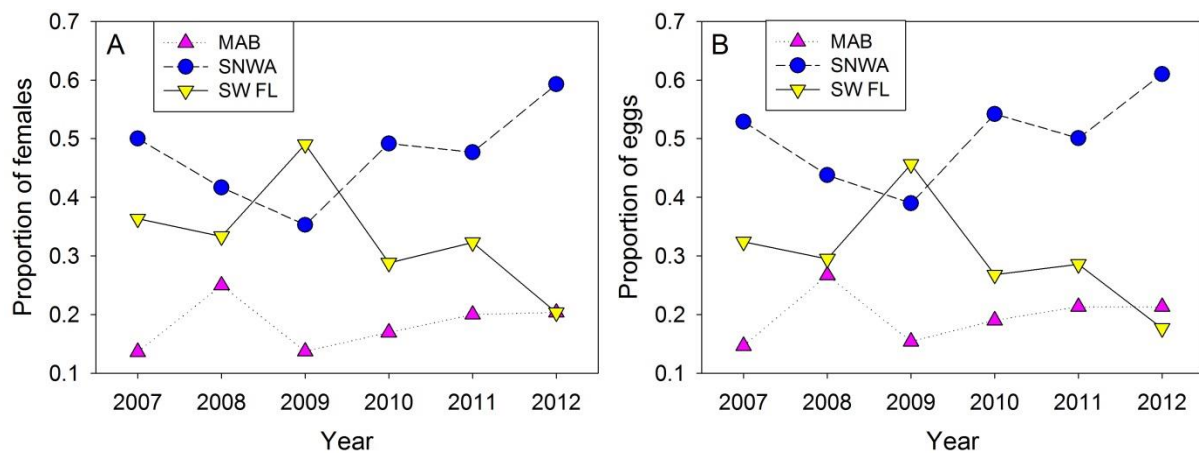


Figure 5.4. Contribution of three foraging areas to the loggerhead assemblage nesting at the ACNWR over a six-year consecutive period (2007 – 2012) expressed as proportion of females (A) and proportion of eggs (B). MAB - Mid Atlantic Bight, SNWA – Subtropical Northwest Atlantic, SW FL – Southwest Florida continental shelf.

Discussion

Assignment to Foraging Grounds and Future Improvements

We collected tissues for stable isotope analysis and a suite of reproductive parameters from a total of 330 nesting loggerheads between 2007 and 2012 to evaluate possible carry-over effects associated with intra-population variation in female foraging strategies. The assignment model was unable to infer the foraging region used by 14.0% ($n = 43$) of the untracked loggerheads sampled. Females that could not be assigned definitively to a foraging region fell mostly in the isotopic space between MAB, SAB and SW FL. Telemetry has shown that several loggerheads nesting along the Florida east coast reside on the SW FL continental shelf (Sasso et al. 2011, Foley et al. 2013, Evans et al. unpublished). Even though previous isotopic studies recognized the importance of SW FL as a foraging area (Ceriani et al. 2012, Pajuelo et al. 2012), the extremely low number of females satellite tagged and sampled for stable isotopes ($n = 1$ in Ceriani et al. 2012, $n = 0$ in Pajuelo et al. 2012) that migrated to SW FL impeded the inclusion of this fourth foraging area in assignment model development. Here, we included an adequate number of females known to feed in SW FL to overcome the limitation of past studies. While the assignment model was able to identify four isotopically distinct regions, females that could not be assigned definitively to a foraging region ($n = 43$, 14% of the 307 untracked) fell in the isotopic space between MAB, SAB and SW FL suggesting that previous estimates of loggerheads using MAB and SAB should be revised (Ceriani et al. 2012, Pajuelo et al. 2012, Vander Zanden et al. 2013). In particular, Ceriani et al. (2012) used 14 satellite loggerheads and 57 untracked turtles at the ACNWR and assigned 61% of the loggerheads to the MAB and SAB,

a result in contradiction with previous flipper tag return (Meylan et al. 1983, Moncada et al. 2010) and telemetry (Dodd and Byles 2003, Foley et al. 2013). However, only one of the satellite tagged loggerheads used to develop the assignment model in Ceriani et al. (2012) migrated to SW FL and, thus, that foraging area could not be characterized isotopically. Our large sample size supports the results of previous flipper tags return (Meylan et al. 1983, Moncada et al. 2010) and satellite telemetry studies (Dodd and Byles 2003, Foley et al. 2013) and confirms the importance of the SW FL continental shelf and the Caribbean (SNWA) as prime foraging areas for loggerheads nesting at the ACNWR. Moreover, our results emphasize the importance of having samples from all known foraging areas in order to develop a meaningful assignment model. Only four of the 23 loggerheads equipped with satellite tags at the ACNWR and vicinity and included in the present study took up residence in the SAB. None of the untracked loggerheads was assigned with adequate posterior probability ($\geq 50\%$) to the SAB; hence, SAB was removed from any comparison of reproductive parameters among foraging areas. Additional markers (e.g. sulfur stable isotope and trace minerals) could help to further discern these three foraging areas (MAB, SAB, SW FL).

Body Size and Reproductive Output Differences

A total of 287 females sampled at the ACNWR were included in the evaluation of possible carry-over effects in this nesting aggregation. We found significant differences in body size and clutch size among females originating from the three feeding areas compared (MAB, SNWA and SW FL). Body size decreased significantly as we moved south from MAB to SW FL (Fig 5.19A). Body size is known to be related to clutch size in sea turtles (Bjorndal and Carr 1989, Broderick

et al. 2003) and reptiles in general (Shine 1992); hence, it was not surprising to find that MAB and SNWA laid significantly larger clutches than females feeding in SW FL (Fig 5.19B). Even though females feeding in the SNWA were significantly smaller than those foraging at higher latitudes in the MAB, clutch size did not differ between the two groups ($p = 0.882$). While body size may set the upper limit to the number of eggs/clutch that a female can lay, this limit may not be reached if conditions are sub-optimal (Shine 1992). Females feeding in the MAB undertake seasonal migrations between highly productive waters in the MAB (Wilkinson et al. 2009), where they forage during summer months, and warmer and less productive waters on the edge of the Gulf Stream or in the SAB where they overwinter (Hawkes et al. 2011, Ceriani et al. 2012, Griffin et al. 2013). On the other hand, loggerheads feeding in the warmer and more constant waters of the SNWA reside there year-round and incur lower energetic costs associated with migration. We hypothesized that the lack of differences in clutch size despite the significant differences in body size between these two groups is the result of differential allocation of energy between the two competing compartments (survival and reproduction) of an adult sea turtle whose growth is negligible (Bjørndal et al. 1983). Even though female diet may vary across marine regions, we found no differences in hatching success among the three foraging areas (Fig 5.19C). The lack of difference in hatching success suggests similar female investment in egg quality (which represents the trade-off between clutch size and quality of individual egg) and no differences in nest-site selection (which has been documented to affect hatching success, Miller et al. 2003) among females originating from the three foraging areas. These hypotheses may be further addressed in future studies.

Loggerheads foraging in the SNWA are larger, lay larger clutches and appear to have a shorter remigration interval; thus, females feeding in the SNWA will contribute more offspring to the overall population assuming no other differences among females originating from the different areas on other reproductive parameters (clutch frequency and years of reproductive activity). While there is evidence supporting natal homing (Miller et al. 2003) and fidelity to foraging sites (e.g., Vander Zanden et al. 2010, Hawkes et al. 2011), it is unknown whether foraging site choice is inherited. Genetic structure has been described in females nesting at the ACNWR (n = 750) and 19 distinct mt-DNA haplotypes (CC-A1.1 and CC-A1.2 being the most common) have been identified (Shamblin et al. 2012). The genetic structure found in females nesting at the ACNWR suggests that the foraging site heritability hypothesis might be investigated combining isotopic and genetic data. Since loggerheads foraging in SNWA have higher reproductive parameters, the contribution of this foraging area will increase over time if foraging site choice is heritable and survival rates are equal among foraging areas.

Trends in Abundance, Implications and Future Directions

Females foraging near the ACNWR contributed disproportionately to the annual make-up of the nesting aggregation: over the six-year analyzed the SNWA contributed on average 47% of the females (range 25% to 59%), while SW FL contributed on average 33% of the individuals (range 20% to 49%). The MAB contributed fewer females in each given year and its contribution appeared more stable over time (Figure 5.4A). The overall contribution of the three foraging areas was relatively stable among the years analyzed with the exclusion of the 2009 nesting season, which saw a spike in the number of females foraging in SW FL compared to SNWA, and

2012, which showed a significant increase in females foraging in the SNWA balanced by a significant decrease in SW FL contribution (Figure 5.4A). SW FL was the second most important foraging area for females nesting at the ACNWR; however, SW FL turtles were significantly smaller and laid significantly smaller clutches than females foraging in the other two areas. Thus, SW FL contribution in terms of eggs (and ultimately hatchling production) in a given year (number of females from SW FL * average SW FL clutch size) was lower than expected based on the number of individual females (Figure 5.4B).

Loggerheads foraging in the SNWA were more likely to have a two-year remigration interval, while females foraging in the other two areas had the same probability of being on a two- or three-year cycle. Therefore, the consistently higher contribution of SNWA to the nesting aggregation at the ACNWR does not necessarily imply differences in female breeding population size among foraging areas. In contrast, the consistently higher contribution of SNWA may be an artifact of the shorter remigration interval. Environmental conditions in the year or two prior to the nesting year have been found to be strongly correlated to the number of nests produced by NWA loggerheads in a given year (Arendt et al. 2013). In addition, physical indices of environmental variation have been used successfully to estimate remigration intervals in Pacific leatherbacks (Saba et al. 2007). A similar climate modeling approach could be applied to loggerheads nesting at the ACNWR, which accounts for approximately 22.5% of all the NWA loggerheads (Ehrhart et al. 2003), in order to better understand present and predict future trends in nesting abundance.

Overall our results suggest that SNWA and SW FL are the most important foraging areas used by loggerheads nesting at the ACNWR, a result in agreement with previous flipper tag returns (Meylan et al. 1983, Moncada et al. 2010) and telemetry studies (Dodd and Byles 2003, Foley et al. 2013). Thus, analysis of threats and development of mitigation measures should focus on these foraging areas of prime importance. The spatial resolution of stable isotope analysis and spatially implicit models (such as the DFA we performed) although informative, can only identify relatively large geographic areas (e.g., the SNWA which include the Bahamas, Cuba and the Florida Keys). Other tools (e.g., telemetry, aerial surveys, isoscapes – spatially explicit models of stable isotopes) should be used to increase spatial resolution, identify hotspots and maximize cost-benefit of conservation measures.

Stable isotope analysis has been increasingly used to infer the origin (foraging location) of nesting sea turtles feeding in spatially and isotopic distinct areas (i.e., Hatase et al. 2002, Caut et al. 2008, Zbinden et al. 2011, Ceriani et al. 2012, Pajuelo et al. 2012, Vander Zanden et al. 2013). This study as well as the one conducted by Vander Zanden et al. (2013) provides strong evidence supporting the use of stable isotope analysis as a low-cost and effective tool to investigate carry-over effects and analyze trends in nesting abundance for NWA loggerheads and sea turtles, in general. Being able to monitor inter-annual contribution of foraging grounds may aid identifying and addressing threats specific to each foraging aggregation. Moreover, sampling for stable isotope analysis as a component of long-term studies will provide the opportunity to address a variety of research questions beyond the scope of this manuscript. For example, a systematic and long-term collection of isotopic data (which can be achieved using unhatched eggs, Ceriani et al. in review, Chapter 4) may be used to investigate whether females from

distinct foraging areas exhibit differences in arrival time to the nesting beach. Arrival time has implication for female reproductive output because nests laid early in the season experience lower sand temperature that, in turn, affects sex ratio and hatching success (Wibbels 2003). In addition, early season nests are less susceptible to hurricanes and storm events that have a negative impact on hatching success (Pike and Stiner 2007). Climate change models predict an increase in the intensity of storm events (Webster et al. 2005) and, thus, if time of arrival to the nesting beach differs among foraging areas, their contribution (i.e. hatchling production) will vary over time. In addition to a temporal component, undertaking systematic sampling for stable isotope analysis may allow addressing spatial scale questions. Loggerheads and sea turtles, in general, exhibit natal homing (Miller 2003) with some degree of individual variation (e.g., loggerhead mean site fidelity = $16.4 \text{ km} \pm 14.6 \text{ km}$, range = 1.8 to 69 km, Tucker 2010). Recently, Reece et al. (2013) found that sections of the ACNWR are eroding at different rates. If females from different foraging areas selectively chose sections of beach under different rates of erosion, the contribution of that foraging area may change over time (assuming high site fidelity).

In conclusion, we found that intra-population variation in foraging strategies affect reproductive output (clutch size and remigration interval) of the most important loggerhead aggregation in the NWA. Using stable isotope analysis to infer foraging areas allowed us to begin exploring inter-annual contribution of foraging areas to the aggregation nesting at the ACNWR and identifying foraging aggregations of greater conservation importance. This study, although based only on six-year of data and relatively few individuals ($n = 287$), provides a proof of concept that the isotopic approach can be used to interpret trends in abundance at nesting beaches and

demographic parameters affecting those trends. An intensive and long-term sampling for stable isotope analysis, which can be achieved easily and non-destructively by collecting unhatched eggs (Ceriani et al. in review, Chapter 4), will improve our understanding of carry-over effects, demographic parameters and trends in nesting abundance. This information, in turn, combined with other techniques (e.g., climate modeling, threat analysis) may aid managing this species of conservation concern more effectively.

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CHAPTER 6: CONCLUSIONS

Connectivity among populations influences their demographics, genetic structure and response to environmental change. Despite the ecological and evolutionary importance of migration, our understanding of the proximate and ultimate causes behind it is still limited. In order to unravel this puzzle and understand life history trade-offs it is critical to adopt a multi-technique approach.

My research contributes to the field studying migratory connectivity and carry-over effects in migratory species. Moreover, I developed new tools to address conservation issues, as understanding geospatial linkages is critical to foster appropriate management and conservation strategies for migratory species. By using a combination of telemetry and stable isotope analysis, I addressed questions at the individual and population level shedding light onto foraging ecology, migratory linkages, reproductive strategy and basic biological information regarding the loggerhead turtle, a species of conservation concern.

My research focused on the Northwest Atlantic (NWA) loggerhead population and, in particular, on the NWA Florida Peninsular Recovery Unit and the breeding aggregation nesting at the Archie Carr National Wildlife Refuge (ACNWR), the largest loggerhead nesting population in the western hemisphere and the second largest in the world (Ehrhart et al. 2003). Using satellite telemetry, I provided the first documentation that the continental shelf of the Mid- and South-Atlantic Bights (MAB and SAB, respectively), within the U.S. Economic Exclusive Zone, are prime foraging areas for adult loggerheads of the NWA Florida Peninsular Recovery Unit as half

(8 of 16) of the females I tracked from the ACNWR took up residence in these areas (Ceriani et al. 2012, Chapter 2). This result is a major difference from prior satellite tracking studies and may reflect the trade-off between high cost and small sample size, a problem common to satellite telemetry studies.

A large component of my research entailed the validation of the use of low-cost intrinsic markers to overcome potential biases associated with the small sample sizes that characterize telemetry studies. As a result, my research contributes significantly to the growing body of literature studying migration and foraging ecology of migratory species using stable isotope analysis of naturally occurring elements. Sea turtles are being increasingly studied using a combination of satellite telemetry and stable isotope analysis, but several assumptions still must be tested to interpret isotopic patterns in the marine realm. To validate the use of the isotopic approach, I sampled a large number of loggerheads in the NWA that were either equipped with satellite tags or sampled at known foraging areas. I then used a combination of satellite telemetry and stable isotope analysis to (1) evaluate the efficacy of stable isotopes to infer loggerhead migratory strategies, and to (2) create loggerhead-specific isotopic base maps (isoscapes). Isoscapes are valuable tools to visualize isotopic geographic patterns, generate hypotheses and explore whether a spatially explicit approach could be used to gain further insight on the ecology of highly migratory species (Ceriani et al. in review, Chapter 3). I used discriminant function analysis (DFA) to examine how well $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ classified loggerhead foraging areas. The DFA model was derived from isotopic signatures of 58 loggerheads equipped with satellite tags to identify foraging locations. I assessed model accuracy with the remaining 156 untracked loggerheads that were treated as unknown and conducted the first external validation of the isotopic method in

marine systems. The results of the external validation (1) confirmed that assignment models based on tracked loggerheads in the NWA are robust and (2) provided the first independent evidence supporting the use of these models for migratory marine organisms. Additionally, I used the same data set to generate loggerhead-specific $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes, the first for a marine predator in the Atlantic Ocean. The isoscapes I developed, though basic, allowed visualizing geographic isotopic patterns at a different spatial scale than any other isoscape developed in the Atlantic (McMahon et al. 2013) and suggest that a spatially explicit approach may provide an additional tool to explore migratory connectivity.

Even though sea turtles spend the greatest majority of their life in the ocean, they are largely studied at breeding areas where they are more accessible to researchers. After providing evidence supporting the use of the isotopic approach to infer foraging strategies and residence areas *in lieu* of more expensive satellite telemetry, I developed a common currency for stable isotope studies at the nesting beach (Ceriani et al. in review, Chapter 4). Isotopic values of several slow-turnover-rate tissues have been used to identify often-distant foraging areas (Caut et al. 2008, Zbinden et al. 2011, Seminoff et al. 2012, Ceriani et al. 2012, Pajuelo et al. 2012a,b, Vander Zanden et al. 2013a, Tucker et al. *in press*) but there was a lack of common protocol among research groups. Thus, I used several commonly-collected tissues (blood, skin, fresh eggs and unhatched eggs) from loggerhead turtles to develop a common currency for stable isotope analysis that may allow future meta-analyses and the elucidation of isotopic patterns across broader spatiotemporal scales. I found highly significant relationships among the tissue signatures and developed equations to convert isotopic values from one tissue to another. Each female lays several clutches during a nesting season; thus, I examined inter- and intra-clutch

isotopic variability and found that a single sampling event over the three-month nesting season adequately defined the female foraging area. Consequently, I proposed using unhatched eggs as a common currency in stable isotope studies of nesting sea turtles. Unhatched eggs represent a non-invasive and non-destructive method that enables more extensive (both numerically and spatially) sampling. Given similar physiologies, these findings are potentially applicable across sea turtle species.

Lastly, I used stable isotope analysis to examine the link between foraging ecology and reproductive output in order to investigate carry-over effects on loggerheads nesting at the ACNWR over a six-year period. There is increasing evidence that arrival time to breeding areas, reproductive success and annual breeding population size are linked to non-breeding habitat quality (Marra et al. 1998, Bearhop et al. 2005, Norris 2005, Vander Zanden et al. 2013b), a phenomenon that is described under the umbrella term of “carry-over effects.” Thus, what we observe at one location is the result of a complex set of interactions occurring at the prior foraging location and migratory route. To understand the biology of any animal, resident or migratory, we need to consider how events in different stages of the life- and the annual-cycle interact and influence subsequent events at the level of the individual and eventually the population (Webster et al. 2002).

The relatively low cost of the isotopic method allowed me to sample a large number of nesting females and obtain information that is more representative at the population level. I then used the assignment model approach developed in previous chapters to infer the putative foraging area used by each female. Next, I examined whether phenotypic variability in female body size or

other reproductive parameters (clutch size, hatching and emerging success, remigration interval) were associated with a female's foraging region. Females nesting at the ACNWR travel from different foraging grounds located at variable distances from the breeding area. In addition to differences in migration distance, females using spatially distinct foraging areas may experience different environmental conditions (e.g., temperature regimes, food availability) that may affect their reproductive output and the time it will take to obtain enough resources to invest in reproduction. Thus, the relative contribution of each foraging area to the annual nesting assemblage may vary among years. My results indicate that foraging area is associated with the size of adult breeding females and fecundity (clutch size and remigration interval). Larger females laid larger clutches, differences in clutch size remained significant after controlling for body size, and females foraging in the Subtropical NWA (SNWA) were more likely to have a two-year remigration interval. Foraging areas near the ACNWR contributed the majority of females to the annual composition of the nesting aggregation: over the six-year study the SNWA and the SW FL continental shelf (SW FL) contributed on average 47% and 33% of the individuals, respectively. Overall my results suggest that SNWA and SW FL are the most important foraging areas used by loggerheads nesting at the ACNWR, a result in agreement with previous flipper tag returns (Meylan et al. 1983, Moncada et al. 2010) and telemetry studies (Dodd and Byles 2003, Ceriani et al. 2012, Foley et al. 2013). Hence, efforts focused on loggerhead female threat reduction at these foraging areas may have the highest conservation impact.

In conclusion, my multi-faceted approach contributes to the field of ecology, basic biology and conservation. Along with sea birds, marine mammals and sharks, sea turtles are caught in large

numbers as a result of fishery by-catch (Hall et al. 2000, Baum et al. 2003, Lewison et al. 2004); thus, a better understanding of their spatial ecology has become a conservation and management priority (Hamann et al. 2010). In addition to conserving *Sargassum* and nesting habitats, essential for oceanic and breeding adult loggerheads, respectively, critical foraging grounds for larger class sizes with high reproductive value (Crouse et al. 1987) must be identified and protected in order to develop a holistic management approach for this imperiled species. Conservation funds are limited and there is a need to prioritize where funds should be spent in order to maximize conservation outcomes. Understanding relative importance of foraging grounds and carry-over effects will allow managers to make more informed decisions by focusing mitigation and by-catch reduction measures to areas that are loggerhead hotspots and on aggregations of higher reproductive value.

Future Research Directions and Remarks

My doctoral work contributed to unraveling issues associated with loggerhead migratory connectivity and shed light onto carry-over effects. However, as a result of this dissertation, new questions and research paths have arisen that I hope to address in the future or that I hope will inspire others to pursue this fascinating line of research.

I conclude with few recommendations and highlighting some future research directions.

1) I would like to emphasize the importance of collecting and banking tissue samples for intrinsic marker analyses (e.g., stable isotopes, trace elements, contaminants) from any sea turtle (and in general, any organism, whenever possible) equipped with tracking devices. Telemetry is an expensive and valuable tool that, in addition to addressing fine-scale movement questions, can be used to validate the use of techniques that will become available in the future.

2) I strongly encourage a systematic collection of samples for stable isotope analysis as it is already done for genetics both at nesting and foraging areas. Despite the large number of data I have collected, I was only able to scratch the surface of possible carry-over effects. Numerically extensive and long-term datasets are necessary to address the relative importance of foraging grounds, how their contribution changes over the years, and what are the reproductive consequences of an individual foraging site choice (carry-over effects). This information will help elucidate the ecological and evolutionary importance of migration and aid understanding life history trade-offs.

3) The single element isoscapes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) I developed are promising, but longer and more extensive datasets are needed to assess isotopic temporal and spatial variability. Future studies should model the contribution of environmental factors (e.g., sea surface temperature, bathymetry) that affect the geographic distribution of isotope signatures and how they could be included to improve the isoscapes (Bowen et al. 2005). Moreover, continuous surface assignment models should incorporate and model several levels of isotopic variation such as (1) within site

variation (namely isotopic variation found in individuals sampled at a given site), (2) isotopic differences between age groups, and (3) inter-annual isotopic variation (Wunder and Norris 2008, Wunder 2010, 2012).

Meanwhile, the dataset I have generated makes it possible to develop a dual-isotope isoscape to further investigate NWA loggerhead foraging strategies. I expect the bivariate approach that integrates the spatial patterns of the two elements will increase the resolution of the loggerhead isoscape in the NWA and, therefore, improve the geospatial assignment accuracy. The development of multi-isotope isoscapes is at the forefront of the field in geospatial assignment of migratory organisms and the results of this approach are promising for locations where isotopic patterns are not well studied (Hobson et al. 2012). The dataset I have collected represents a unique opportunity to test the limits of isoscapes, provides the potential for testing the efficacy of isoscapes for assigning sea turtles to foraging areas, and can be used to test the robustness of recently published isoscape models for the Atlantic Ocean based on plankton (McMahon et al. 2013). Lastly, a dual-isotope loggerhead isoscape will allow modeling continuous-probability surfaces for the assignment of unknown origin individuals that are commonly sampled both on the nesting beaches (Reich et al. 2010, Ceriani et al. 2012, Vander Zanden et al. 2013a,b) and at sea (e.g., the U.S. NMFS fishery observer program). The development of continuous surface assignments based on multiple element isoscapes (currently $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ but potentially more isotopes, trace elements and contaminants in the future) may provide resource managers the ability to identify higher probability areas of interaction with anthropogenic activities (e.g., fishery operations, military activities, oil exploration).

4) During my doctoral work, I found that intra-population variation in foraging strategies affects reproductive output of the most important loggerhead aggregation in the NWA. Females nesting at the ACNWR used distinct foraging areas that may differ in resource availability and quality; thus, female nutritional conditions may vary among areas and these differences may ultimately affect egg quality. Female and egg nutritional analyses should be pursued to further elucidate carry-over effects in this population and provide baseline data necessary for health assessment of wild loggerheads.

5) As others have done before me (Seminoff et al. 2007, Martinez del Rio et al. 2009), I suggest more captive studies should be undertaken to measure isotopic turnover rates and discrimination factors, a step necessary to interpret correctly the data obtained from wild ranging animals. I tried to address these questions in adult loggerheads but the task was more challenging than expected and is a work in progress.

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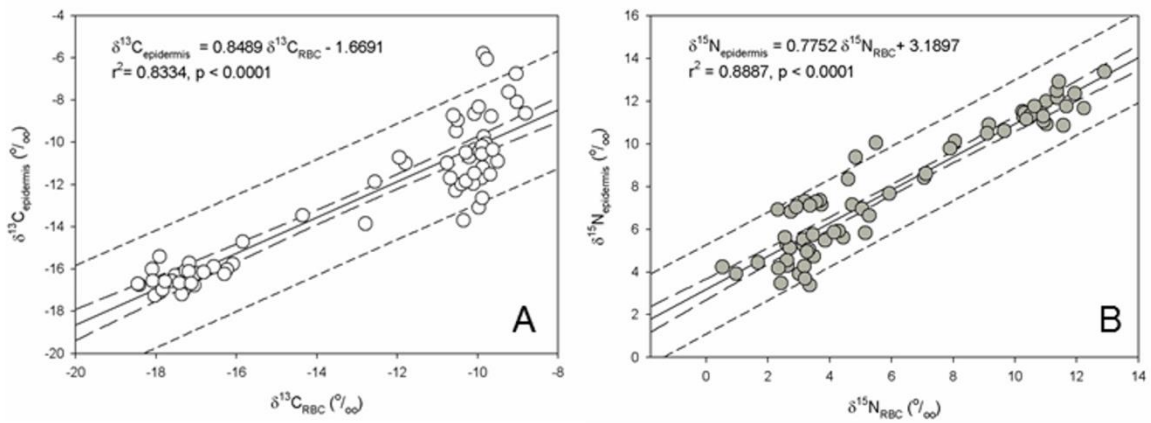
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**APPENDIX A (FOR CHAPTER 3):
RELATIONSHIP BETWEEN EPIDERMIS AND RBC STABLE ISOTOPES**



Relationship between epidermis and RBC stable isotope values of 66 juvenile loggerheads: (A) carbon ($\delta^{13}\text{C}$) and (B) nitrogen ($\delta^{15}\text{N}$). Long dash lines denote 95% confidence interval, short dash lines indicate 95% prediction interval.

**APPENDIX B (FOR CHAPTER 3):
SUMMARY OF BODY SIZE AND STABLE ISOTOPE VALUE**

(A) Summary of body size (expressed as standard Curved Carapace Length, CCL) and (B) stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for the 214 loggerheads included in this study. Thirty-two females were sampled and equipped with satellite tags after nesting and 182 individuals were sampled at foraging grounds. Twenty-six of the 182 loggerheads were equipped with satellite units and transmitted long enough to be included in the training subset ($n = 58$). The remaining 164 were either untracked or their tracking data were too short to derive foraging areas. Female body size and stable isotope values are reported based on the post-nesting destination identified by satellite telemetry. We sampled four foraging areas: the Scotian Shelf, Slope and the abyssal plain (CAN), the Mid-Atlantic Bight (MAB), where we sampled both on the continental shelf, and in North Carolina estuaries (NC estuaries), the South Atlantic Bight (SAB), and, in particular, loggerheads caught of Cape Canaveral (FL), and the Key West National Wildlife Refuge (Key West NWR) in the Subtropical Northwest Atlantic (SNWA).

A

Data Source	Geographic area		CCL (cm)			
			Mean	SD	Min	Max
Nesting ¹ (n=30)		North (n = 10)	102.4	7.8	89.0	111.2
		Central (n = 5)	91.3	1.6	89.0	93.1
		South (n = 15)	100.5	6.4	81.8	108.8
Foraging (n=180)	CAN	CAN (n = 66)	64.2	5.1	51.0	76
	MAB	Continental Shelf (n = 25)	79.3	7.9	63.0	93.0
		NC estuaries ² (n = 18)	67.7	7.8	58.4	88.6
	SAB	Canaveral FL (n = 30)	76.7	11.2	57.5	100.9
	SNWA	Key West NWR (n = 41)	80.1	8.0	66.5	95.6

Note: CCL measurements were missing for two adult females and two juveniles

¹Fourteen of the nesting females were included in Ceriani et al. (2012)

²See McClellan and Read (2007) and McClellan et al. (2010) for further details

B

Data source	Geographic area		$\delta^{13}\text{C}$ (‰)				$\delta^{15}\text{N}$ (‰)			
			Mean	SD	Min	Max	Mean	SD	Min	Max
Nesting ¹ (n=32)		North (n = 11)	-16.1	0.7	-16.9	-14.6	13.8	2.2	10.2	17.0
		Central (n = 5)	-14.7	0.8	-15.9	-13.9	12.4	0.7	11.3	13.1
		South (n = 16)	-11.4	1.4	-13.8	-9.5	7.8	1.7	5.8	11.7
Foraging (n=182)	CAN	CAN (n = 68)	-16.8	0.5	-18.1	-15.1	9.0	0.5	7.4	10.1
	MAB	Continental Shelf (n = 25)	-16.4	0.5	-17.3	-15.4	11.3	1.0	8.4	13.4
		NC estuaries ² (n = 18)	-16.4	0.6	-17.4	-14.8	10.4	1.1	8.9	12.9
	SAB	Canaveral FL (n = 30)	-15.6	1.0	-17.6	-13.9	11.3	1.1	10.0	13.6
	SNWA	Key West NWR (n = 41)	-10.5	2.1	-14.7	-5.8	5.9	1.6	3.4	10.0

¹Fourteen of the nesting females were included in Ceriani et al. (2012)

²See McClellan and Read (2007) and McClellan et al. (2010) for further details

**APPENDIX C (FOR CHAPTER 3):
DIFFERENCES IN BODY SIZE AND THE EFFECT OF FORAGING
AREA**

Examining body size and isotopic patterns in the testing data set ($n = 156$).

We conducted a series of analyses on the test subset that was composed of 156 loggerheads captured at four foraging grounds: (1) the Scotian Shelf, Slope and the abyssal plain (CAN), (2) the Mid Atlantic Bight (MAB), which included loggerheads sampled on the continental shelf ($n = 25$) and within North Carolina estuaries ($n = 18$), (3) the South Atlantic Bight (SAB), which included loggerheads captured in Cape Canaveral (FL) and (4) loggerheads sampled in the Key West NWR in the Subtropical Northwest Atlantic (SNWA). Differences in body size may represent dietary preference differences that could affect the stable isotope ratios of loggerhead tissues. Thus, we tested for differences in body size among the four foraging grounds. Body size measurements were missing for two loggerheads from the CAN aggregation. We found significant differences in body size ($F_{3, 150} = 43.753$, $p < 0.001$) among loggerheads in the four foraging areas sampled. Post hoc Games-Howell (GH) multiple comparison tests indicated that individuals found in Canadian waters were significantly smaller than loggerheads from the other three regions (MAB: $p < 0.001$; SAB: $p = 0.005$; SNWA: $p < 0.001$). We then combined loggerheads from CAN and the MAB to represent the north aggregation and tested for differences in body size among the three groups that were used to develop the DFA: northern, central and southern. We found significant differences in body size ($F_{2, 151} = 24.65$, $p < 0.001$) among groups. Post hoc GH multiple comparison tests indicated that northern individuals were significantly smaller than loggerheads in the southern area ($p < 0.001$).

Since body size differed among foraging areas, we used analysis of covariance (ANCOVA) to determine whether the effect of foraging area was significant after controlling for size. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ differed significantly among foraging grounds ($\delta^{13}\text{C}$, $F_{2, 150} = 277.82$, $p < 0.0001$; $\delta^{15}\text{N}$,

$F_{2,148} = 129.48, p < 0.001$) after accounting for differences in body size. The interaction of loggerhead size and foraging location was significant only for $\delta^{15}\text{N}$ ($F_{2,148} = 9.30, p = 0.0002$).