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Modeling and mapping isotopic patterns in the Northwest Atlantic derived from loggerhead sea turtles

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Abstract. Stable isotope analysis can be used to infer geospatial linkages of highly migratory species. Identifying foraging grounds of marine organisms from their isotopic signatures is becoming de rigueur as it has been with terrestrial organisms. Sea turtles are being increasingly studied using a combination of satellite telemetry and stable isotope analysis; these studies along with those from other charismatic, highly vagile, and widely distributed species (e.g., tuna, billfish, sharks, dolphins, whales) have the potential to yield large datasets to develop methodologies to decipher migratory pathways in the marine realm. We collected tissue samples (epidermis and red blood cells) for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis from 214 individual loggerheads (*Caretta caretta*) in the Northwest Atlantic Ocean (NWA). We used discriminant function analysis (DFA) to examine how well $\delta^{13}C$ and $\delta^{15}N$ classify loggerhead foraging areas. The DFA model was derived from isotopic signatures of 58 loggerheads equipped with satellite tags to identify foraging locations. We assessed model accuracy with the remaining 156 untracked loggerheads that were captured at their foraging locations. The DFA model correctly identified the foraging ground of 93.0% of individuals with a probability greater than 66.7%. The results of the external validation (1) confirm that assignment models based on tracked loggerheads in the NWA are robust and (2) provide the first independent evidence supporting the use of these models for migratory marine organisms. Additionally, we used these data to generate loggerhead-specific $\delta^{13}C$ and $\delta^{15}N$ isoscapes, the first for a predator in the Atlantic Ocean. We found a latitudinal trend of $\delta^{13}C$ values with higher values in the southern region (20–25 °N) and a more complex pattern with $\delta^{15}N$, with intermediate latitudes (30–35 °N) near large coastal estuaries having higher $\delta^{15}N$-enrichment. These results indicate that this method with further refinement may provide a viable, more spatially-explicit option for identifying loggerhead foraging grounds.

Key words: carbon-13; *Caretta caretta*; geographic assignment models; isoscapes; migratory connectivity; Northwest Atlantic; nitrogen-15; satellite telemetry; stable isotopes.

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Introduction

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.

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). Each technique has advantages and limitations; hence, combining complementary techniques may improve our understanding of migratory connectivity (e.g., Rundel et al. 2013). Satellite telemetry provides fine-scale movement information at the individual-level, but the high cost limits the number of individuals that can be tracked, which can lead to biased results. On the other hand, stable isotope analysis of light elements (C, H, N, O, and S) is a relatively cost-effective and rapid tool for studying large-scale migratory connectivity in a variety of taxa allowing population-level questions (Hobson 1999) to be addressed at a coarser spatial resolution. The isotopic approach succeeds because ratios of stable isotopes of naturally occurring elements often change in systematic ways across landscape and continental scales as a result of several biogeochemical processes (Hobson 1999, Ramos and González-Solís 2012, McMahon et al. 2013a). Stable isotope ratios originating at the base of food webs can be discerned at higher trophic levels. Thus, stable isotopes act as forensic tracers, i.e., individuals that move between isotopically distinct landscapes maintain measurable isotopic differences in their tissues that can be related to past locations (Wassenaar 2008, Graham et al. 2010). Stable isotope analysis has helped unravel migratory behaviors of marine species (Killingley 1980, Hobson 1999, Trueman et al. 2012), but despite significant progress, isotopic patterns and their underlying drivers in marine systems are less understood compared to terrestrial systems. Satellite-tracked individuals often constitute training data for the development of models to geographically assign individuals of unknown origin (e.g., Jaeger et al. 2010, Ceriani et al. 2012, Seminoff et al. 2012). However, 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. 2012) 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.

Sea turtle, in particular loggerhead (*Caretta caretta*), migratory connectivity has been increasingly studied using a combination of satellite telemetry and stable isotope analysis (Hatase et al. 2002, Zbinden et al. 2011, Ceriani et al. 2012, Pajuelo et al. 2012, Seminoff et al. 2012). Loggerheads are generalist consumers feeding on a variety of food items, mostly benthos when on the continental shelf (Hopkins-Murphy et al. 2003, but see McClellan et al. 2010). Although a study using longitudinal carapace samples of adult females revealed that individuals feed consistently upon the same mixture of prey items (Vander Zanden et al. 2010), trophic variability may exist among class sizes (Dodd 1988). 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). Breeding females migrate 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 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, Foley et al. 2013): (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 et al. 2003, Broderick et al. 2007, Tucker et al. 2014). NWA loggerheads 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). NWA juveniles 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 large class size loggerheads, i.e., curved carapace length (CCL) $>$ 64 cm (Bjorndal et al. 2000), is better understood than many other marine species, which makes them good candidates to assess existing geographic assignment methods and develop new approaches (e.g., see Wunder 2012 for overview of geographic assignment models).

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 and juveniles sampled at foraging grounds across a broader geographic area. With this more numerically and spatially extensive dataset, we conduct a formal validation of the stable isotope-derived geographic assignments and create loggerhead specific isotopic base maps (i.e., isoscapes) to visualize isotopic geographic patterns to gain further insight into the ecology of this threatened 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 individual loggerheads in the NWA (Fig. 1, Table 1). Our data set is comprised of two subsets: (1) 58 loggerheads equipped with satellite devices at either 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).

We collected a skin biopsy for stable carbon and nitrogen isotope analysis from 32 loggerheads nesting in Florida between 2008 and 2012. For the in-water loggerhead sampling, we collected tissues from four foraging areas in the NWA (Fig. 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. Our sampling encompassed several class sizes representing different life stages.

Living 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 (Avens and Snover 2013). We used the size classification (Stage I to Stage V) proposed by the Turtle Expert Working Group (2009) and adapted by Murray (2011) to create discrete size classes. Little is known about CAN loggerheads, but Stage III juveniles (60.5 $<$ CCL $<$ 75.7 cm), and possibly some Stage II juveniles

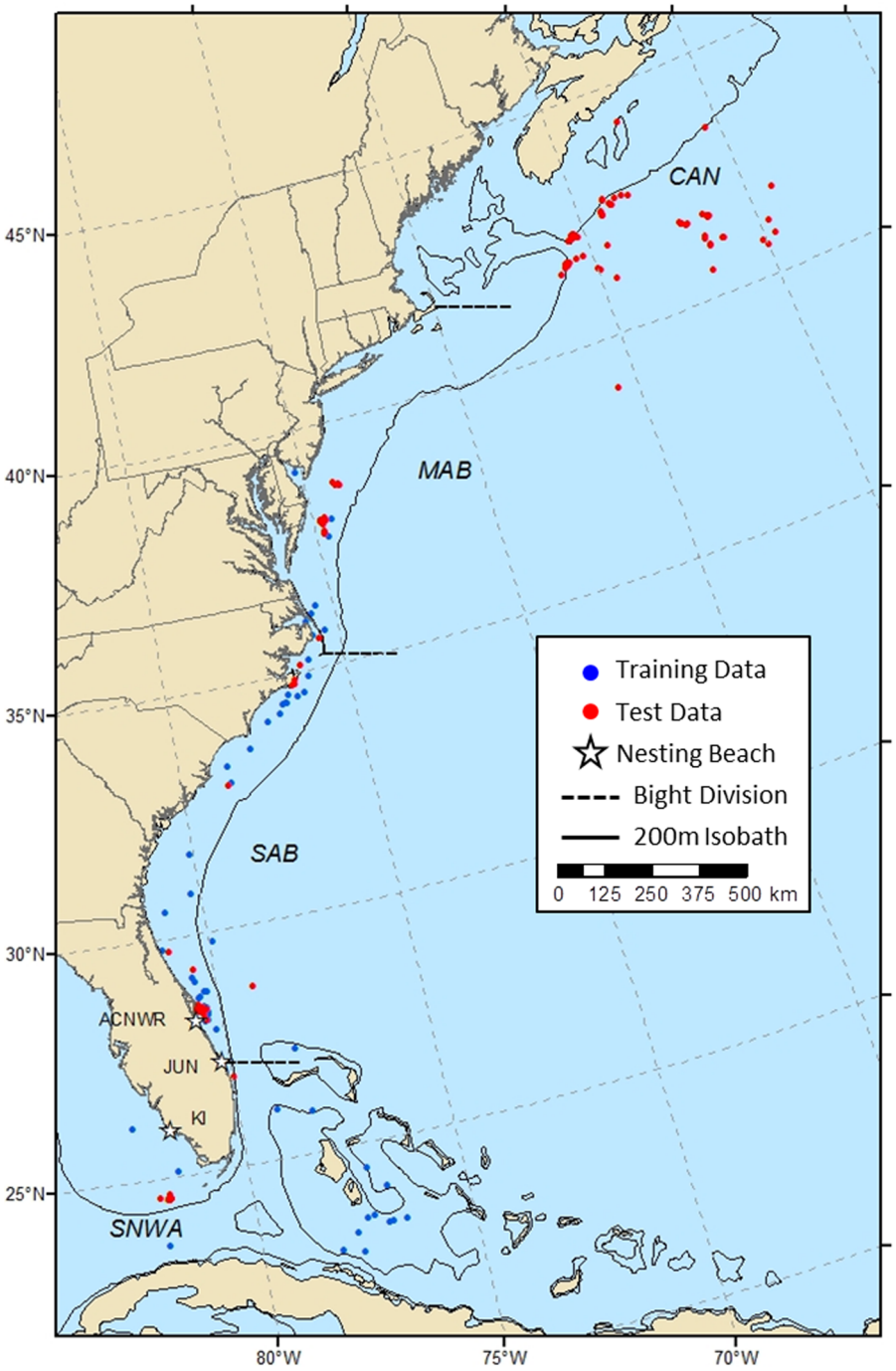

Fig. 1. Foraging area locations of the 205 loggerheads (32 nesting females and 173 individuals captured at foraging grounds) out of 214 total included in this study for which we had specific foraging area geocoordinates. We sampled four geographic areas: the waters off Nova Scotia, Canada (CAN), the 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).

Table 1. Foraging area by encounter type, sample size, year of collection, tissue sampled and data source for the 214 individual loggerheads included in this study.

Foraging area	n	Year	Tissue	Source
Nesting†	32			
MAB	11	2008–2012	Skin, RBC	UCF Marine Turtle Research Group, Sea Turtle Conservancy, NMFS Southeast Fisheries Science Center
SAB	5	2008–2012	Skin, RBC	UCF Marine Turtle Research Group, Sea Turtle Conservancy, NMFS Southeast Fisheries Science Center
SNWA	16	2008–2012	Skin, RBC	UCF Marine Turtle Research Group, Sea Turtle Conservancy, Conservancy of Southwest Florida
Foraging	182			
CAN	68	2011–2012	Skin	Canadian Sea Turtle Network
MAB, Continental Shelf	25	2011	Skin, RBC	Coonamessett Farm Foundation and NMFS Northeast Fisheries Science Center
MAB, NC estuaries‡	18	2002–2004	RBC	McClellan and Read 2007
SAB, Cape Canaveral FL§	30	2013	Skin	NMFS Southeast Fisheries Science Center
SNWA, Key West NWR	41	2010–2011	Skin, RBC	Inwater Research Group

Notes: Abbreviations are: CAN = waters off Nova Scotia, Canada, MAB = Mid-Atlantic Bight, SAB = South Atlantic Bight, SNWA = Subtropical Northwest Atlantic, NC = North Carolina, FL = Florida, Key West NWR = Key West National Wildlife Refuge, RBC = red blood cells.

† Fourteen of the nesting females were included in Ceriani et al. (2012). Nesting females were satellite tagged at Archie Carr National Wildlife Refuge (east central Florida, n = 21), Juno Beach (south Florida; n = 6) and Keewaydin Island (southwest Florida; n = 5).

‡ Thirteen of the 18 loggerheads captured in the NC estuaries were satellite tagged.

§ Thirteen of the 30 loggerheads captured in the Cape Canaveral were satellite tagged.

(16.2 < CCL < 60.5 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, 2007, Musick and Limpus 1997, McClellan and Read 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: in spring and summer, this area 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; J. 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, CCL > 101.5 cm), which have already undergone ontogenetic shifts.

Tissue processing and stable isotope analysis

We measured the stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios of red blood cells (RBC) and epidermis. Tissue turnover rates in sea turtles have not been measured in captivity (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). Thus, RBC and skin samples are assumed to 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 (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 × 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 N_2 and CO_2 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: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X is ^{15}N or ^{13}C, and R is the corresponding ratio ^{15}N:^{14}N or ^{13}C:^{12}C. 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 ±0.14‰ for $\delta^{13}C$ and 0.12‰ for $\delta^{15}N$. 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 (typically <0.5‰) are much smaller than the range of isotopic values sampled (>10‰; Ceriani et al. 2012, Pajuelo et al. 2012).

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; C. R. 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 2011) 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; Fig. 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 (epidermis $\delta^{13}C = 0.8489\ \delta^{13}C_{RBC} - 1.6691$, $r^2 = 0.833$, $p < 0.001$; epidermis $\delta^{15}N = 0.7752\ \delta^{15}N_{RBC} + 3.189$, $r^2 = 0.889$, $p < 0.001$; Appendix A: Fig. A1). Recently, tight relationships between different tissue isotopic values have been found in adult loggerheads and conversion factors have been calculated (Ceriani et al. 2014).

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 $\delta^{13}C$ and $\delta^{15}N$ predict the general location of loggerhead foraging grounds (SPSS v. 19). The $\delta^{13}C$ and $\delta^{15}N$ 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 assign-

ment scenarios based on different probabilities of membership.

Development of isoscapes

Of the 214 samples, we used only 205 that had specific geocoordinate locations associated with foraging areas to generate the $\delta^{13}C$ and $\delta^{15}N$ 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 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 choose a cut-off of 64.0 cm, which is the size at which almost all Atlantic loggerheads are presumed to have been recruited out of the oceanic stage (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 2008) 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 $\delta^{13}C$ 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 ($\delta^{13}C$ and $\delta^{15}N$) isotopic signatures (MANOVA, Pillai's trace test, $F_{4, 110} = 21.128$, $p < 0.001$), and in univariate analyses where both $\delta^{13}C$ (ANOVA, $F_{2,55} = 130.286$, $p < 0.001$) and $\delta^{15}N$ values ($F_{2,55} = 26.305$, $p < 0.001$) differed among foraging aggregations (Fig. 2A). Post hoc GH multiple comparison tests indicated that all aggregations

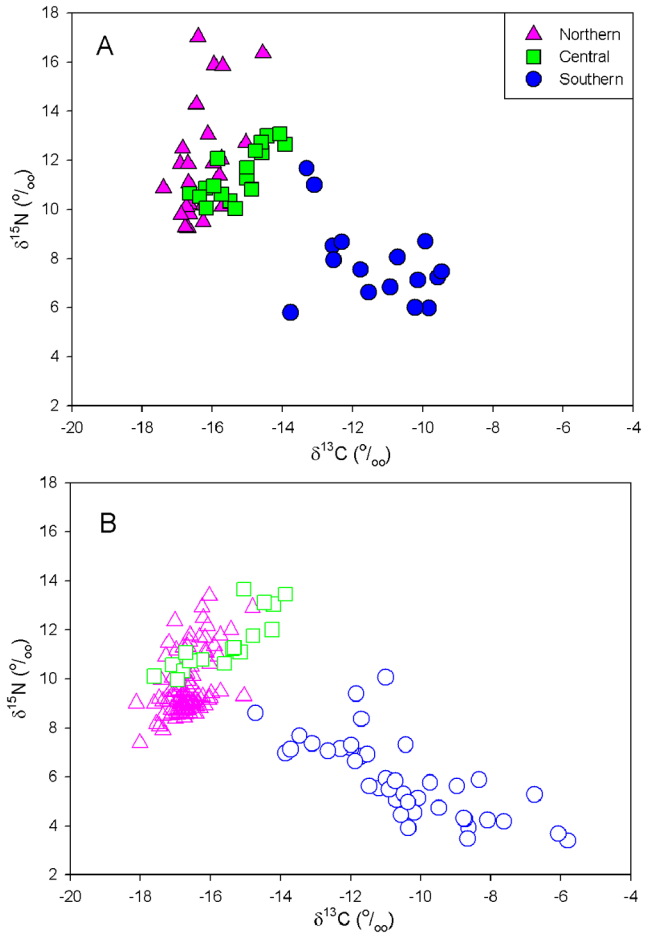

Fig. 2. Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) of (A) the 58 loggerheads equipped with satellite tags (training subset) and (B) the 156 untracked loggerheads (test subset) sampled at foraging areas in the Northwest Atlantic. The Northern area in (B) includes CAN and MAB loggerhead samples. Central is SAB and Southern is SNWA.

differed significantly in $\delta^{13}C$ among each other ($p < 0.001$ in all comparisons). The $\delta^{15}N$ 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 $\delta^{15}N$ ($p = 0.623$). The "unknown" test subset seemed to exhibit similar isotopic patterns (Fig. 2B) as the training subset.

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

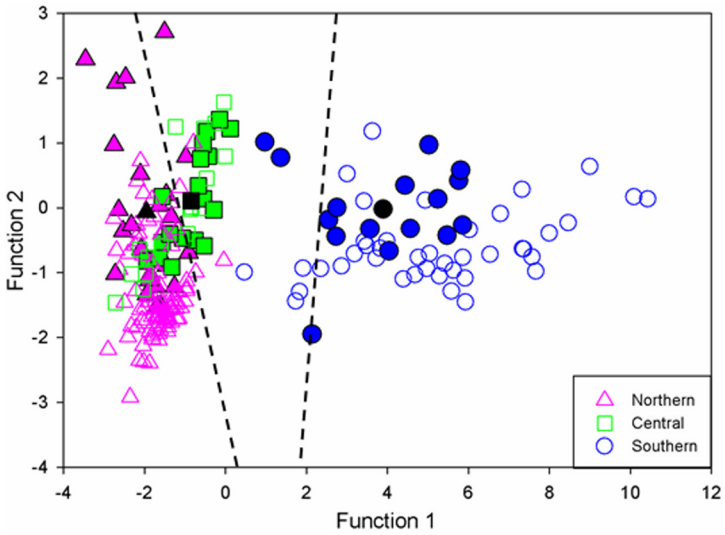

Fig. 3. Discriminant Function Analysis (DFA) of foraging groups based on the stable carbon and nitrogen isotope ratios. The filled markers correspond to the training subset. The empty markers correspond to the test subset. The black symbols correspond to the group centroid. Dashed lines define the DFA territories.

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 $\delta^{13}C$ and $\delta^{15}N$ differed significantly among foraging grounds ($\delta^{13}C$: $F_{2,52} = 94.85$, $p < 0.0001$; $\delta^{15}N$: $F_{2,50} = 4.50$, $p = 0.0160$). $\delta^{13}C$ increased significantly with body size ($F_{1,52} = 4.36$, $p = 0.042$) and, while there was no main effect of size on $\delta^{15}N$ ($F_{1,50} = 0.67$, $p = 0.416$), the interaction of loggerhead size and foraging location was significant for $\delta^{15}N$ ($F_{2,50} = 13.56$, $p < 0.0001$). Summaries of body size and stable isotope ratios for the entire data set are provided in Appendices B and C, respectively. Appendix D 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 ($P >$ 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 ($\delta^{13}C$ and $\delta^{15}N$) became not significant X^2 (1) = 0.301, $p = 0.583$. The $\delta^{13}C$ skin values contributed the most to separation among groups ($\delta^{13}C$ $r = 0.817$, $\delta^{15}N$ $r = -0.673$). The first discriminant function accounted for 99.9% of the between-group variability (Fig. 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, which was based on the above classification functions (Table 2). Foraging areas used by those 156 loggerheads were known. These 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

Table 2. Foraging ground assignment, number and percentage (in parentheses) for the discriminant model based on $\delta^{13}C$ and $\delta^{15}N$ values of loggerhead epidermis.

			Data source		Predicted group membership			
Data type	n	Location	Nesting†	Foraging‡	North	Central	South	Total
Training data	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	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.
† Loggerheads that were sampled and equipped with satellite tags at the nesting beach.
‡ Loggerheads that were sampled at their foraging grounds; the ones used for training were equipped with satellite tags.

different probabilities of membership (Fig. 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 ≥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.

Isoscapes

Both $\delta^{13}C$ and $\delta^{15}N$ varied considerably for loggerheads across the sampled geographic area. Loggerhead $\delta^{13}C$ 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 $\delta^{15}N$ ranged from 3.39‰ to 17.02‰ and exhibited a more complex pattern with depleted values at the lowest latitudes we sampled, intermediate $\delta^{15}N$ 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 $\delta^{13}C$ and $\delta^{15}N$ values of loggerhead epidermal tissue (Fig. 5A and Fig. 6A, respectively) 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 (i.e., observed) values for $\delta^{13}C$ (observed $\delta^{13}C = 1.03 \cdot$predicted $\delta^{13}C + 0.42$‰) and 83% for $\delta^{15}N$ (observed $\delta^{15}N = 1.07 \cdot$predicted $\delta^{15}N - 0.66$‰). 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 $\delta^{13}C$ and $\delta^{15}N$, 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‰ (Fig. 5B) for $\delta^{13}C$ and from 0.18‰ to 3.15‰ (Fig. 6B) for $\delta^{15}N$. We cropped areas beyond 400 km of the sample points which included areas that exhibited high levels of uncertainty.

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 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–

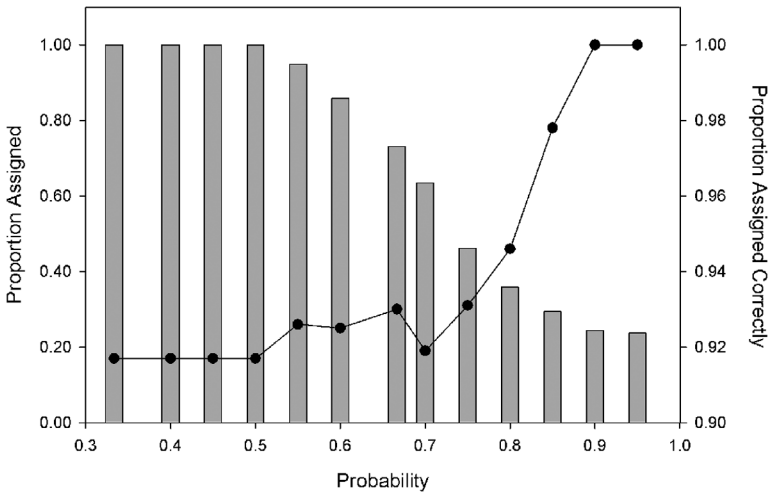

Fig. 4. External validation and evaluation of assignment model performance under different probabilities of membership scenarios. Histogram represents the proportion of the 156 "unknowns" that could be assigned for a given cut-off probability or odds ratio (e.g., 2:3 = 66.66%). The black line indicates what proportion of the "unknown" that met the probability criterion was assigned correctly.

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 $\delta^{13}C$ and $\delta^{15}N$ in the NWA. Latitudinal differences in $\delta^{13}C$ 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; albatrosses Jaeger et al. 2010; Cory's shearwater, Roscales et al. 2011). Latitudinal differences in $\delta^{13}C$ 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 $\delta^{13}C$ values correlate with SST because water temperature affects both cell growth rates and dissolved carbonate concentrations, and thus have a direct effect on the $\delta^{13}C$ values of primary producers. Therefore, an environmental parameter (SST) appears to be a good proxy for phytoplankton $\delta^{13}C$, which, in turn, is reflected in the $\delta^{13}C$ values of marine organisms at higher trophic levels. In addition, the south to north $\delta^{13}C$ 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 $\delta^{13}C$ values falling within the range associated with C_4 metabolism (McMillan et al. 1980, Hemminga and Mateo 1996). Hence ben-

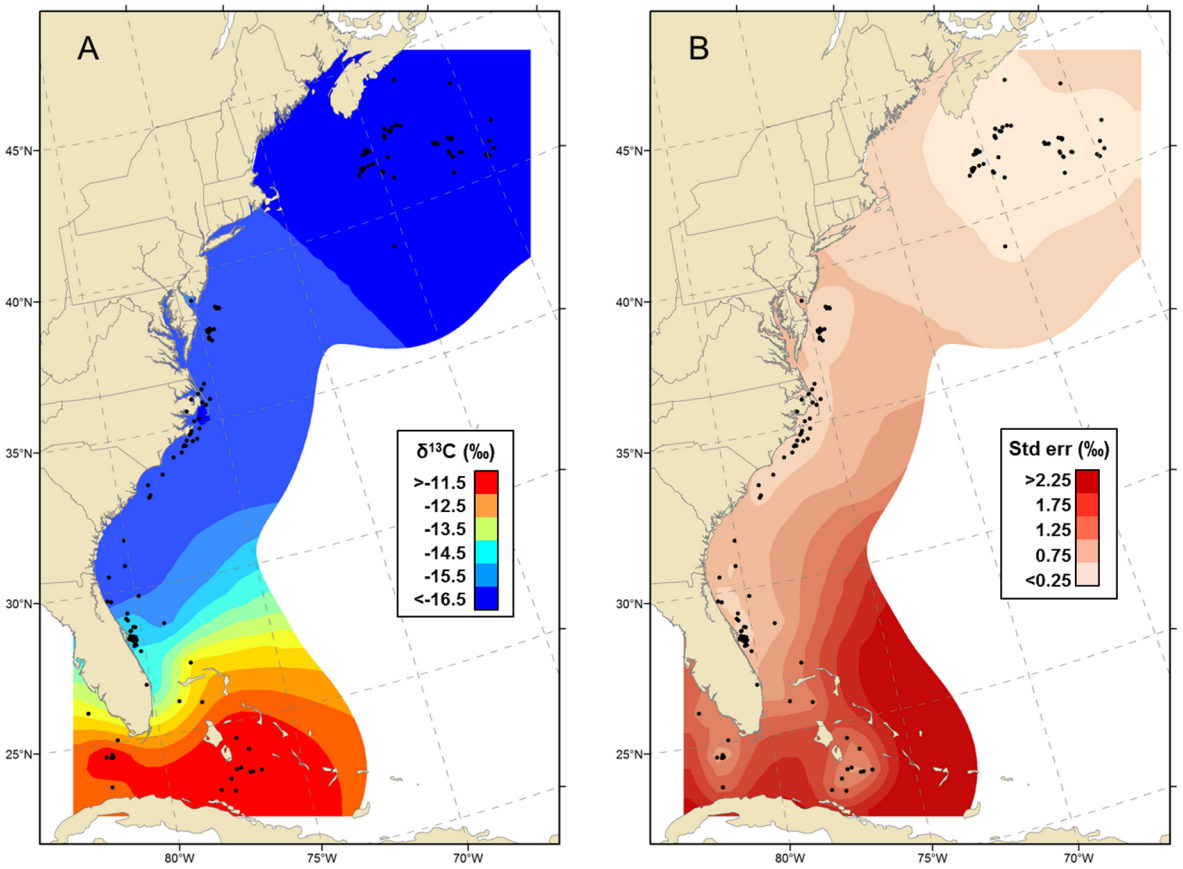

Fig. 5. Isoscape of $\delta^{13}C$ (A) derived from loggerhead epidermal tissue and associated standard error surface (B) based on cross validation of observed and predicted values.

thic seagrass- or macro-algae-based food webs are more ^{13}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 $\delta^{13}C$ in loggerhead tissues are due to a combination of low/high latitudes, nearshore/offshore, benthic/pelagic, and seagrass/phytoplankton-based food webs gradients.

While $\delta^{13}C$ isopleths exhibited a clear latitudinal trend, $\delta^{15}N$ patterns were less linear. We attribute these patterns to a combination of three factors: (1) a baseline shift in primary producer $\delta^{15}N$, (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 $\delta^{15}N$ 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 $\delta^{15}N$ have been attributed to primary producers' shift in nitrogen values (Ceriani et al. 2012, Pajuelo et al. 2012) related to prevailing N cycling regimes that are transferred to higher trophic levels and oceanic/neritic foraging strategies (McClellan et al. 2010). Nitrogen stable isotope ratios of primary producers are a function of $\delta^{15}N$ values of their nutrient pools (e.g., nitrate, ammonium, N_2), biological transformations (e.g., denitrification increases $\delta^{15}N$ while nitrogen fixation lowers $\delta^{15}N$ as these processes preferentially choose

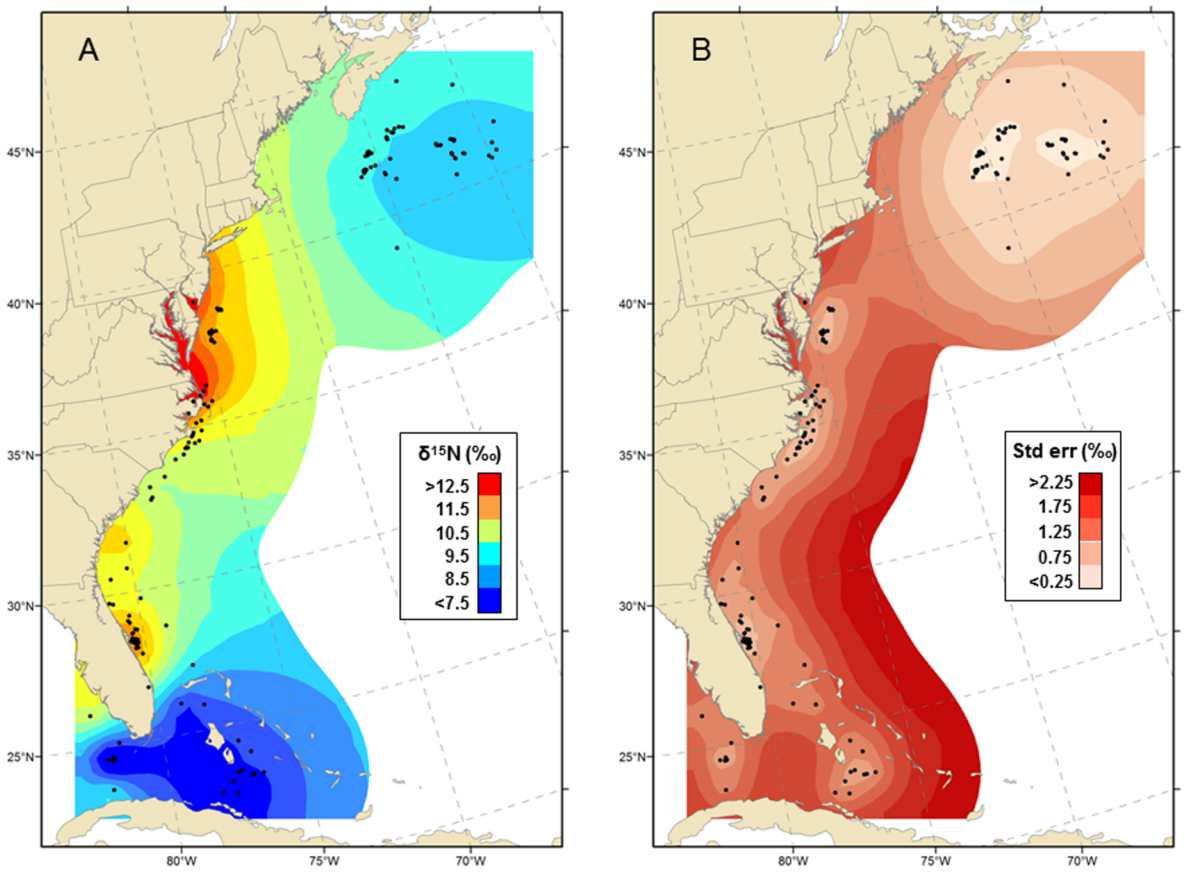

Fig. 6. Isoscape of $\delta^{15}N$ (A) derived from loggerhead epidermal tissue and associated standard error surface (B) based on cross validation of observed and predicted values.

^{14}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 $\delta^{15}N$ (Fennel et al. 2006).

We believe the observed nitrogen patterns are also partially driven by differences in foraging strategies among the aggregations we sampled. 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 C) suggesting that individual loggerheads feed on a similar diet mixture 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 ^{15}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 δ^{15}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 δ^{15}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 δ^{13}C and δ^{15}N isoscapes for the Atlantic Ocean (McMahon et al. 2013b). Contrary to the patters we observed, McMahon et al.'s δ^{13}C isoscape shows little spatial structure within the geographic area we sampled, while their δ^{15}N isoscape indicates a progressive northward enrichment in δ^{15}N values between the SNWA and the Grand Banks. These discrepancies are likely due to differences in scale (ocean basin vs. continental shelf) and resolution (sample locations) of study as well as species (zooplankton-primary consumer vs. loggerhead-high-level consumer).

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. 2012*a*, 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. Overall turtles foraging in northern areas were smaller than those foraging in southern areas. Since it is not possible to age living turtles (Avens and Snover 2013), it is unclear whether differences in body size among foraging areas are due to age or to differences in metabolic rates coupled with water temperature and/or prey abundance.

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 an 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. 2013b), 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 $\delta^{13}C$ and $\delta^{15}N$ over an estimated 4 to 12-year 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 (S. A. 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 (Figs. 5B and 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 interpret 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), model the contribution of environmental factors (e.g., SST, bathymetry) that affect the geographic distribution of isotope signatures, and determine how these factors could be included to improve the isoscapes (Bowen et al. 2005).

Conclusions

Recently, Ramos and González-Solís (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. 2012*a*, *b*, *c*, Ceriani et al. 2012, Hart et al. 2014, Tucker et al. 2014) 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, Vander 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.

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 somewhat basic isoscapes suggest that a spatially 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. 2013b).

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Supplemental Material

Appendix A

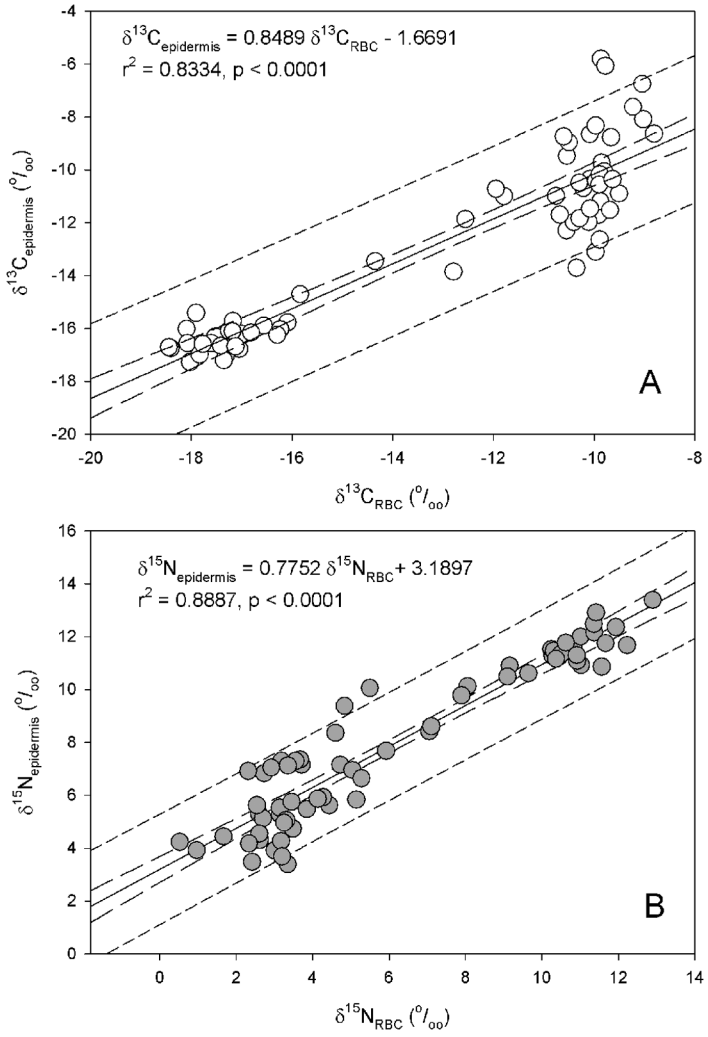

Fig. A1. Relationship between epidermis and RBC stable isotope values of 66 juvenile loggerheads: (A) carbon ($\delta^{13}C$) and (B) nitrogen ($\delta^{15}N$). Long dash lines denote 95% confidence interval, short dash lines indicate 95% prediction interval.

Appendix B

Summary of loggerhead body size

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).

Table B1. Curved carapace length (CCL) for 214 loggerheads included in this study according to nesting and foraging data.

		CCL (cm)			
Foraging area	n	Mean	SE	Min	Max
Nesting†	30				
MAB	10	102.4	2.5	89.0	111.2
SAB	5	91.3	0.7	89.0	93.1
SNWA	15	100.5	1.7	81.8	108.8
Foraging	180				
CAN	66	64.2	0.6	51.0	76
MAB, Continental Shelf	25	79.3	1.6	63.0	93.0
MAB, NC estuaries‡	18	67.7	1.8	58.4	88.6
SAB, Canaveral FL	30	76.7	2.0	57.5	100.9
SNWA, Key West NWR	41	80.1	1.2	66.5	95.6

Note: CCL measurements were missing for two adult females and two juveniles.
† Fourteen of the nesting females were included in Ceriani et al. (2012).
‡See McClellan and Read (2007) and McClellan et al. (2010) for further details.

Appendix C

Table C1. Isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of 214 loggerheads from different foraging areas. Sample details and abbreviations are as in Appendix B.

		$\delta^{13}C$ (‰)				$\delta^{15}N$ (‰)			
Foraging area	n	Mean	SE	Min	Max	Mean	SE	Min	Max
Nesting†	32								
MAB	11	−16.1	0.22	−16.9	−14.6	13.8	0.67	10.2	17.0
SAB	5	−14.7	0.35	−15.9	−13.9	12.4	0.33	11.3	13.1
SNWA	16	−11.4	0.36	−13.8	−9.5	7.8	0.41	5.8	11.7
Foraging	182								
CAN	68	−16.8	0.06	−18.1	−15.1	9.0	0.06	7.4	10.1
MAB, Continental Shelf	25	−16.4	0.10	−17.3	−15.4	11.3	0.2	8.4	13.4
MAB, NC estuaries‡	18	−16.4	0.14	−17.4	−14.8	10.4	0.27	8.9	12.9
SAB, Canaveral FL	30	−15.6	0.18	−17.6	−13.9	11.3	0.20	10.0	13.6
SNWA, Key West NWR	41	−10.5	0.32	−14.7	−5.8	5.9	0.25	3.4	10.0

† Fourteen of the nesting females were included in Ceriani et al. (2012).
‡ See McClellan and Read (2007) and McClellan et al. (2010) for further details.

Appendix D

Examining body size and isotopic patterns in the testing data set

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 significant 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 $\delta^{13}C$ and $\delta^{15}N$ differed significantly among foraging grounds ($\delta^{13}C$, $F_{2, 150} = 277.82$, $p < 0.0001$; $\delta^{15}N$, $F_{2, 148} = 129.48$, $p < 0.001$) after accounting for differences in body size. The interaction of loggerhead size and foraging location was significant only for $\delta^{15}N$ ($F_{2, 148} = 9.30$, $p = 0.0002$).