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ORIGINAL PAPER

Polymodal foraging in adult female loggerheads (*Caretta caretta*)

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Abstract To determine whether loggerhead turtles (*Caretta caretta*) nesting in southeastern USA exhibit polymorphic foraging strategies, we evaluated skin samples for stable isotopes of carbon (δ^{13}C) and nitrogen (δ^{15}N) from 310 loggerheads from four locations on the east coast of Florida and epibionts from 48 loggerheads. We found a dichotomy between a depleted δ^{13}C cluster and an enriched δ^{13}C cluster. Epibionts from oceanic/pelagic or neritic/benthic habitats were largely consistent with this dichotomy. The bimodal distribution of δ^{13}C could reflect a bimodal foraging strategy or—because of the potential for confounding among four gradients of δ^{13}C in marine environments—a polymodal foraging strategy. We integrate our results with results from other stable isotope studies, satellite telemetry, and flipper tags to evaluate potential foraging strategies. Understanding foraging strategies is essential for development of management plans for this endangered species that has suffered a 43% population decline over the last decade.

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Introduction

Loggerhead hatchlings (*Caretta caretta*) emerge from nests on Atlantic coast beaches of the southeastern USA from Florida to North Carolina and enter the North Atlantic. They swim away from shore, are incorporated into offshore currents, and are carried to oceanic foraging areas (Bolten 2003) where they feed primarily on sea jellies and other invertebrates (Bjorndal 1997; Witherington 2002; Frick et al. 2009). Juvenile loggerheads recruit to neritic foraging areas at sizes between 46 and 64 cm curved carapace length, after about 7–12 years in oceanic habitats (Bjorndal et al. 2000, 2003). In neritic habitats, loggerheads shift to a diet primarily composed of hard-shelled, benthic invertebrates (Bjorndal 1997; Seney and Musick 2007). The original hypothesis was that this shift from the oceanic to the neritic environment is a unidirectional ontogenetic niche shift (Carr 1986). However, anecdotal reports (e.g., Eckert and Martins 1989) began to accumulate indicating that some individuals in neritic habitats may return to oceanic habitats and some may never leave oceanic habitats except to reproduce. Thus, the life history model developed by Bolten (2003) included these possibilities as speculative connections. In a study of large juvenile loggerheads captured in estuaries of North Carolina, USA, satellite telemetry revealed that 10 loggerheads moved off the continental shelf and into oceanic habitats to forage, while 13 remained in neritic habitats (McClellan and Read 2007).

Hatase et al. (2002) were the first to confirm that some adult loggerheads use oceanic habitats between nesting seasons. Using stable isotopes ($n = 149$) and satellite telemetry ($n = 5$), they discovered nesting loggerheads from two different nesting beaches in Japan had been foraging in either oceanic or neritic waters. Through the use of satellite telemetry, Hawkes et al. (2006) documented

the same foraging dichotomy for loggerheads ($n = 10$) from the population nesting in the Cape Verde Islands. In both studies, females that foraged in oceanic habitats were significantly smaller than those foraging in neritic habitats, although there was overlap in body size in Japan (Hatase et al. 2002).

Stable isotopes of carbon and nitrogen in the marine environment provide a tool to investigate habitat use and trophic level (Lajtha and Michener 1994; Hobson and Schell 1998; Reich et al. 2007). Four naturally occurring gradients in the ratio of heavy to light carbon isotopes (δ^{13}C) have been identified in marine environments. Habitat gradients from more negative (depleted) δ^{13}C to less negative (enriched) δ^{13}C values extend from oceanic to neritic habitats, from pelagic to benthic zones, and from high latitudes to low latitudes (Lorian et al. 1992; Goericke and Fry 1994; Hobson et al. 1994; France 1995; Michener and Lajtha 2007). The fourth gradient extends from depleted δ^{13}C food webs to enriched δ^{13}C food webs. Food webs based on producers with depleted δ^{13}C values (e.g., phytoplankton) or enriched δ^{13}C values (e.g., seagrasses) maintain depleted or enriched signatures, respectively, throughout the trophic levels (Michener and Kaufman 2007; Newsome et al. 2007). A trophic gradient exists in ratio of heavy to light nitrogen isotopes (δ^{15}N), with δ^{15}N increasing at higher trophic levels (Minagawa and Wada 1984; Macko et al. 1986).

The Atlantic USA nesting population of loggerheads is the largest loggerhead population in the Atlantic system by an order of magnitude (Ehrhart et al. 2003) with more than 70,000 nests per year (National Marine Fisheries Service and U.S. Fish and Wildlife Service 2008). The loggerheads nesting in Florida are about 93% of this population, but Florida's nesting population has declined dramatically by 43% in the last decade (Witherington et al. 2009). This decline makes information on the foraging behavior and locations of foraging grounds for adult Florida loggerheads critical for developing appropriate management strategies. Loggerheads are listed as Endangered on the IUCN Red List (IUCN 2009) and as Threatened under the U.S. Endangered Species Act (National Marine Fisheries Service and U.S. Fish and Wildlife Service 2008).

Do loggerheads nesting in Florida exhibit a polymorphism in foraging strategies? We used two approaches to answer this question and evaluate the foraging strategies of loggerheads before they arrived in Florida to nest. First, we evaluated stable isotopes of C and N in samples of skin collected from 310 loggerheads nesting at four locations on the east coast of Florida. The stable isotope signature in skin represents a temporal integration of the isotopes assimilated during the synthesis of the tissue before the nesting season. Second, we analyzed epibionts from 48 of the 310 loggerheads. Loggerheads serve as a substrate for a diverse array of epibionts (Frick et al. 1998), and these epibiont communities are assumed to reflect the pre-nesting habitat of the host turtle. In addition, we used mtDNA haplotypes to evaluate genetic differences between turtles exhibiting different foraging strategies.

Methods

Sample collection

During the first 6 weeks (2 May–15 June) of the 2003 and 2004 nesting seasons, we collected skin samples from 310 loggerhead turtles nesting in Florida (Fig. 1) on beaches at Canaveral National Seashore (CNS; 28.79°N, 80.73°W; $n = 44$ [2003], 31 [2004]), Melbourne Beach (MEL; 28.01°N, 80.53°W; $n = 60$ [2003], 46 [2004]), Juno Beach (JUN; 26.88°N, 80.04°W; $n = 41$ [2003], 41 [2004]), and Pompano and Ft. Lauderdale beaches in Broward County (BRO; 26.19°N, 80.09°W; $n = 47$ [2003]). Stable isotopes of carbon and nitrogen assimilated from the diet into the skin of juvenile loggerheads have a mean residence time of 44.9 (±3.1) days (Reich et al. 2008). Residence times of isotopes in tissues increase with increasing body mass and with decreasing growth rates (Martínez del Rio et al. 2009). Because adult turtles are two orders of magnitude larger and grow much more slowly than the juveniles used to calculate average residence time in loggerhead skin (Bjorndal et al. 1983; Reich et al. 2008), we are confident that the average residence time in adult turtles is much longer than our sample period (45 days). Therefore, our sample period is appropriate for assessing stable isotope

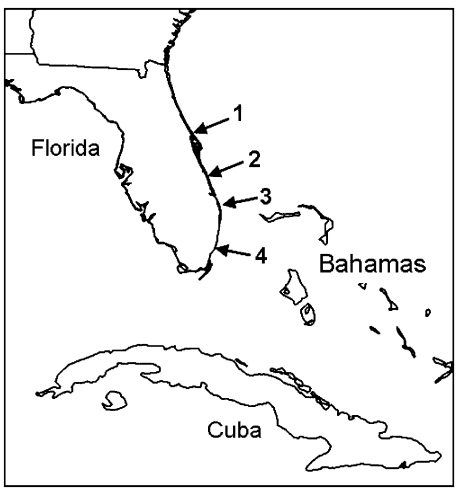

Fig. 1 Locations of the four sampling sites. (*1*) Canaveral National Seashore (28.79°N, 80.73°W); (*2*) Melbourne Beach (28.01°N, 80.53°W); (*3*) Juno Beach (26.88°N, 80.04°W); and (*4*) Broward County (26.19°N, 80.09°W)

signatures of turtles prior to their migration to the nesting grounds.

We used a sterile 6-mm biopsy punch (designed for collecting epidermis samples from humans) to collect samples of non-keratinized skin from the "shoulder" area of each turtle after cleaning the area with alcohol. Skin samples were stored in 70% ethanol at room temperature. Minimum curved carapace length (CCL) was measured from anterior notch to posterior notch; standard flipper tags were applied to both front flippers of untagged turtles to avoid re-sampling individuals.

Skin samples collected from the 192 loggerheads in 2003 were also analyzed for mtDNA haplotype sequences for a study of population genetics of Florida loggerheads (A. Bolten and K. Bjorndal, unpublished data), and the results are used in this study. For details of analytical methods, see Bjorndal and Bolten (2008) except we used primers LCM15382: 5′-GCT TAA CCC TAA AGC ATT GG-3′ and H950 g: 5′-GTC TCG GAT TTA GGG GTT T-3′ (Abreu-Grobois et al. 2006).

Epibionts were collected from the carapace of 48 loggerhead turtles (also sampled for stable isotopes) nesting at CNS. All epibionts present in an area of 20 cm^2 on the posterior right quadrant of the carapace were collected and preserved in 70% ethanol (following Frick et al. 1998). Samples were later sorted and identified to the lowest taxonomic level possible under light microscopy (Pfaller et al. 2008).

Stable isotope analysis

Skin biopsy samples were rinsed in distilled water to remove any epibionts or other organic material and cleaned with isopropyl alcohol swabs. The surface epidermis was removed and homogenized with a scalpel blade. The homogenized sample was dried at 60°C for a minimum of 24 h. After drying, lipids were removed from all samples using an accelerated solvent extractor (ASE) with petroleum ether as the solvent. Approximately 550 μg of each dried, lipid-free sample was loaded into a pre-cleaned 4 mm× 6 mm tin capsule.

All samples were combusted in a COSTECH ECS 4010 elemental analyzer interfaced via a Finnigan-MAT Con-Flow III device (Finnigan-MAT, Breman, Germany) to a Finnigan-MAT DeltaPlus XL (Breman, Germany) isotope ratio mass spectrometer in the light stable isotope lab at the University of Florida, Gainesville, FL, USA. Stable isotope abundances were expressed in delta (δ) notation, defined as parts per thousand (‰) relative to the standard as follows:

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

where R_{sample} and R_{standard} are the corresponding ratios of heavy to light isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in the sample and international standard, respectively. R_{standard} for ^{13}C was Vienna Pee Dee Belemnite (VPDB) and for ^{15}N was atmospheric N_2. Internal standards were inserted in all runs at regular intervals to calibrate the system and assess drift over time. The analytical precision of our measurements, measured as the standard deviation of replicates of standards, was 0.09 for both $\delta^{13}C$ and $\delta^{15}N$ ($n = 88$ and 91, respectively).

Statistical analyses

To determine the number of clusters that best fit the distribution of the stable isotope signatures of individual turtles, we used the function *pam* as the partitioning algorithm in cluster analysis (Kaufman and Rousseeuw 1990; Venables and Ripley 1999). Clusters were evaluated for $\delta^{13}C$ values only, $\delta^{15}N$ values only, and both $\delta^{13}C$ and $\delta^{15}N$ values. Euclidean distance was used to calculate dissimilarities, and mean silhouette width was used to identify the best fit for numbers of clusters. Silhouette width indicates the strength of cluster membership for each observation (Insightful Corporation 2001).

We used chi-square tests to evaluate differences in the proportions of turtles in the two clusters and the proportions of turtles with different haplotypes among the four nesting locations. For these comparisons, we used the 192 loggerheads from 2003 because no samples were collected in BRO in 2004. To compare size differences, we compared CCL of the turtles from the two clusters with a *t*-test.

We analyzed epibiont data to evaluate whether habitat-specific epibionts were consistent with our cluster assignments of nesting loggerheads. Epibiont species were assigned to three categories: (1) oceanic/pelagic, (2) neritic/benthic, or (3) occurring in both categories 1 and 2 (Chace 1951; McCain 1968; Williams 1984; Aoki 1997; Frick et al. 2003, 2004, 2006; Foster et al. 2004). Epibionts in category 3 were excluded from analyses, because they could not help distinguish between the two habitats and are not reported here. We used chi-square tests to evaluate whether the occurrences of oceanic/pelagic epibionts or neritic/benthic epibionts were significantly different between the clusters of loggerheads determined by stable isotopes. We conducted chi-square tests using the computer program CHIRXC (Zaykin and Pudovkin 1993), which calculates probabilities of independence using a Monte Carlo randomization method (1,000 iterations).

Statistical analyses were conducted in S-PLUS (v. 7.0.3) except for CHIRXC. Unless otherwise noted, alpha = 0.05.

Results

We determined that two clusters, based on $\delta^{13}C$ only, best fit the distribution of stable isotope signatures for the 310

Table 1 Determination of numbers of clusters based on average silhouette widths using the *pam* partitioning algorithm

Numbers of clusters	δ^{13}C only	δ^{15}N only	δ^{13}C & δ^{15}N
2	**0.712**	0.620	0.586
3	0.621	0.539	0.503
4	0.584	0.572	0.501
5	0.589	0.572	0.391

Clusters were evaluated for δ^{13}C values only, δ^{15}N values only, and both δ^{13}C and δ^{15}N values. Bold value indicates best fit

nesting females (Table 1; Fig. 2a). The value of 0.712 silhouette width for the two clusters indicates substantial cluster structure (Insightful Corporation 2001). The more depleted δ^{13}C cluster ($n = 167$; mean $\delta^{13}C = -14.82$, SD = 1.08; mean $\delta^{15}N = 11.00$, SD = 2.16) is separated at $\delta^{13}C = -12.37$ from the more enriched δ^{13}C cluster ($n = 143$; mean $\delta^{13}C = -9.84$, SD = 1.26; mean $\delta^{15}N = 7.51$, SD = 1.25).

Thirty-five species of epibionts were present on the 48 loggerheads from CNS (Pfaller et al. 2008). The distributions of 21 species of epibionts (two oceanic/pelagic and 19 neritic/benthic) on 34 loggerheads in the depleted δ^{13}C cluster and 14 loggerheads in the enriched δ^{13}C cluster are presented in Table 2. Although we categorized *Mitrella lunata* as a neritic/benthic epibiont, it was the only neritic/benthic epibiont to have equal and high representation on turtles from both clusters (Table 2; chi-square, $df = 1$, $\chi = 1.07$, $P = 0.301$). The occurrence of neritic/benthic epibionts was significantly higher on females in the enriched δ^{13}C cluster than on females from the depleted δ^{13}C cluster both with *M. lunata* (chi-square, $df = 1$, $\chi = 9.88$, $P = 0.002$) and without *M. lunata* (chi-square, $df = 1$, $\chi = 30.17$, $P < 0.0001$; Fig. 2b). The occurrence of oceanic/pelagic epibionts was significantly higher on females in the depleted δ^{13}C cluster (chi-square, $df = 1$, $\chi = 20.65$, $P < 0.0001$; Fig. 2b). Although the oceanic/pelagic and neritic/benthic epibionts were largely consistent with the depleted δ^{13}C cluster females and the enriched δ^{13}C cluster females, respectively, there are some exceptions (Fig. 2b). Five depleted δ^{13}C females had neritic/benthic epibionts, but four of these females had only one neritic/benthic epibiont species. Neritic/benthic epibionts could colonize depleted δ^{13}C cluster females when these females entered neritic waters during the nesting season. The other depleted δ^{13}C cluster female that had neritic/benthic epibionts hosted five species of neritic/benthic epibionts and did not have oceanic/pelagic epibionts, which suggests she foraged in neritic/benthic habitats. The one enriched δ^{13}C female that had oceanic/pelagic epibionts was very close to the break between the two clusters.

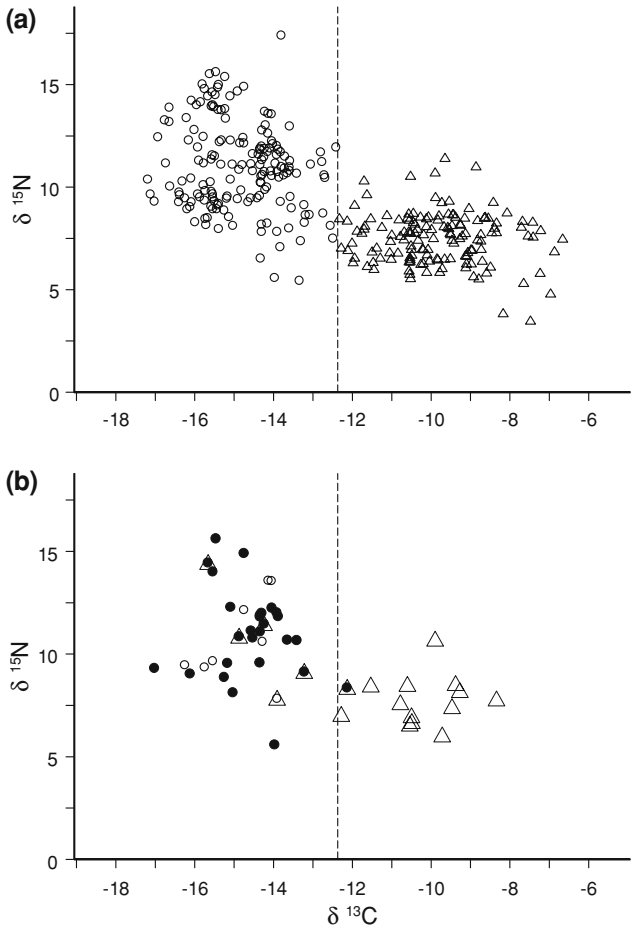

Fig. 2 **a** Distribution of stable isotope values from nesting loggerheads in Florida ($n = 310$). Two clusters, based on δ^{13}C only, best fit the distribution (Table 1) separated at $\delta^{13}C = -12.37$ (*dashed vertical index line*). *Open circles* are the depleted δ^{13}C cluster ($n = 167$); *open triangles* are the enriched δ^{13}C cluster ($n = 143$). **b** Distributions of two species of oceanic/pelagic epibionts (*solid circles*) and 18 species (not including *Mitrella lunata*) of neritic/benthic epibionts (*open triangles*) on 48 nesting loggerheads at CNS (Table 2). *Open circles* are depleted δ^{13}C cluster females that did not have oceanic/pelagic epibionts; all enriched δ^{13}C cluster females had neritic/benthic epibionts. Six females are represented by two symbols. Distributions of oceanic/pelagic and neritic/benthic epibionts are similar to the two clusters of nesting loggerheads determined by the δ^{13}C gradient (see text for statistics)

Females in the depleted δ^{13}C cluster (CCL mean = 97.5 cm, SD = 6.0, $n = 166$) were significantly smaller than females in the enriched δ^{13}C cluster (CCL mean = 100.5 cm, SD = 5.5, $n = 143$; *t*-test, $df = 307$, $t = -4.58$, $P = 0.0001$). There was, however, substantial overlap in CCL between the two groups (Fig. 3).

Among the four nesting locations in 2003, the distributions of females from the depleted δ^{13}C cluster [$n = 33$, 39, 21, 20, from north to south (Fig. 4)] and enriched δ^{13}C cluster [$n = 11$, 21, 20, 27 from north to south (Fig. 4)] were significantly different ($n = 192$, chi-square test,

Table 2 Epibiont species characteristic of either oceanic/pelagic or neritic/benthic habitats identified on loggerheads nesting at Canaveral National Seashore, habitat where each epibiont species is typically found, and the number of turtles (depleted δ^{13}C cluster females or enriched δ^{13}C cluster females) on which the epibiont was identified

Epibiont	Typical habitat of epibiont		Occurrence of epibiont	
	Oceanic/ pelagic	Neritic/ benthic	Depleted δ^{13}C cluster turtles ($n = 34$)	Enriched δ^{13}C cluster turtles ($n = 14$)
Lepas pectinata	×		25	2
Membranipora tuberculata	×		2	0
Anadara transversa		×	0	2
Arbacia punctulata		×	0	2
Bugula fulva		×	0	4
Caprella equilibra		×	0	4
Caprella penantis		×	2	13
Caprella scaura		×	2	2
Conopea galeata		×	0	2
Leptogorgia virgulata		×	1	6
Lytechinus variegatus		×	0	1
Membranipora arborescens		×	1	11
Mitrella lunata		×	17	13
Molgula occidentalis		×	2	14
Obelia dichotoma		×	0	9
Ostrea equestris		×	1	0
Podarke obscura		×	0	3
Ricordia florida		×	0	3
Strombus alatus		×	0	2
Strombus gigas		×	0	1
Thalamoporella floridana		×	0	2

Higher taxonomic designations are given in Pfaller et al. (2008)

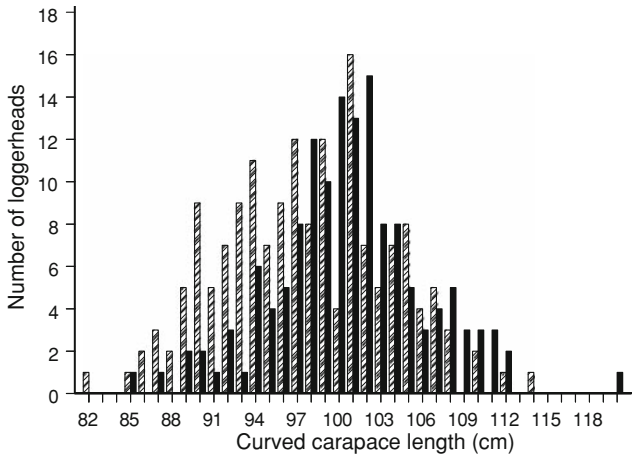

Fig. 3 Size distributions of nesting loggerheads in the depleted δ^{13}C cluster ($n = 166$, mean 97.52 cm, SD 6.0, diagonal hatching) and enriched δ^{13}C cluster ($n = 143$, mean 100.54 cm, SD 5.5; *solid bars*). Mean size of enriched δ^{13}C cluster females is significantly larger than the mean size of depleted δ^{13}C cluster females (*t*-test, $df = 307$, $t = -4.58$, $P = 0.0001$)

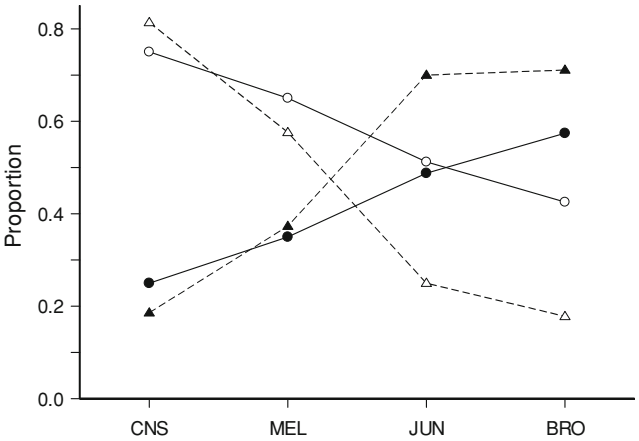

Fig. 4 Latitudinal changes in proportions of depleted δ^{13}C cluster females and enriched δ^{13}C cluster females and in proportions of the two most common haplotypes in female loggerheads nesting at four locations in Florida. *Open triangles* haplotype CC-A1, *closed triangles* haplotype CC-A2, *open circles* depleted δ^{13}C cluster females, and *closed circles* enriched δ^{13}C cluster females. These latitudinal changes are independent (see text for statistics). For location abbreviations, see text

$df = 3$, $\chi = 11.82$, $P = 0.008$). The proportion of females from the depleted δ^{13}C cluster declined from north to south, and the proportion from the enriched δ^{13}C cluster increased from north to south (Fig. 4). Four mtDNA haplotypes were found in the same 192 loggerheads; 95% of the turtles had the common haplotypes CC-A1 and CC-A2. Among the four nesting locations, the distributions of females with CC-A1 and CC-A2 haplotypes were significantly different (chi-square test, $df = 3$, $\chi = 42.41$, $P < 0.0001$). There was a latitudinal trend among the four nesting locations with the proportion of females with the CC-A1 haplotype declining from north to south and with CC-A2 increasing north to south (Fig. 4). To determine whether the latitudinal trend in δ^{13}C is related to the mtDNA trend, we asked if depleted δ^{13}C cluster females were predominantly haplotype CC-A1 and if enriched δ^{13}C cluster females are predominantly CC-A2. There was no significant difference in the distribution of CC-A1 or CC-A2 turtles between depleted or enriched δ^{13}C cluster females (chi-square test, $df = 1$, $\chi = 0.015$, $P = 0.90$).

Therefore, the latitudinal trends in δ^{13}C and mtDNA haplotypes are independent.

Discussion

Analyses of δ^{13}C in skin samples, presence of epibionts, and mtDNA haplotypes reveal that loggerheads nesting in Florida exhibit a dichotomy between δ^{13}C enriched and δ^{13}C depleted groups, which are characterized by different epibiont communities but are genetically homogeneous. We initially interpreted this dichotomy as a bimodal foraging strategy with some females foraging in oceanic habitats and others in neritic habitats. We based our conclusion on the known δ^{13}C gradient of declining δ^{13}C from neritic to oceanic habitats (Lorian et al. 1992; Hobson et al. 1994; Michener and Lajtha 2007; Newsome et al. 2007) and on the distributions of oceanic/pelagic and neritic/benthic epibionts that were largely consistent with the clusters based on δ^{13}C. In addition, this foraging dichotomy was consistent with the oceanic vs neritic dichotomy reported for adult loggerheads nesting in Japan and Cape Verde Islands (Hatase et al. 2002; Hawkes et al. 2006), including smaller body size of oceanic turtles in both locations and an assumed genetic homogeneity between the two groups in Japan (Hatase et al. 2004).

We now believe that the pattern of foraging strategies in Florida nesting loggerheads may be more complex. Although our data on δ^{13}C and epibionts are consistent with an oceanic vs neritic dichotomy, we cannot rule out other possible scenarios. In fact, Florida loggerheads may exhibit a variety of foraging strategies. Polymodal foraging strategies may be disguised in the bimodal distribution of δ^{13}C because of the confounding nature of δ^{13}C gradients, as we discuss later.

In addition, tracking data from satellite telemetry suggest an alternative dichotomy. Data are available on the movements of 19 female loggerheads nesting on the east coast of Florida that were fitted with satellite transmitters between 1988 and 2000 (Dodd and Byles 2003; Foley et al. 2008; Turtle Expert Working Group 2009). Unfortunately, none of these turtles was sampled for stable isotopes. Eight of the 19 loggerheads moved from nesting beaches along the east coast to the Gulf of Mexico, while the other 11 loggerheads remained in the Atlantic, with most moving into the Bahamas Archipelago. Data from standard flipper tags can also inform our discussion of foraging strategies. Loggerheads tagged while nesting on the east coast of Florida have been reported from locations away from the nesting beach (Meylan 1982; Meylan et al. 1983; Guseman and Ehrhart 1992).

We have not relied on δ^{15}N to help distinguish among foraging strategies, because the distribution of δ^{15}N values

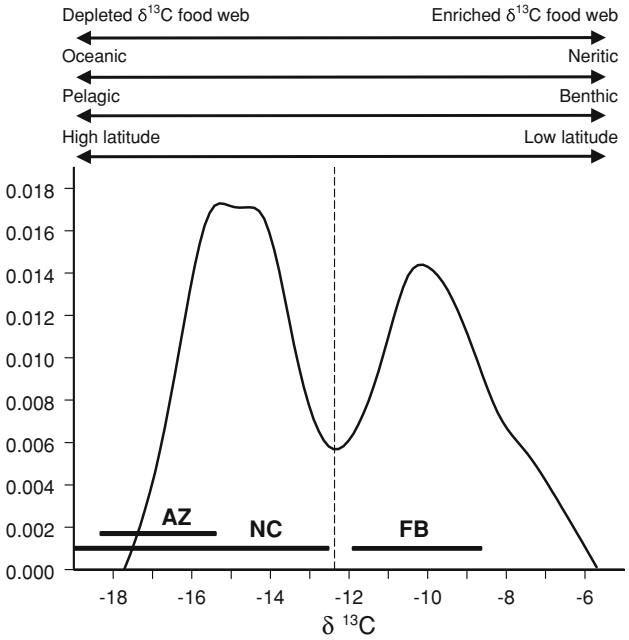

Fig. 5 Proportional distribution of nesting loggerheads in Florida based on δ^{13}C stable isotope signatures (‰, $n = 310$, *solid curved line* is a smoothing spline with $df = 10$ and interval width = 0.1‰). The depleted δ^{13}C cluster is separated at $\delta^{13}\text{C} = -12.37$‰ (*dashed vertical index line*) from the enriched δ^{13}C cluster (see text). The *solid horizontal lines* (*AZ* Azores, *FB* Florida Bay, and *NC* North Carolina) represent δ^{13}C ranges from three loggerhead foraging aggregations. The *four horizontal lines* at the *top* of the figure represent four known gradients in δ^{13}C (see text). AZ data modified from Reich et al. (2007); FB data from B. Schroeder, A. Foley, B. Witherington, K. Bjorndal, A. Bolten, and K. Reich, unpublished data; and NC data from Wallace et al. (2009) without three outliers <-19 δ^{13}C (B. Wallace, personal communication)

is unimodal for our entire sample as well as within each δ^{13}C cluster. Therefore, there is no clear division between foraging strategies based on δ^{15}N.

δ^{13}C gradients

Four gradients from depleted to enriched δ^{13}C are known to exist in marine habitats (Lorian et al. 1992; Goericke and Fry 1994; Hobson et al. 1994; France 1995; Michener and Kaufman 2007; Michener and Lajtha 2007), and the δ^{13}C gradient exhibited by the loggerheads in our study could result from one gradient or any combination of these gradients, as illustrated in Fig. 5. These gradients can be characterized as scenopoetic (derived from habitat use), bionomic (derived from resource use), or both (Newsome et al. 2007). We will discuss each gradient and the data that support the contribution of each gradient in Florida nesting loggerheads.

The first gradient extends from oceanic (depleted δ^{13}C) to neritic (enriched δ^{13}C) habitats and is a scenopoetic gradient. Our epibiont results are consistent not only with this gradient, but also with the pelagic to benthic gradient

(see later). The depleted $\delta^{13}C$ values from small juvenile loggerheads in oceanic habitats around the Azores islands (modified from Reich et al. 2007) and the enriched $\delta^{13}C$ values from large sub-adult loggerheads from neritic habitats in Florida Bay, Florida, USA (B. Schroeder, A. Foley, B. Witherington, K. Bjorndal, A. Bolten, and K. Reich, unpublished data) are consistent with this gradient (Fig. 5). However, as we discuss later, the depleted $\delta^{13}C$ signature of immature loggerheads in neritic habitats off North Carolina, USA (Wallace et al. 2009), is not consistent with this gradient. All flipper tag returns from Florida nesting turtles of which we are aware have come from neritic habitats (Meylan 1982; Meylan et al. 1983; Guseman and Ehrhart 1992). However, the number of published returns is relatively low, and the opportunities for capturing loggerheads in oceanic habitats are many fewer than in neritic habitats.

The second gradient is from pelagic (depleted $\delta^{13}C$) to benthic (enriched $\delta^{13}C$) zones within the same habitat and is both a scenopoetic and bionomic gradient. Our epibiont data are consistent with this gradient as well as with the oceanic to neritic gradient. Standard flipper tags and satellite transmitters that do not record dive depths cannot be used to distinguish positions of turtles along this depth gradient.

The scenopoetic gradient from high latitudes (depleted $\delta^{13}C$) to low latitudes (enriched $\delta^{13}C$) is the third gradient that may play a role in the distribution of $\delta^{13}C$ in Florida nesting loggerheads. At least some of the difference between the $\delta^{13}C$ signatures of the two populations of neritic loggerheads—higher latitude North Carolina (35.0°N) and lower latitude Florida Bay (24.9°N)—may result from the latitudinal gradient (Fig. 5). We know of no data that indicate that loggerheads that nest in Florida move into the North Carolina estuarine complex from which the stable isotope data are derived (Wallace et al. 2009). However, returns of tags attached to loggerheads nesting along the east coast of Florida range over a latitudinal spread of 22° from Atlantic City, NJ, USA (39.35°N) to Belize (~17°N) (Meylan 1982). If females from northern foraging grounds tend to nest in northern stretches of Florida beaches and females from southern foraging grounds nest in southern stretches, the latitudinal trend that we observed in $\delta^{13}C$ in females at four locations (Fig. 4) could be a result of, at least in part, the scenopoetic latitudinal gradient in $\delta^{13}C$.

The last gradient is the bionomic gradient from depleted $\delta^{13}C$ food webs to enriched $\delta^{13}C$ food webs. Marine food webs based on phytoplankton are typically more depleted in $\delta^{13}C$ than those based on benthic plants, and algae-based food webs are usually more depleted in $\delta^{13}C$ than those based on seagrasses (Michener and Kaufman 2007). This gradient can reinforce the scenopoetic gradients described earlier. The dichotomy in our study could result from this gradient; perhaps the loggerheads in our study are divided between neritic habitats characterized by either depleted $\delta^{13}C$ or enriched $\delta^{13}C$ food webs. This gradient could explain the differences between the $\delta^{13}C$ signatures of the neritic foraging ground aggregations in North Carolina and Florida Bay (Fig. 5) as well as the similarities between the oceanic Azores aggregation and the neritic North Carolina aggregation.

Conclusions

Our study has revealed a bimodal distribution of $\delta^{13}C$ values in loggerheads nesting in Florida. However, the continuum of depleted to enriched $\delta^{13}C$ can result from one or more $\delta^{13}C$ gradients. Our results are consistent with the bimodal strategy representing an oceanic vs neritic dichotomy as reported for nesting loggerheads in Japan and Cape Verde Islands (Hatase et al. 2002; Hawkes et al. 2006), but our results cannot preclude effects from other gradients.

Stable isotope analysis is a powerful tool in ecological studies. However, as with any tool, there are limitations. Newsome et al. (2007) refer to the "myopia" of stable isotopes that results from the inability to distinguish between different resources or habitats unless those resources/habitats have different isotopic signatures. The overlap of $\delta^{13}C$ values between the oceanic foraging aggregation in the Azores and the neritic foraging aggregation in the estuarine complex on the North Carolina coast (Fig. 5) is an excellent example of how individuals in very different habitats, feeding on very different diets (Wallace et al. 2009; Frick et al. 2009) can share stable isotope values. Such overlap can result in low levels of accuracy in studies of animal movements (Rubenstein and Hobson 2004; Wunder et al. 2005).

Knowledge of habitats and diets of both female and male loggerheads between their reproductive migrations to the nesting beaches in Florida is essential for the management of this large population that has incurred a 43% decline over the past 10 years (Witherington et al. 2009). Integrated studies, in which sufficient numbers of individual turtles are fitted with satellite transmitters and passive tags, and sampled for carbon and nitrogen stable isotopes, epibionts, and other biomarkers (such as sulfur stable isotopes and trace elements), will be needed to evaluate further the foraging strategies and foraging habitats of loggerheads that nest in Florida.

Because of the large size of the Florida loggerhead population and the large proportion of females in the depleted $\delta^{13}C$ cluster (= oceanic foragers?), our results may cause a major paradigm shift in the perceived roles of

loggerheads in marine ecosystems and in the appropriate management plans for the conservation of this endangered species. Loggerheads are major marine predators (Bjorndal 2003), but we now know that adults may not be supported entirely within neritic/benthic food webs, as previously supposed. Management agencies recognize the importance of adult loggerheads potentially using oceanic habitats as seen in the shift of emphasis from earlier recovery plans to the most recent (National Marine Fisheries Service and U.S. Fish and Wildlife Service 1991, 2008).

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