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Patterns of loggerhead turtle ontogenetic shifts revealed through isotopic analysis of annual skeletal growth increments

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Abstract. Ontogenetic changes in resource use often delimit transitions between life stages. Ecological and individual factors can cause variation in the timing and consistency of these transitions, ultimately affecting community and population dynamics through changes in growth and survival. Therefore, it is important to document and understand behavioral and life history polymorphisms, and the processes that drive intraspecific variation in them. To evaluate juvenile loggerhead sea turtle (*Caretta caretta*) life history variation and to detect shifts in habitat and diet that occur during an oceanic-to-neritic ontogenetic shift, we sequentially analyzed the stable isotope composition of humerus bone growth increments from turtles that stranded dead on Southeastern U.S. beaches between 1997 and 2013 ($n = 84$). In one-half of the sampled turtles, growth increment-specific nitrogen stable isotope ($\delta^{15}N$) data showed significant increases in $\delta^{15}N$ values over each turtle's life. These data were used to provide a new line of evidence that juvenile Northwest Atlantic loggerheads exhibit two major ontogenetic shift patterns: discrete shifts ($n = 24$), which were completed within one year, and facultative shifts ($n = 14$), which were completed over multiple years (up to five). The mean difference in pre- and post-ontogenetic shift $\delta^{15}N$ values was 4.3‰. Differences in isotopic baselines between neritic and oceanic habitats of the Northwest Atlantic Ocean make it likely these patterns are driven by a coupled change in both habitat and diet, and that facultative shifters utilize both neritic and oceanic resources within transitional growth years. Mean size and age at transition between habitats (54.2 cm straightline carapace length, SCL; 11.98 yr) was within the range of previous estimates and did not differ between discrete and facultative shifters. Our results further expand our understanding of loggerhead sea turtle life history polymorphisms and demonstrate the value of bone tissue analysis to the study of this variation. Sequential analysis of annual skeletal growth increments provides a valuable method for reconstructing long-term ontogenetic changes in foraging ecology and habitat use in long-lived, cryptic marine species.

Key words: *Caretta caretta*; growth rates; life history variation; loggerhead sea turtle; nitrogen stable isotopes; Northwest Atlantic; ontogenetic shift; skeletochronology.

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Introduction

Ontogenetic changes in resource use are widespread ecological phenomena among vertebrates that result in complex interactions within food webs (Werner and Gilliam 1984). These transitions are predicted to occur with increasing body size to maximize fitness, whereby individuals select habitats and diets that provide optimal growth conditions at the lowest risk of predation (Werner and Gilliam 1984, Dahlgren and Eggleston 2000). Among marine organisms, shifts in habitat and diet between life stages have been observed across most major taxonomic groups, often manifesting as a biphasic life history characterized by separate pelagic and benthic life stages (e.g., invertebrates, Moksnes et al. 1998; fishes, Eggleston 1995, Estrada et al. 2006; mammals, Newsome et al. 2009; reptiles, Musick and Limpus 1997). Because environmental influences on growth and survival vary by habitat, differential habitat use associated with ontogenetic shifts may ultimately have profound effects on species interactions, community dynamics, and population growth rates.

Intraspecific variation in the timing of habitat and diet transitions further complicate our understanding of species life history, and have been tied to a suite of environmental, biological, and genetic factors (e.g., hatching date, body size, larval growth; see Sponaugle and Cowen 1997, Post 2003, Pechenik 2006). Furthermore, fidelity to alternative habitat and diet shifts is not always fixed within species (Skulason and Smith 1995, Bolnick et al. 2003). For example, amphibians can respond facultatively to the presence or absence of predators, prey, and conspecifics, with delayed metamorphosis and changes in movement patterns (Skelly and Werner 1990, Newman 1992). Similar behavioral polymorphisms have been observed in various invertebrate and fish species (Werner and Hall 1988, Miller 1993, McCormick 1999), and more recently in sea turtles (Hatase et al. 2006, McClellan and Read 2007). These species, in effect, can make instantaneous resource use decisions based on current ecological conditions or individual state (Werner and Gilliam 1984). The consequences of facultative responses to biological and environmental stimuli in large marine vertebrates are not well understood, although changes in growth and survival of individuals at critical life stages may ultimately affect recruitment and population dynamics (Heppell et al. 2002).

Sea turtles undergo extensive, transoceanic migrations throughout their ontogeny that were long believed to culminate in permanent residency in neritic habitats after an oceanic life stage for most species (for review see Musick and Limpus 1997, Plotkin 2003). However, mounting evidence suggests sea turtle life histories can be polymorphic, such that habitat and diet shifts may be facultative in some species and populations (e.g., Hatase et al. 2002, 2006, Hawkes et al. 2006). Facultative ontogenetic shifts in sea turtles are characterized by contrasting oceanic, neritic, or mixed resource use patterns among individuals of the same population in later life stages (i.e., large juvenile, subadult, adult), and are particularly well documented in Northwest Atlantic loggerhead sea turtles (*Caretta caretta*; Witzell 2002, McClellan and Read 2007, Mansfield et al. 2009, McClellan et al. 2010). McClellan and Read (2007) and Mansfield et al. (2009) used satellite telemetry to observe juvenile loggerheads migrating from seasonal neritic foraging grounds in North Carolina to offshore, oceanic habitats for up to three years. Northwest Atlantic loggerhead sea turtles occupy oceanic habitats for the first decade of their life (Bjorndal et al. 2000, Avens et al. 2013); therefore, the initial presence of these turtles in nearshore habitats suggests they had already completed the initial oceanic-to-neritic habitat shift. The patterns of these return migrations varied, with some turtles spending a year or more in oceanic habitats, and others making multiple migrations between oceanic and neritic habitats within three years (McClellan and Read 2007, Mansfield et al. 2009). Coupled stable isotope analyses of consumer and prey soft tissues showed this alternative habitat use is associated with a neritic/oceanic prey foraging dichotomy (McClellan et al. 2010).

Despite these recent observations, methodological limitations have impeded our ability to robustly assess the duration and prevalence of alternative life history patterns in sea turtles. Satellite telemetry is costly, time consuming, and resource intensive, which can restrict sample sizes, whereas long-term diet histories are impossible to obtain via isotopic analysis of soft tissues due to high isotopic turnover and low

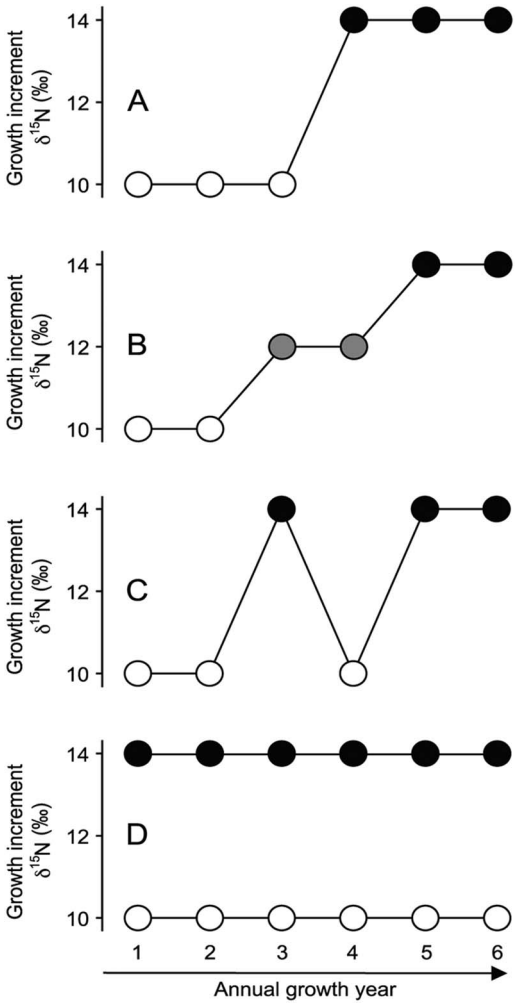

Fig. 1. Conceptual model of predicted ontogenetic shift patterns for juvenile loggerhead sea turtles. Lines track individual resource use through time. Each circle represents the nitrogen stable isotope value of an annual skeletal growth increment (i.e., the integration of diet and habitat use over a single year). Black circles represent forage on neritic species, white circles represent forage on oceanic species, and gray circles represent forage on both neritic and oceanic species. (A) Discrete ontogenetic shift: single year, one-way transition from oceanic to neritic lifestyle; (B) facultative ontogenetic shift: gradual transition from oceanic to neritic lifestyle over multiple years, characterized by utilization of both oceanic and neritic resources in transitional growth years; (C) continual ontogenetic shift: multiple, discrete transitions between oceanic and neritic lifestyles for entire growth years; (D) no ontogenetic shift: continuous neritic or oceanic lifestyles.

recapture rates of tagged wild animals (Hobson 2007). Reconstruction of life history through stable isotope analyses of sequentially deposited tissues may provide a means to overcome these limitations (e.g., hair, Cherel et al. 2009; scute, Reich et al. 2007; teeth, Newsome et al. 2009; vertebrae, Estrada et al. 2006). The isotopic composition of consumer tissues ultimately reflects that of cumulative prey consumption and habitat occupation (Hobson 2007), such that nitrogen stable isotope ratios (^{15}N:^{14}N, $\delta^{15}N$) can characterize trophic relationships within communities (DeNiro and Epstein 1981, Post 2002), while carbon stable isotope ratios (^{13}C:^{12}C, $\delta^{13}C$) reflect differences in baseline primary productivity between habitats (Rau et al. 1982, Cherel and Hobson 2007). Some sea turtle species are known to deposit annual growth layers in their humerus bones composed of cortical bone (Avens and Snover 2013), a relatively inert tissue with minimal cellular turnover. These growth layers can be sequentially sampled for stable isotopes (Avens et al. 2013), and thus may allow for the study of facultative ontogenetic shifts in sea turtles, limited only by the amount of bone resorbed in the central, vascularized portion of the bone (Zug et al. 1986).

In the present study, we sequentially analyzed the cortical tissue of juvenile loggerhead sea turtle humerus bones for nitrogen and carbon stable isotope ratios to identify patterns of ontogenetic changes in resource use. This study focused on the transition that occurs as loggerheads migrate from oceanic to neritic habitats, which coincides with a simultaneous change in diet from epipelagic invertebrates clustered in floating *Sargassum* to large benthic invertebrates (Bjorndal 1997, Seney and Musick 2007). Previous Northwest Atlantic loggerhead turtle studies found neritic prey species have higher $\delta^{15}N$ values than oceanic prey species, whereas $\delta^{13}C$ values are either similar, or slightly higher in neritic prey species (Wallace et al. 2009, McClellan et al. 2010, Snover et al. 2010). Therefore, we expected prey-mediated differences in nitrogen, and possibly carbon, stable isotope ratios to be evident in skeletal analyses. Fig. 1 presents a conceptual model of four predicted ontogenetic shift patterns for juvenile loggerhead sea turtles that integrates these prey nitrogen stable isotope patterns with the current understanding of

loggerhead sea turtle life history variation. We asked the following questions: (1) what is the prevalence of alternative life history patterns among individuals, (2) over what length of time do facultative ontogenetic shifts occur, and (3) do turtles that display different life history patterns undergo ontogenetic shifts at similar points in their life history? By quantifying intraspecific variation in the prevalence, duration, and timing of ontogenetic shifts in sea turtles researchers can begin to address how life history variation may affect sea turtle population vital rates and conservation strategies.

Methods

Sample collection

Humerus bones were collected from juvenile loggerhead sea turtles that stranded dead on beaches along the eastern U.S. from 1997 to 2013, obtained by the National Marine Fisheries Service through the National Sea Turtle Stranding and Salvage Network. Turtles stranded in North Carolina ($n = 62$), Virginia ($n = 14$), Maryland ($n = 4$), and New Jersey ($n = 4$). One front flipper was collected from each turtle and prepared for skeletochronological and stable isotope analyses. For each stranded animal, body size, stranding location, and sex (determined by necropsy) were recorded. Straightline carapace length (SCL) measurements, the straightline distance from the nuchal notch to the posterior end of the posterior marginal scute of the turtle carapace, were used as an indicator of body size in this study. When only curved carapace length (CCL) was recorded, it was converted to SCL as described by Snover et al. (2010).

Skeletochronology

This study utilized newly collected ($n = 26$) and previously processed ($n = 58$) humerus bones that were histologically prepared as described by Snover and Hohn (2004), Goshe et al. (2009), and Avens et al. (2012). Skeletochronology data for previously processed humerus bones were also presented in Snover et al. (2010) and Avens et al. (2013). Two sequential cross-sections (2–3 mm thick) were cut from each bone, with one used for skeletochronology and the second for paired stable isotope analyses. For full description and review of histological processing of sea turtle humeri see Avens and Snover (2013). Histologically prepared stained thin sections of the skeletochronology section were mounted onto microscope slides, digitally imaged using a CCD digital camera in conjunction with Microsuite image analysis software (Olympus America), and analyzed in Adobe Photoshop (Adobe Systems) to determine the location and number of lines of arrested growth (LAGs) that delimit the outer edges of each skeletal growth increment (Fig. 2A). Assuming annual LAG deposition (validated by Bjorndal et al. 2003, Snover and Hohn 2004), a calendar year was assigned to each LAG based on date of stranding. The diameters of observable LAGs were measured for each turtle and used to back-calculate SCLs for each successive growth increment (for back-calculation method see Snover et al. 2007). A mean SCL was generated for each pair of successive LAGs that was used in all analyses.

Growth increment-specific age estimates were quantified following Parham and Zug (1997) and Avens et al. (2012). As juvenile loggerhead sea turtles age and grow, bone resorption in the core of their bones results in the loss of early growth increments, such that typically only the most recent five to ten growth increments remain completely intact at any moment in time. Therefore, to assign age estimates to individual LAGs it was first necessary to estimate the number of LAGs lost to resorption for each turtle (see Parham and Zug 1997). This estimate was then added to the number of observed LAGs for each bone to give an initial age estimate for each turtle at stranding. Initial age estimates were used to back-assign an age estimate to each visible LAG. A final age estimate at stranding was determined for each turtle by adjusting the initial age estimate to the nearest 0.25 years based on the mean hatch date for the population (August/September) and individual stranding date (see Avens et al. 2013).

Stable isotope analysis

Bone sections cut for stable isotope analyses were mounted onto microscope slides with the side originally proximal to the skeletochronology section oriented upwards for sampling. Humerus bone cross-sections were micro-milled using a New Wave Research Micromill (ESI), which consists of a Leica GZ6 StereoZoom microscope

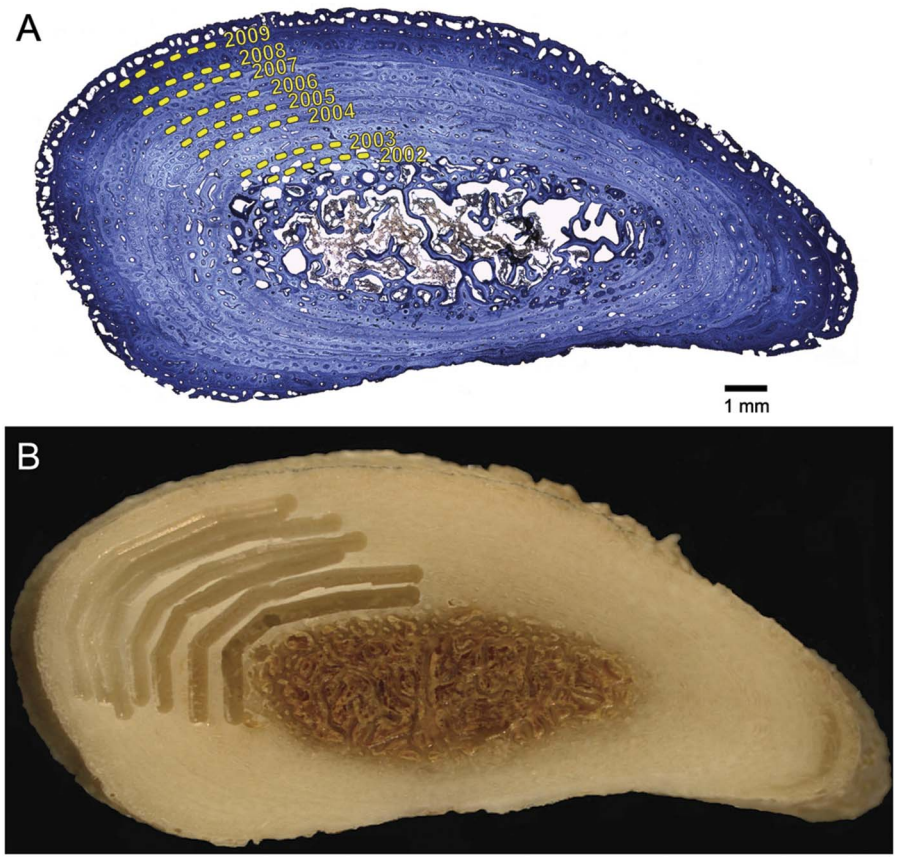

Fig. 2. (A) Histologically processed and (B) micro-milled humerus bone cross-sections from a juvenile loggerhead sea turtle that stranded dead on 7 December 2009, with a measured straightline carapace length (SCL) of 51.2 cm and an estimated age of 11.25 yr. Dashed lines delimit the outer edge of each skeletal growth increment, or line of arrested growth (LAG). The calendar year associated with each LAG is provided.

fitted with a S-video color CCD video camera, fine resolution (0.25 μm) computer-guided X, Y, and Z stages, a high torque DC milling chuck with adjustable speed, and a 0.1 mm diameter carbide dentist drill bit (Brasseler). MicroMill software was used in conjunction with a computer monitor to display a live video image of the sample area. To ensure milling of individual growth increments, LAGs were traced on the corresponding digital skeletochronology image and printed onto transparency film, which was then overlaid on the computer monitor image of the stable isotope cross-section and used to guide precision drilling between paired LAGs to a depth of no more than 1.0 mm (Fig. 2B). Approximately 1.6 mg of bone dust was collected from each annual growth increment and analyzed for $\delta^{15}N$ and $\delta^{13}C$ values by a continuous-flow isotope-ratio mass spectrometer at Oregon State University, Corvallis, Oregon, USA (see Appendix A for more details). In some cases composite samples of two or three narrow growth increments were collected due to our inability to individually sample the narrowest growth increments (see Appendix A). Composite samples were only used for life history pattern classification and were excluded from all further analyses. Each sample was considered an integration of information over each growth year, or set of growth years for composite samples.

Isotopic analyses of bone tissue are typically performed on isolated bone collagen, the organic component of bone assimilated from protein constituents of a consumer's diet (Koch 2007). However, for sea turtles it is not possible to isolate bone collagen from individual growth

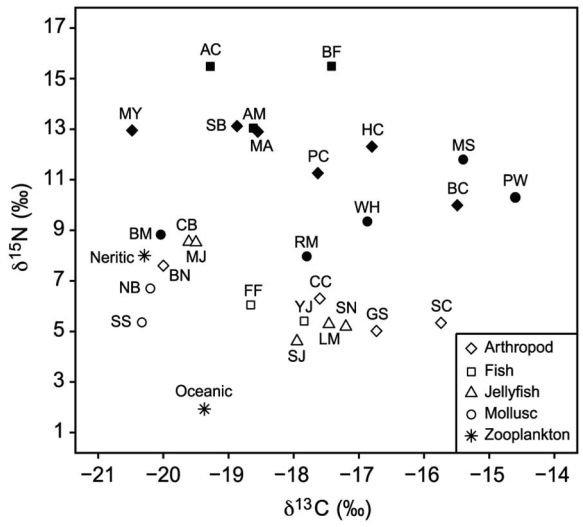

Fig. 3. Weighted mean prey isotope values by taxonomic group (shapes) and habitat (neritic = black, oceanic = white). See Appendix A: Table A1 for full list of species, isotopic values, and sources. Species codes: AC = Atlantic croaker, AM = Atlantic menhaden, BC = Blue crab, BF = Bluefish, BM = Blue mussel, BN = Barnacle, CB = Cannonball jellyfish, CC = Columbus crab, FF = Filefish, GS = Brown grass shrimp, HC = Horseshoe Crab, LM = Lion's mane jellyfish, MA = Mantis shrimp, MJ = Moon jellyfish, MS = Moon snail, MY = Mysid shrimp, NB = Nudibranch, PC = Spider crab, PW = Periwinkle, RM = Ribbed mussel, SB = Sevenspine bay shrimp, SC = Sargassum swimming crab, SJ = Mauve stinger jellyfish, SN = Sea nettle jellyfish, SS = Sea snail, WH = Whelk, YJ = Yellow jack.

increments in sufficient quantities for isotopic analyses. Nitrogen stable isotope ratios do not differ between bone collagen and bulk bone tissue in sea turtles (Medeiros et al. 2015, Turner Tomaszewicz et al. 2015). Therefore, growth increment-specific $\delta^{15}N$ values of bulk bone tissue were assumed to reflect that of bone collagen and thus prey consumed at the time of bone deposition. Bulk bone carbon stable isotope data were mathematically corrected using the linear equation developed by Turner Tomaszewicz et al. (2015) for Northwest Atlantic loggerhead sea turtles (bone collagen $\delta^{13}C$ value = 1.1 × bulk bone $\delta^{13}C$ value + 0.2).

Variation in $\delta^{15}N$ and $\delta^{13}C$ values within turtles may be driven by forage at different trophic levels, geographic differences in isotopic baselines, or both. To identify the most probable mechanism that drives the reconstructed sea turtle life history patterns, we compiled and compared published $\delta^{15}N$ and $\delta^{13}C$ data for common Northwest Atlantic loggerhead prey from neritic and oceanic habitats (Fig. 3; Appendix A: Table A1). Isotopic data for zooplankton were also collected from the literature to represent the base of the food web.

Life history patterns

In the marine environment, carbon stable isotope ratios are often used to reconstruct animal migratory patterns because they vary by latitude (Rau et al. 1982) and can also discriminate between neritic/oceanic foraging strategies (Hobson et al. 1994, Burton and Koch 1999). However, in the Northwest Atlantic neritic and oceanic prey species display similar and overlapping $\delta^{13}C$ values (Fig. 3), which limited our ability to robustly identify life history patterns using this biogeochemical marker. Consequently, to assess loggerhead life history variation we focused our analyses on $\delta^{15}N$ values because they are generally distinct between neritic and oceanic prey species, although $\delta^{13}C$ data was collected and is available for reference in Appendix A.

To objectively characterize sea turtle life history variation, we developed a classification method that iteratively assigned individuals to one of four predicted life history pattern groups—discrete shifter, facultative shifter, continual shifter, non-shifter—based on the pattern of their reconstructed $\delta^{15}N$ transect and a series of threshold $\Delta\delta^{15}N$ values (see Appendix B for details of the method). A threshold $\Delta\delta^{15}N$ value of +3.0‰ was determined to be the most conservative, and least biased to reclassification of individuals to a different life history pattern. This method was chosen to avoid uncertainties associated with classification based on habitat-specific mean prey isotope values (e.g., diet specializations, temporal and spatial heterogeneity), and because the $\delta^{15}N$ diet-tissue fractionation factor for sea turtle bone tissue has yet to be determined.

The beginning of an ontogenetic shift was identified as the first growth increment along a $\delta^{15}N$ transect where the $\delta^{15}N$ value surpassed 11.0‰, or increased by at least 1.0‰ relative to

the previous growth increment. The number of growth increments required for the $\delta^{15}N$ values to cumulatively increase by greater than 3.0‰ was used to designate duration of ontogenetic shift for each turtle. All turtles were predicted to show evidence of an ontogenetic shift because they likely died in nearshore habitats and were the minimum size of turtles found in these areas (Epperly et al. 2007). We acknowledge, however, that it is possible that the smallest stranded turtles had only recently shifted to neritic habitats and would not yet show an isotopic change in their bone tissue, and that the largest turtles had likely lost transitional growth increments to bone resorption.

Discrete shifters were turtles that exhibited a sharp increase in $\delta^{15}N$ value greater than 3.0‰ within one year, which would be expected for turtles that followed the traditional life history paradigm of a one-way, single-year transition from oceanic to fully neritic prey and habitats (Fig. 1A). Facultative shifters were turtles that exhibited a gradual increase in $\delta^{15}N$ values over multiple years as would be expected for turtles that consume mixed oceanic and neritic prey or occupy transitional habitats between isotopically distinct regions (Fig. 1B). Continual shifters were turtles that exhibited multiple sharp increases and decreases in $\delta^{15}N$ values over multiple years (Fig. 1C). Non-shifters were turtles that exhibited consistent $\delta^{15}N$ values that did not increase by a magnitude necessary to surpass the threshold $\Delta\delta^{15}N$ value (Fig. 1D). A fifth life history pattern group, indeterminate shifters, was created for turtles that could not confidently be classified into one of the four predicted life history patterns.

Statistical analyses

A cluster analysis was performed to identify structure within the stable isotope data and to allow for assignment of growth increment-specific stable isotope and turtle data (e.g., SCL, age) to likely foraging habitats. Clusters were evaluated for $\delta^{15}N$ values only, $\delta^{13}C$ values only, and both $\delta^{15}N$ and $\delta^{13}C$ values using the function *pam* from the *cluster* package in R (Maechler et al. 2015). The method seeks to minimize the sum of dissimilarities between observations and allows for the use of silhouette widths, a measure of clustering validity, to determine the optimum number of clusters in a dataset (Kaufman and Rousseeuw 1990).

Mean SCL and age at transition from oceanic to neritic habitats was quantified in two ways. The first approach estimated mean SCL and age at the beginning of an ontogenetic shift based on turtle-specific $\delta^{15}N$ data. Non-parametric Mann-Whitney U tests were used to compare SCL and age at transition among life history pattern groups and by shift duration. The second approach used logistic regression analyses to estimate SCL and age at transition. Based on the best fit-cluster from the cluster analysis, sampled skeletal growth increments of all turtles were placed into neritic (^{15}N-enriched cluster) or oceanic (^{15}N-depleted cluster) categories to create a categorical response variable. Because SCL and age are highly correlated in sea turtles ($r^2 > 0.80$), they were regressed against the categorical response variable in separate models, with the predicted values at the inflection point (i.e., 50% probability) used as estimates of SCL and age at transition between foraging habitats. Only data from turtles that exhibited an ontogenetic shift were included in the regression analyses to allow for comparison with estimates from the first approach. All analyses were performed using program R (version 3.0.2; R Core Team 2015).

Results

For the loggerhead turtles used in this study ($n = 84$), straightline carapace length (SCL) and age at stranding ranged from 51.2 to 88.6 cm SCL (mean ± SD = 67.8 ± 9.9 cm SCL) and 11.0 to 29.75 yr (mean ± SD = 17.7 ± 4.5 yr). Sex was not included as a covariate in analyses due to the limited number of positive identifications (male: $n = 16$, female: $n = 29$, unknown: $n = 39$). A total of 596 bone samples were milled and analyzed for stable isotopes from all turtles (n = 4–12 samples per turtle; mean = 7 per turtle), 40 of which were composite samples of two ($n = 37$) or three ($n = 3$) skeletal growth increments.

Stable isotope ratios of bone tissue and prey

The $\delta^{15}N$ and $\delta^{13}C$ values of sampled skeletal growth increments ranged from 7.31‰ to 18.92‰, and −19.00‰ to −11.68‰, respectively. In general, $\delta^{15}N$ and $\delta^{13}C$ values increased with increasing SCL (Fig. 4). Two clusters, based on

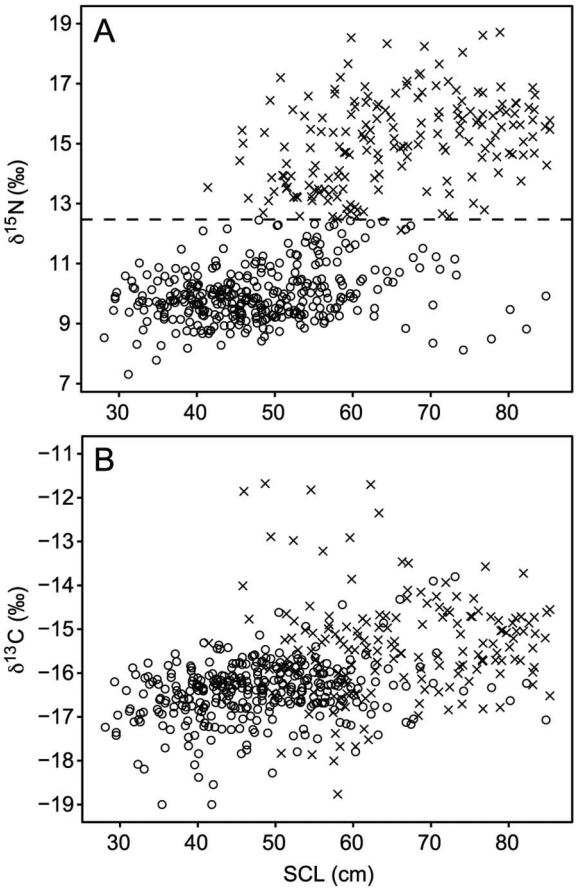

Fig. 4. Comparison of (A) $\delta^{15}N$ and (B) $\delta^{13}C$ values with straightline carapace length (SCL) of annual skeletal growth increments ($n = 556$) sampled from juvenile loggerhead sea turtles ($n = 84$). Two clusters, based on $\delta^{15}N$ only, best fit the data. Dashed horizontal line separates ^{15}N-depleted data cluster (circles) and ^{15}N-enriched data cluster (exes) at $\delta^{15}N = 12.47‰$. SCL is the mean back-calculated straightline carapace length for the growth increment.

$\delta^{15}N$ only, optimally fit the stable isotope data. Average silhouette width was 0.722, indicative of strong structure within the dataset (Kaufman and Rousseeuw 1990). The ^{15}N-depleted cluster (mean $\delta^{15}N$ value = 9.99‰, mean $\delta^{13}C$ value = −16.42‰) was separated from the ^{15}N-enriched cluster (mean $\delta^{15}N$ value = 15.04‰, mean $\delta^{13}C$ value = −15.42‰) at a $\delta^{15}N$ value of 12.47‰ (see Fig. 4A). The C:N ratios of all micromilled bone samples were below 3.5 (see Appendix A: Fig. A1), and thus characteristic of pure, unaltered protein (Koch et al. 1994) with low lipid content (Post et al. 2007).

Mean $\delta^{15}N$ and $\delta^{13}C$ values for zooplankton and loggerhead prey species in oceanic and neritic habitats are presented in Fig. 3 and Appendix A: Table A1. Zooplankton and prey $\delta^{15}N$ values were generally greater in neritic versus oceanic habitats, whereas $\delta^{13}C$ values were similar.

Classification into life history pattern groups

Juvenile loggerhead sea turtles were divided into four groups based on the pattern of their $\delta^{15}N$ transect (Fig. 5). Mean growth increment-specific $\delta^{15}N$ values one year prior to and year of completion of ontogenetic shifts by life history pattern are presented in Table 1. Associated $\delta^{13}C$ data can be found in Appendix A: Table A2, Fig. A2. Discrete shifters ($n = 24$) exhibited sharp increases in $\delta^{15}N$ values that surpassed the +3.0‰ threshold $\Delta\delta^{15}N$ value in one year, while facultative shifters ($n = 14$) exhibited gradual increases in $\delta^{15}N$ values that cumulatively increased by 3.0‰ over two to five years. No turtles exhibited the predicted continual shifter life history pattern (Fig. 1C). Among turtles that exhibited an ontogenetic shift ($n = 38$), 37% were facultative shifters whereas 63% were discrete shifters.

Twenty-four turtles were classified as non-shifters because they did not display any marked increase in $\delta^{15}N$ value indicative of an ontogenetic shift. Non-shifters were sub-classified into two groups, with those that exhibited consistently lower $\delta^{15}N$ values termed *oceanic non-shifters* ($n = 16$) and those that exhibited consistently higher $\delta^{15}N$ values termed *neritic non-shifters* ($n = 8$). These turtles exhibited $\delta^{15}N$ values above (neritic non-shifters) or below (oceanic non-shifters) the 12.47‰ cutoff identified by the cluster analysis (Fig. 5).

Twenty-two turtles were classified as indeterminate shifters. Of these, eight had $\delta^{15}N$ transects similar to those of discrete or facultative shifters, but could not be accurately classified to either group because growth increments at points critical to life history pattern classification either were too narrow to sample ($n = 3$), were partially resorbed in the core of the bone ($n = 3$), or could only be sampled as a composite of multiple growth increments ($n = 2$). The other 14 indeterminate shifters exhibited evidence of an

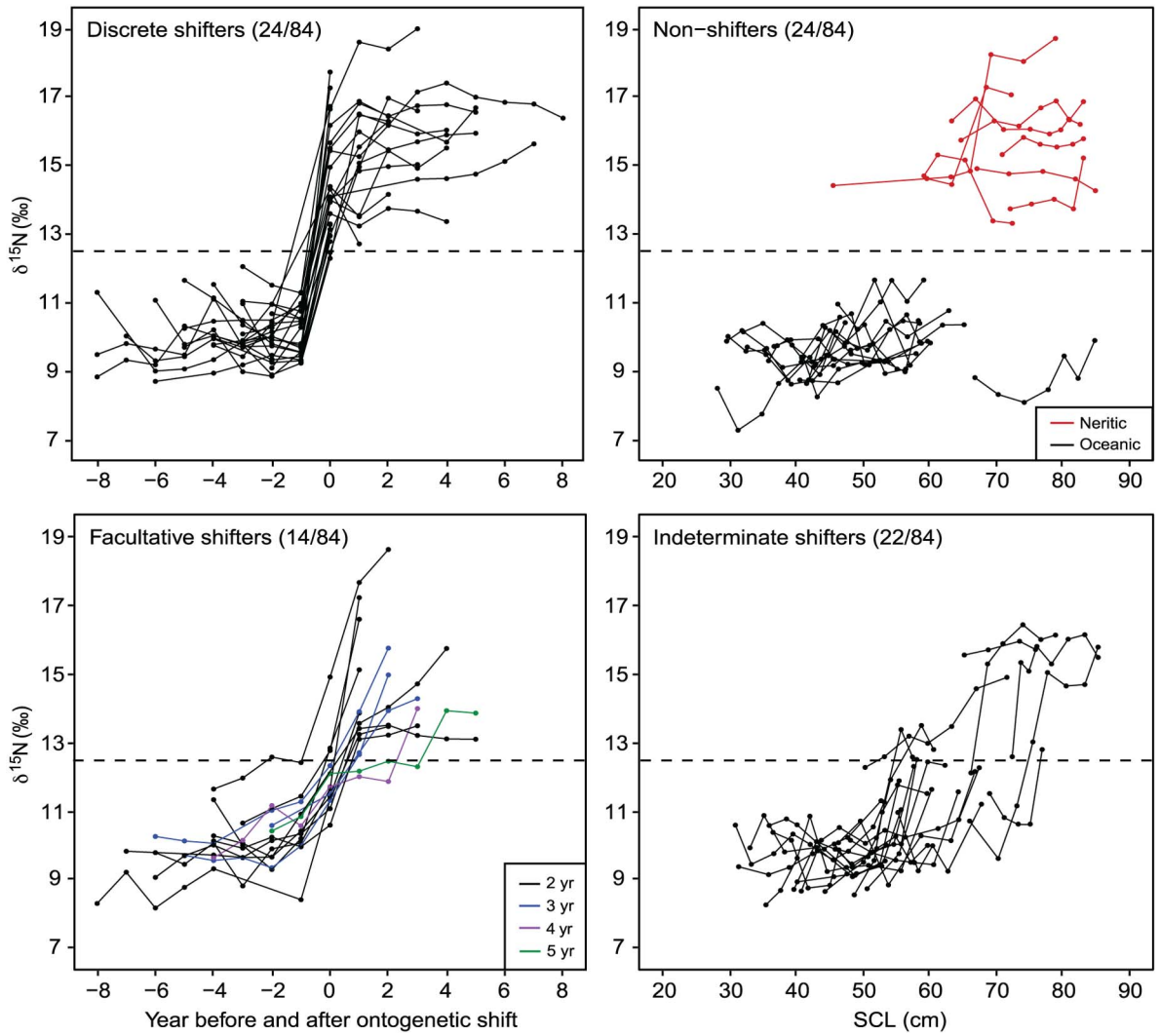

Fig. 5. Turtle-specific nitrogen stable isotope transects by life history pattern. Plots represent sampled growth increments (points) within turtles (lines). Discrete and facultative shifters showed evidence of an ontogenetic shift in diet and habitat (i.e., ≥3‰ increases in $\delta^{15}N$ values over one, discrete shifter, or more, facultative shifter, years). Non-shifters exhibited no ontogenetic shift, while indeterminate shifters could not be classified due to insufficient data. Dashed horizontal line separates ^{15}N-depleted data cluster and ^{15}N-enriched data cluster at $\delta^{15}N = 12.47‰$.

incomplete ontogenetic shift characterized by an elevation in $\delta^{15}N$ values greater than 1.0‰, but less than the threshold $\Delta\delta^{15}N$ value of 3.0‰. Non-shifters and indeterminate shifters were excluded from further analyses.

Size and age at transition to nearshore habitats

Mean size and age at transition for each life history pattern based on turtle-specific $\delta^{15}N$ transects are presented in Table 2, summarized by duration of ontogenetic shift (i.e., years to surpass the +3.0 ‰ threshold $\Delta\delta^{15}N$ value). Mean transition estimates presented for non-shifters and indeterminate shifters were based on size and age at stranding. SCL and age at transition did not differ between discrete and facultative shifters (Mann-Whitney U test; SCL model: $W = 175.5$, $P = 0.405$; Age model: $W = 208$, $P = 0.422$),

Table 1. Mean (± SD) skeletal growth increment-specific $\delta^{15}N$ values by life history pattern and shift duration for juvenile loggerhead sea turtles.

Life history pattern	*n*	Pre-shift (‰)	Post-shift (‰)	Difference (‰)
All ontogenetic shifters†	38	10.24 ± 0.80	14.58 ±1.54	4.34 ± 1.30
Discrete shifters	24	10.10 ± 0.72	14.53 ±1.60	4.43 ± 1.40
Facultative shifters	14			
All durations	14	10.44 ± 0.89	14.65 ±1.50	4.21 ± 1.16
2 years	9	10.34 ± 1.05	14.72 ±1.78	4.38 ± 1.31
3 years	3	10.61 ± 0.64	14.87 ± 0.91	4.26 ± 0.84
4 years	1	10.57	13.98	3.41
5 years	1	10.84	13.92	3.08
Non-shifters‡	24			
Oceanic	16	8.59 ± 0.70	10.63 ± 0.59	...
Neritic	8	14.54 ± 0.96	16.39 ± 1.29	...
Indeterminate shifters‡	22	9.69 ± 1.43	12.92 ± 1.85	...

† Combined data for discrete and facultative shifters.
‡ Presented are mean minimum and maximum $\delta^{15}N$ values of sampled growth increments within turtles.

and did not vary by duration of ontogenetic shift (Kruskal-Wallis test; SCL model: $\chi^2 = 0.88$, df = 2, $P = 0.643$; Age model: $\chi^2 = 0.28$, df = 2, $P = 0.870$). Data for facultative shifters with shift durations greater than three years were excluded from analyses related to shift duration due to low sample size ($n = 2$). The logistic regression models for size and age at transition based on the cluster analysis showed high correlation between the categorical response variable (neritic/oceanic) and explanatory variables (SCL model: $\chi^2 = 237.71$, df = 1, $P < 0.001$; age model: $\chi^2 = 209.29$, df = 1, $P < 0.001$). The model predicted transition to occur at 54.4 cm SCL (95% CI: 52.8–56.0 cm SCL; Fig. 6A) and 12.07 years of age (95% CI: 11.53–12.61 years; Fig. 6B).

Discussion

Interpretation of isotopic shifts in bone growth increments

We found a strong relationship between $\delta^{15}N$ values, $\delta^{13}C$ values, and back-calculated SCL estimates as would be expected with movements to neritic habitats and/or trophic increases in diet (Michener and Schell 1994, Post 2002). These results suggest that sequential isotopic analysis of skeletal growth increments can be used to reconstruct dietary and habitat use histories of sea turtles. However, growth increment-specific $\delta^{13}C$ clusters displayed high overlap, the patterns of reconstructed $\delta^{13}C$ were inconsistent within life history pattern groups, and we found little to no difference in $\delta^{13}C$ values for both zooplankton

Table 2. Estimated straightline carapace length (SCL) and age at transition from oceanic to neritic habitats by life history pattern and shift duration for juvenile loggerhead sea turtles.

		SCL (cm)				Age (yr)			
Life history pattern	*n*	Mean	SD	Min	Max	Mean	SD	Max	Min
All ontogenetic shifters†	38	54.2	7.3	40.8	73.8	11.98	2.23	8.75	17.75
Discrete shifters	24	55.1	7.6	41.4	73.8	12.17	2.17	8.75	17.75
Facultative shifters	14	...	...	...	...	...	...	...	...
All shift durations	14	53.0	7.1	40.8	66.5	11.68	2.37	8.75	15.75
2 years	9	54.8	6.8	45.4	66.5	12.19	2.19	9.75	15.75
3 years	3	51.4	7.0	43.4	55.7	11.75	3.61	8.75	15.75
4 years	1	53.9	...	...	...	9.75	...	...	...
5 years	1	40.8	...	...	...	8.75	...	...	...
Non-shifters‡	24	...	...	...	...	...	...	...	...
Oceanic	16	62.4	8.8	51.2	86.8	16.13	3.75	11.25	25.0
Neritic	8	82.7	4.7	74.8	87.2	24.00	3.60	18.75	27.75
Indeterminate shifters‡	22	67.6	10.6	50.3	88.6	18.93	5.06	11.75	29.75

Note: Ontogenetic shift identified using skeletal growth increment-specific $\delta^{15}N$ values.
† Combined data for discrete and facultative shifters.
‡ Based on SCL and age at stranding.

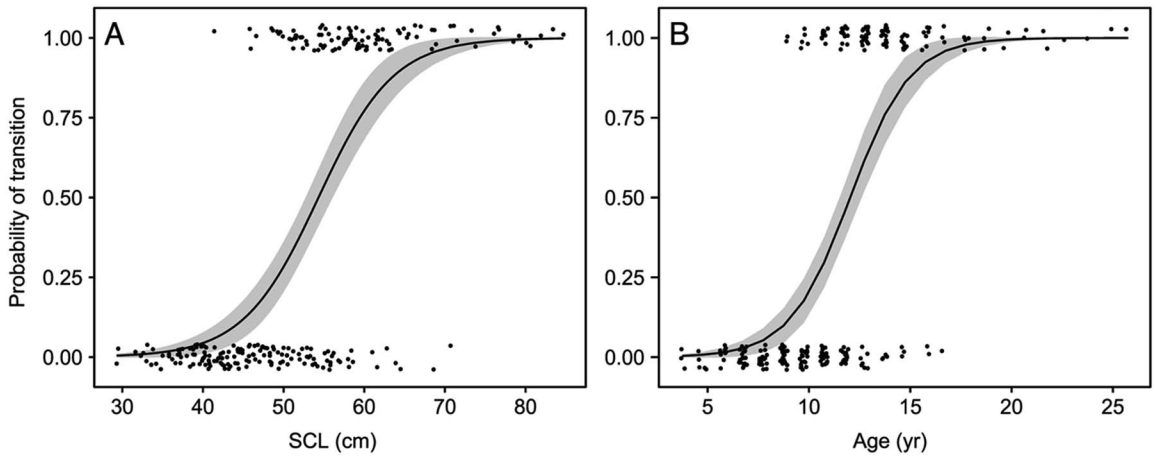

Fig. 6. Probability of ontogenetic shift vs. (A) straightline carapace length (SCL) and (B) age for juvenile loggerhead sea turtles. The thick black line is the predicted relationship from a logistic regression and is bounded by 95% confidence intervals. The model predicts transition from oceanic to neritic habitats (i.e., 50% probability of transition) to occur at 54.4 cm SCL (95% CI: 52.8–56.0 cm SCL) and 12.07 years of age (95% CI: 11.53–12.61 years).

and loggerhead prey from neritic and oceanic habitats. Taken together, these findings provide justification for focusing analyses herein on $\delta^{15}N$ values only, and suggest carbon isotope analyses may be of limited value to the study of ontogenetic shifts in Northwest Atlantic loggerhead sea turtles.

The mean difference in pre- and post-shift $\delta^{15}N$ values presented here (4.3‰) was greater than previously reported (3.1‰, Snover et al. 2010; 2.5‰, Avens et al. 2013). Mean pre-shift $\delta^{15}N$ values in the present study were intermediate to previous studies (10.2‰, herein; 11.0‰, Snover et al. 2010; 9.7‰, Avens et al. 2013), whereas mean post-shift $\delta^{15}N$ values were greater (14.6‰, herein; 14.1‰, Snover et al. 2010; 12.1‰, Avens et al. 201). Because loggerheads are long-term resource use specialists (Avens et al. 2003, Vander Zanden et al. 2010), such differences may be caused by the inclusion of turtles with alternative diet preferences within each study, or possibly spatiotemporal variation in baseline $\delta^{15}N$ values within and among habitats (e.g., Ohman et al. 2012, Pajuelo et al. 2012). Alternatively, discrepancies among these studies may be methodological. It is possible the classification method employed in this study inflated mean difference and post-shift $\delta^{15}N$ values relative to those of previous studies where no such criterion was used, although greater sample sizes of ontogenetic shifters herein may have also allowed us to capture a broader range of variation in loggerhead life history ($n = 38$, herein; $n = 23$, Snover et al. 2010; $n = 8$, Avens et al. 2013). In addition, Snover et al. (2010) based inferences on a two-point sampling method rather than through sequential analysis of all growth increments, thus differences between these two studies may also relate to differences in sampling resolution.

Mechanisms to explain variance in stable isotope ratios

The significant changes in $\delta^{15}N$ values within turtles associated with ontogenetic shifts may ultimately be driven by forage at different trophic levels, differences in habitat-specific isotopic baselines, or both. The mean difference in pre- and post-shift $\delta^{15}N$ values within turtles (4.3‰) was consistent with the regularly observed 3–5‰ enrichment in ^{15}N per trophic level within foodwebs (Schoeninger and DeNiro 1984, Post 2002), which provided some evidence that trophic effects may explain the observed isotopic patterns. However, the $\delta^{15}N$ values of zooplankton were ~6‰ greater in neritic versus oceanic habitats (Fig. 3), indicative of broadscale differences in baseline $\delta^{15}N$ values. Indeed, isotopic mapping previously predicted $\delta^{15}N$ values to be

greater along the U.S. continental shelf than in the Sargasso Sea and Tropical Atlantic (McMahon et al. 2013). Because the difference in $\delta^{15}N$ values between pre– and post–shift $\delta^{15}N$ values for turtles (~4.3‰), difference in growth increment-specific $\delta^{15}N$ clusters (~5‰), and difference in neritic and oceanic prey species (~5‰) and zooplankton (~6‰) were similar, we propose the increases in $\delta^{15}N$ observed within juvenile loggerhead humerus bones were driven by a coupled diet and habitat shift mediated by isotopic differences at the base of the food web.

Given that oceanic prey species can become entrained in continental shelf waters via eddies and meanders, and can ultimately enter neritic habitats, we cannot rule out the possibility that the observed $\delta^{15}N$ patterns within turtles are due to a diet shift irrespective of habitat, whereby turtles shift from forage on oceanic to neritic prey species within neritic habitats. However, because size and age at transition estimates herein are similar to those of other studies and to minimum size observations of turtles in nearshore waters (Epperly et al. 2007, Avens et al. 2013), it is more likely that the observed patterns are due to a coupled habitat and diet change. This conclusion is in line with the current understanding of Northwest Atlantic loggerhead sea turtle life history, which includes a post-hatchling oceanic life stage (Musick and Limpus 1997).

While an isotopic difference at the base of the food web is the most likely mechanism driving the observed isotopic patterns among the turtles in this study, there is evidence of an additive trophic level effect for some turtles. Although gut content data were not collected for the majority of turtles in this study, three turtles had fish bones in their stomachs at time of death. Two of these turtles displayed the highest growth increment-specific $\delta^{15}N$ values of all turtles (18.61‰, 18.92‰), which were over 8‰ greater than mean pre-shift $\delta^{15}N$ values (Table 1). It is likely then that some turtles, particularly those that forage on fish or fish discards, have multiple drivers that influence the $\delta^{15}N$ values observed in their tissues.

Although isotopic maps are beginning to be developed for predictive modeling in the Atlantic Ocean (McMahon et al. 2013, Ceriani et al. 2014, Vander Zanden et al. 2015), a greater understanding of the spatiotemporal variability of isotopic baselines in the ocean is needed to better evaluate historical diet and habitat use of sea turtles. Inclusion of other isotopic (e.g., $\delta^{34}S$, $\delta^{18}O$, compound specific) and trace element analyses in future studies may aid in interpreting these patterns. Additionally, the lack of a diet–bulk bone tissue fractionation factor for sea turtles is a major limitation to the interpretation of turtle bone stable isotope data. Diet-tissue fractionation factors can vary between 0.5‰ and 5.5‰ (Post 2002), or more. Therefore, quantification of this metric for sea turtle bone tissue, and the influence of diet, growth, and physiology on this factor, will be critical to future studies that employ these methods.

Alternative sea turtle life histories

This study adds a new line of evidence that facultative ontogenetic shifts are prevalent among juvenile loggerhead sea turtles (Witzell 2002, McClellan and Read 2007, Mansfield et al. 2009, McClellan et al. 2010), and is the first to reconstruct and assess the patterns and duration of these changes with multiple years of retrospective individual life history. The prevalence and duration estimates of facultative ontogenetic shifts quantified herein were similar to those from previous studies. McClellan and Read (2007) and Mansfield et al. (2009) found that up to 43% of satellite tagged turtles returned to oceanic habitats from neritic habitats for up to three years, whereas we found that 37% ($n = 14$ of 38) of turtles exhibited this alternative life history pattern for up to five years. Estimates of SCL and age at transition herein (life history pattern, 54.2 cm SCL, 11.98 yr; logistic regression, 54.4 cm SCL, 12.07 yr) were similar to those based on growth increment-specific $\delta^{15}N$ values from Avens et al. (2013; 55.3 cm SCL, 12.4 yr), and overlapped to some extent with the range of estimates based on length frequency and skeletochronology methods (Bjorndal et al. 2000, 42.4–59.5 cm SCL, 6.5–11.5 yr; Snover et al. 2010, 43.6–47.4 cm SCL; see Avens et al. 2013 for review).

Our results demonstrate that discrete and facultative shifters begin ontogenetic shifts at similar sizes and ages. Such similarities in size at transition among life history patterns have previously been observed for juvenile loggerhead sea turtles in the Northwest Atlantic (McClellan

and Read 2007, Mansfield et al. 2009), but contrast with adult loggerhead populations in other regions that show a size-based dichotomy in habitat use (Japan, Hatase et al. 2002; Cape Verde, Hawkes et al. 2006). This agreement between life history patterns suggests other ecological or non-ecological factors influence when turtles make this habitat shift. Snover et al. (2010) provided evidence that juvenile loggerheads experience an increase in growth rate at the time of this habitat shift, thus changes in growth may signal these transitions (Werner and Gilliam 1984). In addition, other factors, such as prey availability, physiology, and oceanographic processes may differentially influence individual behavior and mediate these transitions, or it may be that life history patterns in sea turtles are genetically fixed. Further research into these and other factors is needed to better understand the mechanisms driving intraspecific variation in the timing and duration of ontogenetic shifts in sea turtles.

We propose intermediate nitrogen stable isotope values (i.e., ~11–14‰) observed within facultative shifters are indicative of forage in and occupancy of both oceanic and neritic habitats within individual growth years, and are consistent with a gradual transition to completely benthic diets in neritic habitats over multiple years. Similar inferences have been made in studies of other marine organisms known to occupy and migrate between alternative isotopically distinct areas (e.g., Smith et al. 1996, Burton and Koch 1999, Angerbjörn et al. 2006). Still, it is possible these turtles may occupy transitional habitats along the continental shelf or Gulf Stream that allow access to both neritic and oceanic prey species, as there is regular exchange of water between the continental shelf and Sargasso Sea via entrainments, meanders, and eddies (Olson 2001). These patterns may also be indicative of indirect, gradual transitions between the Sargasso Sea and U.S. East Coast through the Bahamas. The Sargasso Sea and tropical Atlantic both tend to have low $\delta^{15}N$ values relative to the continental shelf, as they are both areas of high N_2-fixation (Montoya 2007, Mompean et al. 2013). Therefore, occupation of and movement between these habitats may be indistinguishable. Ultimately, such behavioral polymorphisms may be best assessed through satellite telemetry or archival tag studies.

Based on sizes at stranding, it is likely that most of the non-shifters either recently transitioned to (oceanic non-shifters) or had been resident in (neritic non-shifters) neritic habitats. All neritic non-shifters were older, larger turtles (mean SCL at stranding = 82.7 cm), and thus would have lost inner, earlier growth increments with transitional $\delta^{15}N$ values to bone resorption, which effectively limits sampling to the most recent five to ten growth layers for juvenile loggerheads. All but one of the oceanic non-shifters were younger, smaller turtles (mean SCL at stranding = 62.4 cm). Because these individuals stranded at SCLs just above the mean SCL at transition (54.2 cm SCL), it is likely that oceanic non-shifters died within one year of transition to neritic habitats, or died offshore during an oceanic life stage and were carried by currents into the nearshore. However, it is also possible that oceanic non-shifters are long-term oceanic residents, as has been suggested for adults in other populations (Japan, Hatase et al. 2002; Cape Verde, Hawkes et al. 2006). Indeed, the single oceanic non-shifter that stranded at 86.8 cm SCL may have been an oceanic resident. However, it is also possible this and other oceanic non-shifters occupied neritic habitats in the tropical Atlantic with characteristically low $\delta^{15}N$ values prior to their stranding (Ceriani et al. 2014). Future studies should analyze additional ecological factors (e.g., other stable isotopes, trace elements, growth rates) to better clarify whether non-shifters herein represent a previously unidentified life history pattern for Northwest Atlantic loggerheads.

Implications for sea turtle conservation

Facultative ontogenetic shifts may ultimately have profound effects on sea turtle population dynamics and conservation. Fisheries interactions are a persistent threat to sea turtles in the Northwest Atlantic due to spatial overlap in optimal fishing and turtle foraging areas (Witzell 1999), and because many fisheries disproportionately impact large juveniles and sub-adults, stage classes with high reproductive value and strong effects on population growth rates (Crowder et al. 1994, Heppell et al. 2002). The source and magnitude of natural and anthropogenic mortality vary between oceanic and neritic habitats

(Bolten et al. 2011, Lewison et al. 2014). Therefore, turtles that return to oceanic habitats for extended periods of time or make multiple transitions between oceanic and neritic habitats may have altered survival probabilities. A greater understanding of how these alternative life history patterns are maintained in sea turtles and their effects on growth and survival are needed to better determine their role in shaping population dynamics, and management and conservation priorities (National Research Council 2010).

Conclusion

This study highlights the utility of combined skeletal and stable isotope analyses to the study of sea turtle ecology. We propose that these methods provide a means to study habitat and diet specializations that can be used to robustly quantify the prevalence, duration, and timing of alternative sea turtle life history patterns. These methods may be critical to the study of cryptic species and life stages, as some may never be logistically feasible to study via traditional tracking methods, such as the oceanic-to-neritic transition in loggerhead sea turtles. Studies that examine differential growth and survival between these habitats would be useful for investigating how alternative life history patterns are maintained in populations and how they influence population dynamics. More robust studies that possibly incorporate samples from historical collections are needed to better evaluate the prevalence of facultative ontogenetic shifts in sea turtles through time. Sequential analysis of annual skeletal growth increments is a valuable method for reconstructing ontogenetic changes in foraging ecology and habitat use of long-lived marine vertebrates.

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Supplemental Material

Ecological Archives

Appendices A and B are available online: http://dx.doi.org/10.1890/ES15-00255.1.sm