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Ontogenetic niche theory predicts that as organisms grow they make size-specific changes in habitat use and diet to optimize growth and survival. A variety of factors contribute to growth and survival in different habitats, ultimately leading to variation in life history that can affect population dynamics. An understanding of the variation in timing of habitat shifts and fidelity to those habitats is critical for population dynamics modeling and evaluation of conservation strategies, especially for species whose population vital rates are sensitive to changes in growth and survival of critical life stages, such as the loggerhead sea turtle (*Caretta caretta*). Isotopic analysis of sequentially deposited structures, such as sea turtle humerus bone, provides a means of studying intraspecific life history variation. I sequentially analyzed the annual humerus bone growth increments of 84 juvenile loggerhead sea turtles for stable isotopes ($\delta^{13}C$, $\delta^{15}N$) to reconstruct the diet and habitat use histories of turtles undergoing an oceanic-to-neritic ontogenetic shift. I also used skeletochronological methods to evaluate the growth dynamics surrounding this transition.

Generated isotopic transects were used to classify individuals into alternative life history pattern groups and were combined with body size and growth data obtained from skeletal analyses to evaluate differences in the duration, timing, and growth dynamics of ontogenetic shifts. Sea turtles that displayed increases in nitrogen stable isotope ratios ($\delta^{15}N$) greater than 3.0‰ over one or more years were presumed to have transitioned from oceanic to neritic diets and/or habitats based on oceanic and neritic prey isotopic information collected from the literature, and were classified into one of two life history pattern groups: *discrete shifters* (*n* = 23) completed this transition within year, while *facultative shifters* (*n* = 16) completed this transition in up to eight years. As differences in isotopic values between neritic and oceanic prey are most likely driven by differences in isotopic baselines, I propose the gradual increases in $\delta^{15}N$ values within *facultative shifters* over multiple years is indicative of foraging in both oceanic and neritic habitats within growth years. Size-at-transition between habitats was similar between *discrete shifters* (55.1 ± 7.6 cm straightline carapace length, SCL) and *facultative shifters* (52.8 ± 6.9 cm SCL).

Growth variance was higher for *facultative shifters* versus *discrete shifters*. Yet, mean size at transition, size-at-age relationships, and mean increment-specific growth rates were similar between turtles with alternative life history patterns. Annual growth rates generally peaked within one year of transition (31/38 of turtles), providing support for a short-term (i.e., 1-2 year) ontogenetic shift-associated growth advantage. However, there was considerable variation in the

timing of maximal growth rate among turtles with some individuals exhibiting maximal growth in years prior to the ontogenetic shift (14/38 turtles). The lack of substantial differences in the timing of transition and growth dynamics between *discrete* and *facultative shifters* likely limits the influence of these alternative life history patterns on time to sexual maturity in this species, though differences in habitat-specific survival probabilities could affect loggerhead population dynamics. This study demonstrates the value of paired isotopic and skeletal analyses to the study of long-term sea turtle life history variation and its affect on growth.

Sequential Isotopic Analysis to Characterize Ontogenetic Shifts and Growth Dynamics of Loggerhead Sea Turtles (*Caretta caretta*)

by
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Matthew D. Ramirez, Author

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CONTRIBUTION OF AUTHORS

Dr. Larisa Avens provided humerus bone samples, growth data, training, and guidance on project design, implementation, and data interpretation. Dr. Jeffrey Seminoff provided training and guidance on data interpretation. Lisa Goshe provided training. All co-authors contributed to improving manuscripts for publication.

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CHAPTER 1: GENERAL INTRODUCTION

Life history theory dictates that because resources are limited there exist biological tradeoffs to maximize fitness. In pre-reproductive animals, fitness is best maximized through reduced time and increased survival to sexual maturity (i.e., increased growth rates, decreased mortality rates; Werner and Gilliam 1984, Snover 2008). Many organisms make one or more changes in habitat or diet throughout their ontogeny, termed ontogenetic shifts, to maintain optimal growth rates. These organisms, in effect, make instantaneous resource use decisions based on current ecological and environmental conditions to select habitats that balance the drive for optimal growth with the need for survival. Ultimately animals are predicted to select habitats that minimize the ratio of mortality risk to growth rate, which may lead to the use of potentially suboptimal growth habitats where predation risk is low until critical sizes are reache (Werner and Gilliam 1984). Empirical studies in freshwater and marine systems support this hypothesis and show these transitions can infer a growth advantage in the new habitat (e.g., Salvanes et al. 1994, Dahlgren and Eggleston 2000, Snover et al. 2010, Grol et al. 2011, Kimirei et al. 2013).

Intraspecific variation in the timing of and fidelity to changes in habitat and diet complicate our understanding of species ontogenetic shifts (Bolnick et al. 2003, Post 2003, Snover 2008). Previous studies have tied this variation to a suite of environmental, biological, and genetic factors (Sponaugle and Cowen 1997, Post 2003, Pechenik 2006), and have shown individuals can respond facultatively, or reversibly, to changes in resource availability and predation risk (Werner and Hall

1988, Skelly and Werner 1990). As the factors that influence growth and survival vary spatially and temporally across ocean basins, differential habitat use associated with ontogenetic shifts may have profound effects on community and population dynamics, especially in long-lived, late-maturing species, such as the loggerhead sea turtle (*Caretta caretta*), whose population vital rates are sensitive to changes in growth and survival of critical life stages.

Loggerhead sea turtles have complex life histories that lead to the occupancy of multiple developmental, foraging, and reproductive habitats throughout their ontogeny (for review see Musick and Limpus 1997, Plotkin 2003). After hatching from beaches in the Southeastern U.S., individuals enter the Gulf Stream and are transported to the Azores, Madeira, and Canary Islands where they take up temporary residency (<10yrs; Bolten 2003). Throughout this oceanic migration turtles associate with drifting *Sargassum* and forage on epipelagic invertebrates (Bjorndal 1997, Musick and Limpus 1997). At critical sizes, individuals transition to neritic habitats in what was once considered a discrete, one-way transition (for review see Musick and Limpus 1997, Bolten 2003). However, satellite telemetry and stable isotope studies show these transitions to be facultative, in that some individuals return to oceanic habitats for up to three years, but possibly longer, after the initial oceanic-to-neritic ontogenetic shif (McClellan and Read 2007, Mansfield et al. 2009, McClellan et al. 2010). If growth rates differ between habitats, turtles undergoing facultative ontogenetic shifts may exhibit altered growth trajectories

and time to sexual maturity. Differences in habitat-specific survival probabilities may also affect species population dynamics.

Despite the observation of this life history variation, the short-term nature of data collection through traditional sea turtle study methods, e.g. satellite telemetry, stable isotope analyses of soft tissue, has largely limited our ability to robustly assess the duration and prevalence of these alternative life history patterns. Analysis of sea turtle humerus bones for stable isotopes may allow us to overcome these limitations in order to examine long-term resource use and life history variation. My research uses sequential isotopic analysis of annual humerus bone growth increments and paired skeletochronological analyses to characterize patterns of ontogenetic changes in habitat use, diet, and growth of juvenile loggerhead sea turtles that undergo an oceanic-to-neritic ontogenetic shift. Isotopic transects from individuals are used in Chapter 2 to evaluate the applicability of this method to observe facultative ontogenetic shifts and to quantify the duration, prevalence, and timing of alternative life history patterns in loggerhead sea turtles. Chapter 3 is an in-depth analysis of the ontogenetic growth dynamics of juvenile loggerhead sea turtles in light of individual retrospective life history, and evaluates ontogenetic niche theory as it applies to loggerhead sea turtles. My study highlights the utility of combining skeletal and stable isotope analyses to refine our understanding of intraspecific life history variation in sea turtles.

CHAPTER 2: PATTERNS OF ONTOGENETIC SHIFTS IN JUVENILE LOGGERHEAD SEA TURTLES OF THE NORTHWEST ATLANTIC OCEAN

ABSTRACT

Ontogenetic changes in resource use often mark transitions in life stage that can affect community and population dynamics. Intraspecific variation in the timing and duration in these transitions further confounds our understanding of these processes and the factors that contribute to growth and survival. To evaluate variation in the patterns of an oceanic-to-neritic transition in juvenile Northwest Atlantic loggerhead sea turtles (*Caretta caretta*), we sequentially sampled humerus bone growth layers for stable isotopes ($\delta^{13}C$, $\delta^{15}N$) to produce a long-term record of life history. Isotopic data showed significan increases in $\delta^{15}N$ values over one or more years, with a mean difference in pre- and post-ontogenetic shift $\delta^{15}N$ values of 4.4‰ (min = 3.1‰, max = 8.4‰). Additionally, isotopic values verified that juvenile loggerhead ontogenetic shifts follow one of two patterns (*discrete shifters*, *n* = 23, complete the oceanic-to-neritic transition within one year; *facultative shifters*, *n* = 16, complete the transition over multiple years). Over one third of sampled individuals exhibit extended ontogenetic shifts that lasted up to eight years. Differences in the isotopic baselines between neritic and oceanic habitats make it likely these patterns are driven by a habitat shift, and that *facultative shifters* migrate between both neritic and oceanic foraging habitats within growth years. Mean size at transition between habitats (54.1 cm straightline carapace length, SCL) was within the range of previous estimates and did not differ between *discrete*

shifters (55.1 cm SCL) and *facultative shifters* (52.8 cm SCL). Sequential analysis of annual skeletal growth increments in sea turtles provides a valuable method for reconstructing long-term ontogenetic changes in foraging ecology and habitat use.

INTRODUCTION

Ontogenetic changes in resource use are widespread ecological phenomena among vertebrates that result in complex interactions within food webs (Werner and Gilliam 1984, Schmitz et al. 1997). 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, Snover 2008). Among marine organisms, shifts in habitat and diet between life stages have been observed across most major taxonomic groups (e.g., fish, Eggleston 1995; sharks, Estrada et al. 2006; mammals, Mendes et al. 2007), often manifesting as a biphasic life history characterized by separate pelagic and benthic life stages (e.g., Moksnes et al. 1998, Dahlgren and Eggleston 2000, Snover 2008). As the factors that influence growth and survival vary spatially and temporally across ocean basins, differential habitat use associated with ontogenetic shifts may ultimately have profound effects on species interactions, community dynamics, and population vital rates.

Individual variation in timing of and fidelity to ontogenetic shifts further complicates our understanding of species life history. Intraspecific variation in the timing of resource transitions has 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), though data are generally lacking for large marine vertebrates. Furthermore, the fidelity to alternative habitats and diets are 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 (e.g., Skelly and Werner 1990, Newman 1992). Similar behaviors have been observed in various invertebrate and fish species (e.g., Werner and Hall 1988, Miller 1993, McCormick 1999), and more recently in juvenile loggerhead sea turtles (McClellan and Read 2007, Mansfield et al. 2009). These species, in effect, can make instantaneous resource use decisions based on current ecological conditions (Werner and Gilliam 1984). The consequences of facultative responses to biological and environmental stimuli in large marine vertebrates are not well understood. However, changes in growth and survival of individuals at critical life stages may ultimately affect recruitment and population dynamics (Crouse 1999, Snover 2008).

Sea turtles undergo extensive, transoceanic migrations throughout their ontogeny that were long considered to be discrete for most species, whereby individuals were thought to permanently migrate to neritic habitats at some critical size after an oceanic life stage (for review see Musick and Limpus 1997, Plotkin 2003). However, mounting evidence shows these transitions to be facultative across species (Hawkes et al. 2006, Hatase et al. 2006), populations (Hatase et al. 2002,

Casale et al. 2008), and stage classes (Witzell 2002, Reich et al. 2010). Facultative ontogenetic shifts are particularly well documented in Northwest Atlantic loggerhead sea turtles (*Caretta caretta*) (e.g., Witzell 2002, McClellan and Read 2007, Mansfield et al. 2009, Reich et al. 2010, Vander Zanden et al. 2010, McClellan et al. 2010), and are characterized by movements from neritic foraging areas back to deep, open ocean habitats after an initial immigration to neritic habitats. Among these studies, facultative ontogenetic shifts have largely been assessed via satellite telemetry and, more recently, through stable isotope analyses of soft tissues (e.g. blood, skin). 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. Their initial presence in nearshore habitats indicates these turtles had already completed the initial oceanic-to-neritic habitat shift. McClellan et al. (2010) used stable isotope analyses to show this alternative habitat use is coupled with a neritic/oceanic prey foraging dichotomy, and similar foraging dichotomies have been observed in adults (Hatase et al. 2002, Reich et al. 2010, Vander Zanden et al. 2010). Despite these recent gains, 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 often makes it difficult to collect adequate sample sizes, whereas long-term diet histories are impossible to obtain via isotopic analysis of soft tissues due to high isotopic turnover and low recapture rates of tagged wild animals (Hobson 2007).

Isotopic analysis of sequentially deposited tissue may provide a means to overcome these limitations as it can allow for the reconstruction of long-term trophic and habitat use histories (e.g., teeth, Walker and Macko 1999; vertebrae, Estrada et al. 2006; bones, Avens et al. 2013). For example, Avens et al. (2013) demonstrated that the humerus bones of stranded loggerhead turtles could be sequentially sampled for stable isotopes to assess the timing of an ontogenetic shift. Skeletal growth increment deposition in juvenile loggerheads is annual (Snover and Hohn 2004), thus sequential analysis of growth increments in humerus bone tissue may allow for the study of facultative ontogenetic shifts in sea turtles, limited only by the amount of bone resorption that occurs in the metabolically active cor (Zug et al. 1986). The utility of these methods in ecological studies is derived from the fact that the isotopic composition of consumer tissues ultimately reflects that of cumulative prey consumption and habitat occupation (Peterson and Fry 1987, Hobson 2007). Nitrogen isotope ratios (^{15}N:^{14}N, $\delta^{15}N$) are commonly used to study trophic relationships because consumers are enriched, on average, by ~3-5‰ relative to their prey (DeNiro and Epstein 1981, Schoeninger and DeNiro 1984, Post 2002). Nitrogen isotopes vary spatially based on localized oceanographic processes (Montoya 2007, McMahon et al. 2013); therefore, a thorough knowledge of prey and baseline $\delta^{15}N$ values is necessary to characterize trophic relationships (see Cabana and Rasmussen 1996). Carbon isotope ratios (^{13}C:^{12}C, $\delta^{13}C$), meanwhile, are used to trace migratory patterns because they vary minimally between trophic levels

(<1‰; DeNiro and Epstein 1978), and thus reflect localized differences in primary productivity.

In the present study, I sequentially analyzed sea turtle humerus bones for $\delta^{15}N$ and $\delta^{13}C$ to identify the patterns of ontogenetic changes in resource use in juvenile loggerhead sea turtles from the Northwest Atlantic Ocean. This study focused on the transition that occurs as juvenile loggerheads migrate from oceanic to neritic habitats, which coincides with a simultaneous change in diet from epipelagic to benthic prey (Bjorndal 1997). Previous stable isotope studies of loggerhead sea turtles and their principal prey have found $\delta^{15}N$ and $\delta^{13}C$ values are generally 4-5‰ and 1-2‰ higher, respectively, for prey in neritic habitats than for prey in oceanic habitats in the Northwest Atlantic (Wallace et al. 2009, McClellan et al. 2010, Snover et al. 2010); therefore, I expected prey-mediated differences in $\delta^{15}N$ and $\delta^{13}C$ values to be evident in skeletal analyses. I asked the following questions: (1) is there evidence of facultative ontogenetic shifts in sea turtle skeletal tissue; (2) if so, over what time periods do these transitions occur; (3) what is the prevalence of facultative ontogenetic shifts among individuals; and, (4) does the timing of ontogenetic shifts differ between individuals that display alternative life history patterns (discrete vs. facultative)? By quantifying intraspecific variation in the timing, duration, and prevalence of ontogenetic shifts in sea turtles researchers can begin to address how life history variation may affect sea turtle population vital rates.

MATERIALS AND METHODS

Sample collection and preparation

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 (STSSN). One or both front flippers were collected from each turtle and prepared for skeletochronological and stable isotope analyses. In most cases, only the left flipper was taken for consistency, though in five cases only the right flipper was available for analysis. For each animal, body size, stranding location, and sex were recorded. Straightline carapace length (straightline distance from the nuchal notch to the tip of longest posterior marginal of the carapace, SCL) was used as a metric for 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 used newly collected and previously processed humerus bones that were histologically prepared as described by Snover and Hohn (2004), Goshe et al. (2009), and Avens et al. (2012). Two sequential cross-sections (2 - 3 mm thick) were taken from each humerus bone, with one used for skeletochronology and the second for paired stable isotope analyses. Histological thin sections 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 mark (Avens et al. 2012). Assuming annual LAG deposition (Bjorndal et al. 2003, Snover and Hohn 2004, Snover et al. 2007), a calendar year was assigned to each measureable skeletal growth mark based on date of stranding counting backwards from the most external (newest) skeletal growth mark to the most internal (oldest). Estimates of SCL for each successive growth increment were then calculated using LAG diameters following Snover et al. (2007). A mean SCL was generated for each pair of successive LAGs that was used in all further analyses.

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 sections were micro-milled at Oregon State University using a New Wave Research Micromill (ESI), which consists of a Leica GZ6 StereoZoom microscope 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 paired digital skeletochronology images, printed onto transparency film, 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. In some cases composite samples of two narrow growth increments were collected due to my inability to individually sample the narrowest growth increments. 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 (Newsome et al. 2009, Avens et al. 2013), or set of growth years for composite samples.

Approximately 1.6 mg of bone dust was collected from each annual growth increment and analyzed for $\delta^{15}N$ and $\delta^{13}C$ by a continuous-flow isotope-ratio mass spectrometer in the Stable Isotope Lab at Oregon State University, Corvallis, OR. The system consists of a Carlo Erba NA1500 elemental analyzer interfaced with a DeltaPlusXL isotope-ratio mass spectrometer (Finnigan MAT, Bremen, Germany). Stable isotope ratios of samples relative to the standard are presented in the standard delta (δ) notation as follows:

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

where X is ^{15}N or ^{13}C and R is the ratio of heavy to light isotopes ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) in the sample and standard, respectively. $R_{standard}$ was IAEA 600 (Caffeine) for both nitrogen and carbon. USGS 40-glutamic acid ($\delta^{15}N$ = -4.52‰, $\delta^{13}C$=-26.39‰), IAEA N2 ammonium sulfate ($\delta^{15}N$ = +20.3‰), and ANU sucrose ($\delta^{13}C$ = -10.45‰) were used for calibration. Precision was 0.10‰ for $\delta^{15}N$ and 0.09‰ for $\delta^{13}C$. In addition to stable isotope ratios, %N and %C were calculated using mass 28

and mass 44 peak areas, respectively, with a precision of 0.46% for %N and 0.61% for %C.

Life History Patterns

To characterize the breadth of isotopic values loggerhead sea turtles may display in their bone tissue before and after ontogenetic shifts, I compiled isotopic data ($\delta^{13}C$, $\delta^{15}N$) from the literature for principal loggerhead prey and zooplankton from neritic (U.S. East Coast) and oceanic (Sargasso Sea) habitats in the Northwest Atlantic Ocean (Figure 2.1, Table B1). Oceanic juvenile loggerheads primarily consume epipelagic invertebrates clustered in floating sargassum, while neritic juveniles primarily forage on large benthic invertebrates (Bjorndal 1997, Seney and Musick 2007). In the marine environment, carbon isotope ratios are often used to reconstruct animal migratory patterns (e.g., Rau et al. 1982, Burton and Koch 1999). However, overlap in $\delta^{13}C$ values between neritic and oceanic loggerhead prey species limited my ability to infer migratory patterns based on $\delta^{13}C$ values (see Figure 2.1, Table B1). Consequently, to robustly assess loggerhead life-history variation I focused analyses hereafter on nitrogen isotope ratios ($\delta^{15}N$) because they are generally distinct between neritic and oceanic prey species (see Figure 2.1, Table B1). Nitrogen isotope ratios do not differ between bone collagen and bulk bone tissue (*personal observation*), and are assumed to reflect the $\delta^{15}N$ values of loggerhead prey (DeNiro and Epstein 1981). A conceptual model of the $\delta^{15}N$ values

associated with habitat-specific foraging opportunities for loggerhead turtles is presented in Figure 2.2a.

Nitrogen isotope ratio transects were reconstructed for each turtle and were used in conjunction with a pre-determined $\Delta\delta^{15}N$ threshold (+3.0‰; see 'Appendix A') to assign individuals to one of four predicted life-history patterns: *discrete shifter, facultative shifter, non-shifter*, and *indeterminate shifter*. All of my bone samples were predicted to show evidence of a diet shift because the samples were collected from turtles that likely died in nearshore habitat. *Discrete shifters* were predicted to exhibit a sharp increase in $\delta^{15}N$ greater than or equal to the $\Delta\delta^{15}N$ threshold within one year. This pattern would be expected for turtles that follow the traditional life history of a one-way, single-year transition from oceanic to fully neritic prey (Lutcavage and Musick 1985, Avens et al. 2003). *Facultative shifters* were predicted to exhibit a gradual increase in $\delta^{15}N$ values as would be expected for turtles that consume mixed oceanic and neritic prey over multiple years or occupy transitional habitats between isotopically distinct regions. Duration of ontogenetic shift was quantified for each *facultative shifter* based on the number of years it took the $\delta^{15}N$ values to surpass the $\Delta\delta^{15}N$ threshold. *Non-shifters* were turtles that exhibited consistent $\delta^{15}N$ values that did not increase by a magnitude necessary to surpass a given threshold. *Indeterminate shifters* were turtles that could not be classified due to insufficient data (e.g., missing data points, incomplete ontogenetic shift).

Trophic Position Estimation

Variation in $\delta^{15}N$ values within Northwest Atlantic loggerhead turtles may be driven by forage at different trophic levels, geographic differences in isotopic baselines, or both. Figure 2.2b presents a conceptual model of hypothesized isotopic patterns that reflect these effects. In order to identify the most probable mechanism driving the observed sea turtle life-history patterns we quantified and compared baseline $\delta^{15}N$ values and estimated trophic positions between neritic and oceanic loggerhead prey species (Figure 2.1, Table B1). We also quantified and compared trophic position estimates for turtles by averaging $\delta^{15}N$ values across all sampled growth increments before and after ontogenetic shifts to generate mean pre-shift (i.e., oceanic) and post-shift (i.e., neritic) $\delta^{15}N$ values for each turtle. Variation in $\delta^{15}N$ driven by trophic level effects would be supported by higher trophic position estimates for neritic prey consumed by turtles post-ontogenetic shift as compared to oceanic prey consumed by turtles pre-ontogenetic shift, while similar trophic assignments coupled with a large difference in $\delta^{15}N$ values between baseline organisms (i.e. zooplankton) would support the hypothesis that variation in $\delta^{15}N$ is driven by geographical differences (Figure 2.2b).

I used the following equation to estimate the trophic position of potential prey items and individual sea turtles pre- and post-ontogenetic shift (see Vander Zanden et al. 1997):

$$\text{trophic position} = \lambda + (\delta^{15}N_{consumer} - \delta^{15}N_{base})/\Delta_n$$

where λ is the trophic position of the organism used to represent $\delta^{15}N_{base}$, $\delta^{15}N_{consumer}$ is the measurement of $\delta^{15}N$ for the species of interest, and Δ_n is the enrichment in $\delta^{15}N$ per trophic level for the system (Post 2002). A trophic enrichment factor (Δ_n) of 3.3‰ was used for all estimations (Schoeninge and DeNiro 1984). Zooplankton were chosen to represent $\delta^{15}N_{base}$ due to availability of published data and were assigned a trophic level of 2.0 (i.e., $\lambda = 2$), typical of primary consumers.

Statistical Analyses

A cluster analysis was performed to determine the optimum number of clusters that best fit the distribution of turtle stable isotope data. 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. 2013). The method seeks to minimize the sum of dissimilarities between observations and allows for the use of silhouette widths, a measure of the clustering validity, to determine the optimum number of clusters in a dataset (Kaufman and Rousseeuw 1990). Mean SCL at transition from oceanic to neritic diets was quantified in two ways. The first approach estimated mean SCL at the start of an ontogenetic shift (i.e., growth increment with initial increase in $\delta^{15}N$) by life history pattern, with estimates for *facultative shifters* summarized by duration of shift. Non-parametric Mann-Whitney U tests were used to compare SCL at transition among life history pattern groups and by shift duration. For the second approach we performed a logistic regression

with the categorical response variable of whether a growth increment exhibited neritic ($\delta^{15}N > 12.47‰$) or oceanic ($\delta^{15}N < 12.47‰$) $\delta^{15}N$ values. This $\delta^{15}N$ cutoff was based on the best fit-cluster from the cluster analysis. Body size was regressed against the categorical response, with the predicted values at the inflection point (i.e., 50% probability of transition) used as estimates of SCL at transition from oceanic to neritic diets. Estimated trophic positions of oceanic and neritic prey were compared using a Mann-Whitney U test, while within-turtle estimated trophic position before and after a perceived ontogenetic shift were compared using a non-parametric Wilcoxon signed-rank test. All analyses were performed using program R (version 3.0.2; R Core Team 2013).

RESULTS

Straightline carapace length (SCL) at stranding ranged from 51.2 to 88.6 cm SCL (mean ± standard deviation = 67.8 ± 9.9 cm SCL) for turtles (n = 84) sampled from North Carolina (n = 62), Virginia (n = 14), Maryland (n = 4), and New Jersey (n = 4). Sex was not included as a covariate in analyses due to the limited number of positive identifications via necropsy analysis (male: n = 16, female: n = 29, unknown: n = 39).

Stable isotope ratios in bone tissue

A total of 599 bone samples were milled and analyzed for stable isotopes from all turtles (n = 4–12 samples per turtle; mean = 7 per turtle). Of these, 559

were sampled from individual growth increments (i.e., annuli) while 40 were composites of two (n = 37) or three (n = 3) growth increments. Of the 40 composite samples analyzed, only five affected life history pattern classification (see 'Appendix A'). Two clusters, based on $\delta^{15}N$ only, optimally fit the stable isotope data. Average silhouette width equaled 0.722, indicative of strong structure within the dataset (see Figure 2.3a,b; Kaufman and Rousseeuw 1990). The more depleted $\delta^{15}N$ cluster (n = 353, $\delta^{15}N$ = 9.99 ± 0.94‰, $\delta^{13}C$ = -15.11 ± 0.65‰) was separated at $\delta^{15}N$ = 12.47‰ from the more enriched $\delta^{15}N$ cluster (n = 186, $\delta^{15}N$ = 15.04 ± 1.47‰, $\delta^{13}C$ = -14.21 ± 1.05‰). In general, $\delta^{15}N$ and $\delta^{13}C$ increased with body size (Figure 2.3a,c), and $\delta^{15}N$ increased with $\delta^{13}C$ (Figure 2.3b), as would be expected with movements to neritic habitats and/or trophic increases in diet (Michener and Schell 1994, Burton and Koch 1999, Post 2002).

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. *Discrete shifters* (*n* = 23) exhibited sharp increases in $\delta^{15}N$ values that surpassed the $\Delta\delta^{15}N$ threshold (≥3.0‰) in one year (Figure 2.4a), while *facultative shifters* (*n* = 16) exhibited gradual increases in $\delta^{15}N$ values that took two to eight years to surpass the $\Delta\delta^{15}N$ threshold (Figure 2.4c). Mean growth increment-specific $\delta^{15}N$ values at the start and end of habitat shifts by life history pattern are presented in Table 2.1. Among turtles that exhibited an ontogenetic shift, 41% were *facultative shifters* whereas 59% were *discrete shifters*. Within the

facultative shifter group, 62% of turtles completed an ontogenetic shift in two years, 19% completed an ontogenetic shift in three years, and 19% took four years or more to complete an ontogenetic shift.

Twenty-eight turtles were classified as *non-shifters* because they did not display any marked increase in $\delta^{15}N$ values indicative of a shift in diet (Figure 2.4b, Table 2.1). *Non-shifters* were sub-classified into two groups, with thos that exhibited consistently lower $\delta^{15}N$ values labeled *oceanic non-shifters* (n = 20, $\delta^{15}N$ = 9.69 ± 0.81‰) and those that exhibited consistently higher $\delta^{15}N$ values labeled *neritic non-shifters* (n = 8, $\delta^{15}N$ = 15.51 ± 1.22‰). However, their presence in coastal waters suggests these turtles either recently transitioned to (*oceanic non-shifters*) or had been resident in (*neritic non-shifters*) neritic habitats. Younger, smaller turtles that died within one year of transition would not have deposited bone tissue with $\delta^{15}N$ values representative of a neritic lifestyle, while older, larger turtles that died after long-term residency in neritic habitats may have lost inner, earlier growth increments with transitional $\delta^{15}N$ values to bone resorption. Seventeen turtles could not be reliably classified into one of the other three life history pattern groups and were thus classified as *indeterminate shifters* (Figure 2.4d) due to missing data points (n = 5), the occurrence of composite $\delta^{15}N$ values at points in $\delta^{15}N$ transects critical to life history pattern classification (n = 3), or evidence of an incomplete ontogenetic shift (n = 9) characterized by an elevation in $\delta^{15}N$ values greater than 1.0‰, but less than the $\Delta\delta^{15}N$ threshold (+3.0‰). *Non-shifters* and *indeterminate shifters* were excluded from further analyses.

Size at transition to nearshore habitats

To analyze size at transition from oceanic to neritic habitats based on life history pattern, I assigned the transition year to the inner LAG of the growth increment exhibiting the initial increase in $\delta^{15}N$ value (≥ 3.0‰). Growth increment-specific $\delta^{15}N$ values within turtles were assigned 'ontogenetic positions' to allow for comparisons across turtles. The LAG associated with the $\delta^{15}N$ value at the start of an ontogenetic shift was assigned an ontogenetic position of 'zero', while previous and subsequent LAGs were assigned decreasing (e.g., -1, -2, -3, etc.) and increasing (e.g., 1, 2, 3, etc.) ontogenetic positions, respectively, to signify years to and from the ontogenetic shift (Figure 2.4a,c).

Mean size at transition for each life history pattern is presented in Table 2.1 and was summarized by duration of ontogenetic shift (i.e., years needed fo $\Delta\delta^{15}N$ to cumulatively increase by ≥ 3.0‰; see 'Appendix A'). Mean SCL estimates presented for *non-shifters* and *indeterminate shifters* in Table 2.1 were based on size at stranding. SCL did not differ between *discrete* and *facultative shifters* at the beginning (Mann-Whitney U test, W = 175.5, P = 0.405) of ontogenetic shifts, and did not vary by duration of ontogenetic shift (Kruskal-Wallis test, H = 0.8, df = 2, P = 0.643). Data from *facultative shifters* with shift durations greater than three years (n = 3) were excluded from analyses related to shift duration due to low sample size. With the exception of a single turtle that was 85.8 cm SCL, all *oceanic non-shifters* were <74 cm SCL at stranding. All *neritic non-shifters* were >74 cm SCL at stranding. The logistic regression model for size at transition showed high correlation between

the categorical response variable (neritic/oceanic) and explanatory variable (Overall χ^2 = 155.29, df = 2, P < 0.001; z-value for individual predictors: SCL = 4.010, P = <0.001). The model predicted transition to occur at 56.0 cm SCL (95% CI: 52.8 to 59.2 cm SCL, Figure 2.5), slightly larger than those presented based on life history pattern (Table 2.1, 51.4 – 55.1 cm SCL).

Turtle and prey trophic position

Mean $\delta^{15}N$ and $\delta^{13}C$ values and estimated trophic positions for zooplankton and principal loggerhead prey species i oceanic and neritic habitats are presented in Figure 2.1 and Table B1. Zooplankton $\delta^{15}N$ values were higher in neritic (7.92 ± 1.40‰) versus oceanic habitats (1.93 ± 1.17‰), while $\delta^{13}C$ values were similar between habitats (mean oceanic zooplankton: -19.37 ± 0.98‰, mean neritic zooplankton: -20.65 ± 2.11‰). Prey isotopic values showed a similar pattern with $\delta^{15}N$ values being higher in neritic habitats (11.84 ± 2.61‰) compared to oceanic habitats (6.15 ± 1.81‰) and $\delta^{13}C$ values not being isotopically distinct between habitats (mean neritic prey: -18.17 ± 1.42‰, mean oceanic prey: -18.14 ± 1.2‰, Mann-Whitney U test, W = 68.5, P = 0.953). Median trophic positions of all prey in oceanic and neritic habitats were the same (Mann-Whitney U test, W = 103, P = 0.770; Figure 2.6a). Median turtle trophic positions before and after an ontogenetic shift were significantly different (Wilcoxon signed rank test, Z =3.9, P < 0.001), with estimated trophic position post-ontogenetic shift (mean = 4.12) lower on average than pre-ontogenetic shift (mean = 4.48; Figure 2.6b).

DISCUSSION

The ontogenetic diet and habitat shifts that occur as juvenile loggerhead sea turtles recruit from oceanic to neritic habitats mark a critical, yet complex, transition in life stage that is theorized to occur in order to increase fitness (Werner and Gilliam 1984, Post 2003). My sequential isotopic analysis of annual bone growth increments revealed that juvenile loggerhead ontogenetic shifts follow one of two patterns (*discrete shifters*, *facultative shifters*, Figure 2.4a,c). My results suggest that as many as one third of turtles may take up to eight years to complete these transitions (Table 2.1), surpassing previous estimates of up to three years (Mansfield et al. 2009), and indicate that while a majority of these facultative transitions are brief (2 yrs, n = 10), many are completed over a more extended period of time (3 to 8 yrs, n = 6). I found similar means and variances in body size at transition between individuals exhibiting alternative life history patterns (i.e., *discrete* vs. *facultative shifter*; Table 2.1), supporting previous conclusions from satellite telemetry studies that intraspecific variation in the life history patterns of juvenile loggerheads in the Northwest Atlantic are not well explained by body size (McClellan and Read 2007, Mansfield et al. 2009).

Interpretation of isotopic shifts in bone layers

Through sequential isotopic analysis of humerus bone tissue I found a strong relationship between $\delta^{15}N$, $\delta^{13}C$, and back-calculated body size estimates (Figure 2.3a-c) as would be expected for loggerheads following the known life history of a

transition from oceanic to neritic habitats and prey (Figure 2.1, Table B1). These results suggest that sequential analysis of humerus bone cross-sections can be used to reconstruct the diet and habitat use histories of sea turtles. However, high overlap in $\delta^{13}C$ values between turtle growth increment-specific isotope values and between $\delta^{13}C$ values of both neritic and oceanic zooplankton and loggerhead prey impeded the use of $\Delta\delta^{13}C$ values to mark changes in habitat. McClellan et al. (2010) observed similar carbon isotope patterns between oceanic and neritic loggerhead soft tissues and prey. Taken together, these studies 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-ontogenetic shift $\delta^{15}N$ values presented here (mean = 4.2‰) is greater than that reported by Avens et al. (2013) (mean = 2.5 ‰) and Snover et al. (2010) (mean = 3.1‰). Absolute pre-ontogenetic shift $\delta^{15}N$ values in the present study (mean pre-shift = 10.2‰) were intermediate to those presented by Snover et al. (2010) (mean pre-shift = 11.0‰) and Avens et al. (2013) (mean pre-shift = 9.7‰), while post-ontogenetic shift $\delta^{15}N$ values (mean post-shift = 14.6‰) were greater (Snover et al. (2010): mean post-shift = 14.1‰; Avens et al. (2013): mean post-shift = 12.1‰). Avens et al. (2013) suggested such differences might be due to temporal and spatial variation in baseline $\delta^{15}N$ values or variation in turtle-specific foraging strategies (Seney and Musick 2007, Ohman et al. 2012, Ceriani et al. 2014). Both juvenile and adult loggerhead sea turtles display

strong foraging site fidelity (e.g., Avens et al. 2003, Broderick et al. 2007) and have been shown to be long-term diet specialists (Vander Zanden et al. 2010). Therefore, it is possible turtles included in each study displayed alternative diet preferences and foraging strategies. Nevertheless, discrepancies among these studies may also be due to the utilization of disparate sampling and classification techniques. In the present study, classification of life history pattern based on a $\Delta\delta^{15}N$ threshold resulted in the exclusion of seven turtles from analyses that exhibited $\Delta\delta^{15}N$ values greater than 1.0‰ but less than 3.0‰. This may have inflated mean difference and post-ontogenetic shift $\delta^{15}N$ values relative to those of previous studies that used no such criterion, though greater sample sizes of ontogenetic shifters herein (n = 39) may have also allowed me to capture a broader range of variation in loggerhead life history patterns (Snover et al. 2010, n = 23; Avens et al. 2013, n = 8). Furthermore, Snover et al. (2010) based inferences on isotopic samples taken on either side of a transitional growth increment rather than through sequential analysis of all growth increments, thus differences between these two studies may also relate to differences in resolution between sampling methods.

Mechanisms to explain variance in stable isotope ratios

The significant enrichments in $\delta^{15}N$ within turtles associated with ontogenetic shifts may ultimately be driven by one of two mechanisms: (1) forage at different trophic levels, or (2) differences in isotopic baselines (see Figure 2.2b). First, it is possible that the observed increases in $\delta^{15}N$ within turtles are due to

individuals foraging at higher trophic levels. Measured $\delta^{15}N$ values within turtles increased by an average of 4.4 ± 1.3‰ (min = 3.1‰, max = 8.4‰), consistent with the regularly observed 3 to 5‰ enrichment in $\delta^{15}N$ per trophic level within foodwebs (Post 2002). However, trophic position estimates did not differ between prey in oceanic and neritic habitats (Figure 2.6a) and were in fact lower on average in turtles post-ontogenetic shift (Figure 2.6b), which suggests juvenile turtles forage at similar trophic levels in these alternative habitats. On the other hand, zooplankton and turtle prey species from neritic habitats in the eastern U.S. had $\delta^{15}N$ values 5 to ‰ higher than oceanic species sampled from the Sargasso Sea (Figure 2.1, Table B1). Because the mean enrichment in $\delta^{15}N$ between turtle increment-specific isotope clusters (~5‰) tracked those of both zooplankton and known prey, I propose the enrichments in $\delta^{15}N$ observed within juvenile loggerhead bones is driven by differential forage on oceanic and neritic prey. Furthermore, since $\delta^{15}N$ values are predicted to be higher along the continental U.S. relative to the Sargasso Sea and Tropical Atlantic (McMahon et al. 2013), I suggest these enrichments are due to a coupled change in both diet and habitat.

Given that oceanic prey can become entrained in continental shelf waters via eddies and meanders I cannot rule out the possibility that the observed $\Delta\delta^{15}N$ patterns within turtles are due to diet shifts irrespective of habitat. However, because size at transition estimates in the present study are similar to those of other studies and to minimum size observations of turtles in nearshore waters (Table 2.1, Epperly et al. 2007, Avens et al. 2013), it is likely that the observed patterns are due

to a coupled habitat and diet change. Our understanding of isotopic baselines in the ocean is limited, largely hindered by the cost and logistical difficulty of accessing remote areas (McMahon et al. 2013). Undoubtedly, a greater understanding of the spatial and temporal variability of isotopic baselines in the ocean is needed to better evaluate historical diet and habitat use of sea turtles. Inclusion of other isotopic and trace element analyses (e.g., $\delta^{34}S$, $\delta^{18}O$) in future studies may aid in better understanding these patterns.

Alternative sea turtle life histories

This study adds to the mounting 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 ontogenetic changes in light of retrospective individual life history. Th prevalence and duration of facultative ontogenetic shifts quantified herein are 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. Here, greater than one third (n = 16 of 39) of turtles exhibited this alternative life history pattern, with estimated shift durations consistent with these previous studies (i.e., ≤5 years), though one turtle took 8 years to complete this transition.

As the large increases in $\delta^{15}N$ within *discrete shifters* appear to be driven by isotopic baseline differences and a coupled diet and habitat shift, I propose the intermediate isotope values (i.e., ~11-14‰) observed within *facultative shifters* (Figure 2.4c) are indicative of foraging in oceanic and neritic habitats within individual growth years as observed by McClellan and Read (2007) and Mansfield et al. (2009), 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 marine animals known to occupy alternative isotopically distinct areas (Smith et al. 1996, Angerbjörn et al. 2006, McClellan et al. 2010). Still, I cannot rule out the possibility of these turtles occupying transitional habitats along the continental shelf or Gulf Stream, which would allow access to both neritic and oceanic resources. The Gulf Stream regularly exchanges water between the continental shelf and Sargasso Sea via entrainments, meanders, and eddies (Olson 2001); therefore, turtles that forage along the continental shelf and Gulf Stream may have access to prey carried along and across this barrier. Ultimately, such differences in behavior may best be assessed through satellite telemetry or archival tag studies.

These results show that *discrete* and *facultative shifters* begin and complete ontogenetic shifts at similar sizes, and that the variance associated with these parameters is similar between the two life history patterns (Table 2.1). Such similarities have previously been observed in juvenile loggerhead sea turtles in the Northwest Atlantic (McClellan and Read 2007, Mansfield et al. 2009), but contrast with other loggerhead populations that show a size-based dichotomy in habitat use

(Hatase et al. 2002, Hawkes et al. 2006), although these differences were found among postnesting females. Estimates of body size at transition herein (life history pattern: 54.1 cm SCL; logistic regression: 56.0 cm SCL) are similar to those based on growth increment-specific δ^{15}N values from Avens et al. (2013) (55.3 cm SCL), and overlap to some extent with the range of estimates based on length frequency and skeletochronology methods (Bjorndal et al. 2000: 42. - 59.5 cm SCL; Snover et al. 2010: 43.6 - 47.4 cm SCL). Differences in estimates among these studies have been suggested to reflect a temporal change in size at transition (Avens et al. 2013). Here, there is weak evidence of a temporal shift in size at transition where turtles that transitioned to neritic habitats in the 2000s (mean SCL = 56.5 cm, mean year = 2004, *n* = 18) were larger than turtles that transitioned in the 1990s (mean SCL = 52.2 cm, mean year = 1996, *n* = 21). Further research is needed to determine whether these patterns are indicative of a temporal shift in size at transition or are an artifact of small sample sizes. Nevertheless, these results further highlight the need to better understand the mechanisms driving intraspecific variation in the timing and duration of ontogenetic shifts in sea turtles.

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 of optimal fishing and turtle foraging areas (Witzell 1999), and 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, Crouse 1999, Heppell et al. 2002). As the sources and magnitude of natural and anthropogenic mortality likely vary between oceanic and neritic habitats and foraging strategies (Bolten et al. 2011, Lewison et al. 2014), turtles that return to oceanic habitats for extended periods of time and 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 decisions.

Conclusion

This study highlights the utility of combined skeletal and stable isotope analyses to the study of sea turtle ecology. I propose these methods can be used to assess variation in sea turtle life history and diet specializations, and can potentially provide a means of robustly quantifying the prevalence, duration, and timing of alternative sea turtle life history patterns. My study further confirms that a significant proportion of turtles exhibit facultative ontogenetic shifts that extend over multiple years. Studies that examine differential growth and survival between these habitats would be useful for investigating how such life history patters are maintained in populations and how they influence sea turtle population dynamics. I

provide initial evidence of a temporal shift in size at transition among turtles, but more robust studies that incorporate samples from historical collections are needed to better evaluate temporal variation in the timing of ontogenetic shifts and the prevalence of facultative ontogenetic shifts through time. Sequential analysis of annual growth increments in bone tissue is a valuable method for reconstructing ontogenetic changes in foraging ecology and habitat use of sea turtles.

TABLES AND FIGURES

Table 2.1. Estimated straightline carapac length (SCL and $\delta^{15}N$ values a the oceanic-to-neritic transition fo juvenile loggerhead sea turtles by life-history pattern. Values are presented as mean ± SD (range). Estimates and ranges for *facultative shifters* are summarized by shift duration. *n* = sample size.

Life-history Pattern	*n*	SC (cm)		$\delta^{15}N$ (‰)		
		Start of Shift	End of Shift	Start of Shift	End of Shift	$\Delta\ \delta^{15}N$
All shifters[a]	39	54.1 7.3 (40.8 – 73.8)		10.25 0.79	14.64 ± 1.47	4.39 ± 1.28
Discrete shifters	23	55.1 7.6 (41.4 – 73.8)		10.14 0.71	14.63 ± 1.56	4.49 ± 1.40
Facultative shifters						
All durations	16	52.8 6.9 (40.8 – 66.5)	59.0 6.2 (49.9 – 71.1)	10.38 0.88	14.66 1.40	4.28 ± 1.15
2 years	10	54.8 6.8 (45.4 – 66.5)	58.4 6.5 (49.9 – 71.1)	10.24 1.05	14.74 1.69	4.50 ± 1.30
3 years	3	51.4 7.0 (43.4 – 55.7)	59.2 5.6 (55.2 – 63.1)	10.61 0.64	14.87 0.91	4.26 ± 0.84
4 years	1	53.9	63.4	10.57	13.98	3.41
5 years	1	40.8	52.1	10.84	13.92	3.08
8 years	1	50.3	67.0	10.56	14.56	4.00
Non-shifters[b]	28					
Oceanic	20	62.9 8.3 (51.2 – 85.8)		9.69 0.81		
Neritic	8	82.7 4.7 (74.8 – 87.2)		15.51 1.22		
Indeterminate shifters[b]	17	69.2 10.7 (57.1 – 88.6)		11.24 2.24		

[a]Combined data from *discrete shifters* and *facultative shifters*
[b]Based on SCL at stranding and $\delta^{15}N$ values of all sampled growth increments

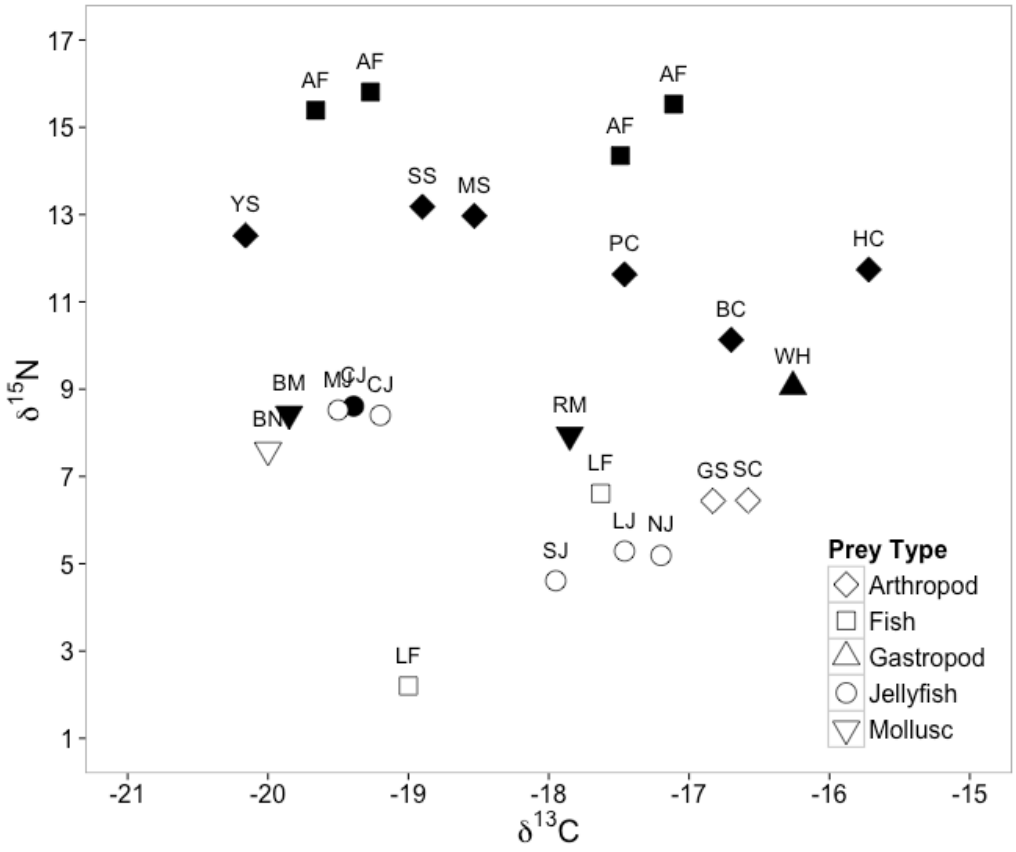

Figure 2.1. Mean prey isotope values by taxonomic group (shapes) and habitat (neritic = black, oceanic = white). Species codes: AF = Adult fish (bycatch), BC = Blue crab, BM = Blue mussel, BN = Barnacle, CJ = Cannonball jellyfish, GS = Brown grass shrimp, HC = Horseshoe Crab, LF = Larval fish, LJ = Lion's mane jellyfish, MJ = Moon jellyfish, MS = Mantis shrimp, NJ = Sea nettle jellyfish, PC = Spider crab, RB = Ribbed mussel, SC = Sargassum crab, SJ = Mauve stinger jellyfish, SS = Sand shrimp, WH = Whelk, YS = Mysid shrimp. See Table B1 for full list of species and isotopic values.

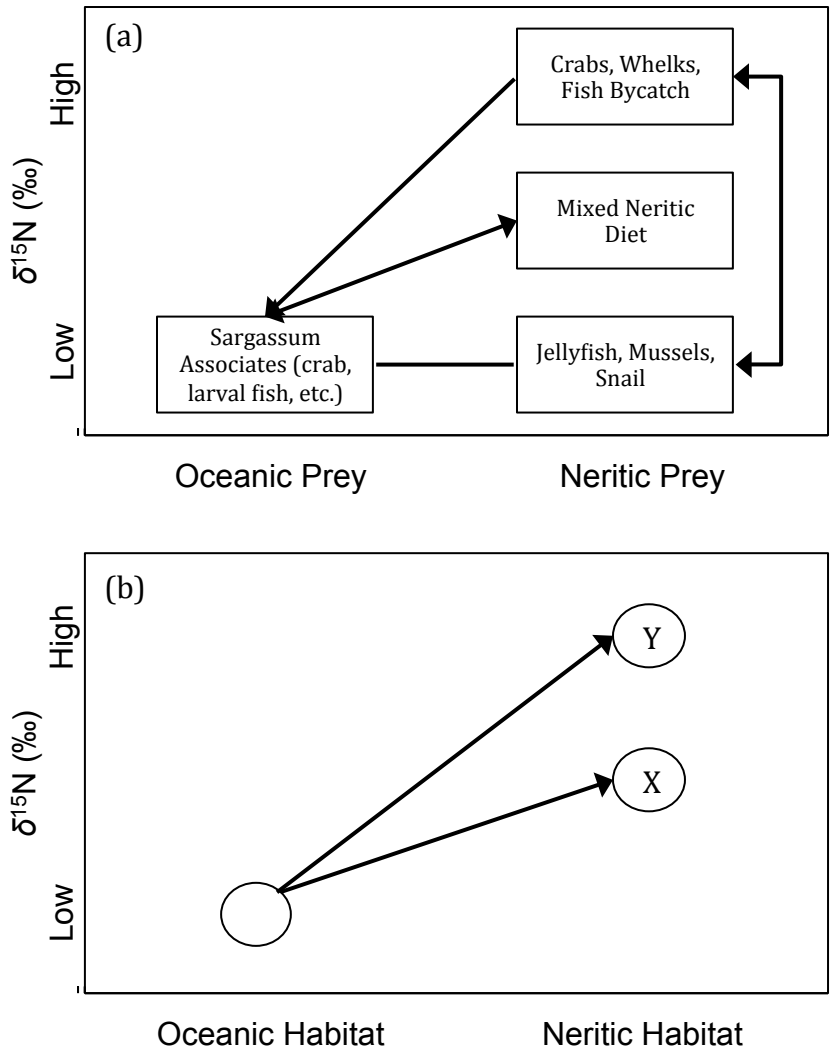

Figure 2.2. (a) Conceptual model of the $\delta^{15}N$ values associated with habitat-specific foraging opportunities for loggerhead turtles. Potential oceanic prey species have relatively low $\delta^{15}N$ values, but potential prey found in neritic habitats show wide variance in possible $\delta^{15}N$ values. Arrows track all possible diet transitions. (b) Conceptual model of two nitrogen isotope patterns predicted for changes in baseline $\delta^{15}N$ and/or foraging trophic level between habitats. Arrows track the two patterns, and circles represent the $\delta^{15}N$ values in each habitat. (X) higher $\delta^{15}N$ baseline in neritic habitats and same foraging trophic level between habitats, or same $\delta^{15}N$ baseline between habitats and higher trophic level in neritic habitats; (Y) highe $\delta^{15}N$ and foraging trophic level in neritic habitats.

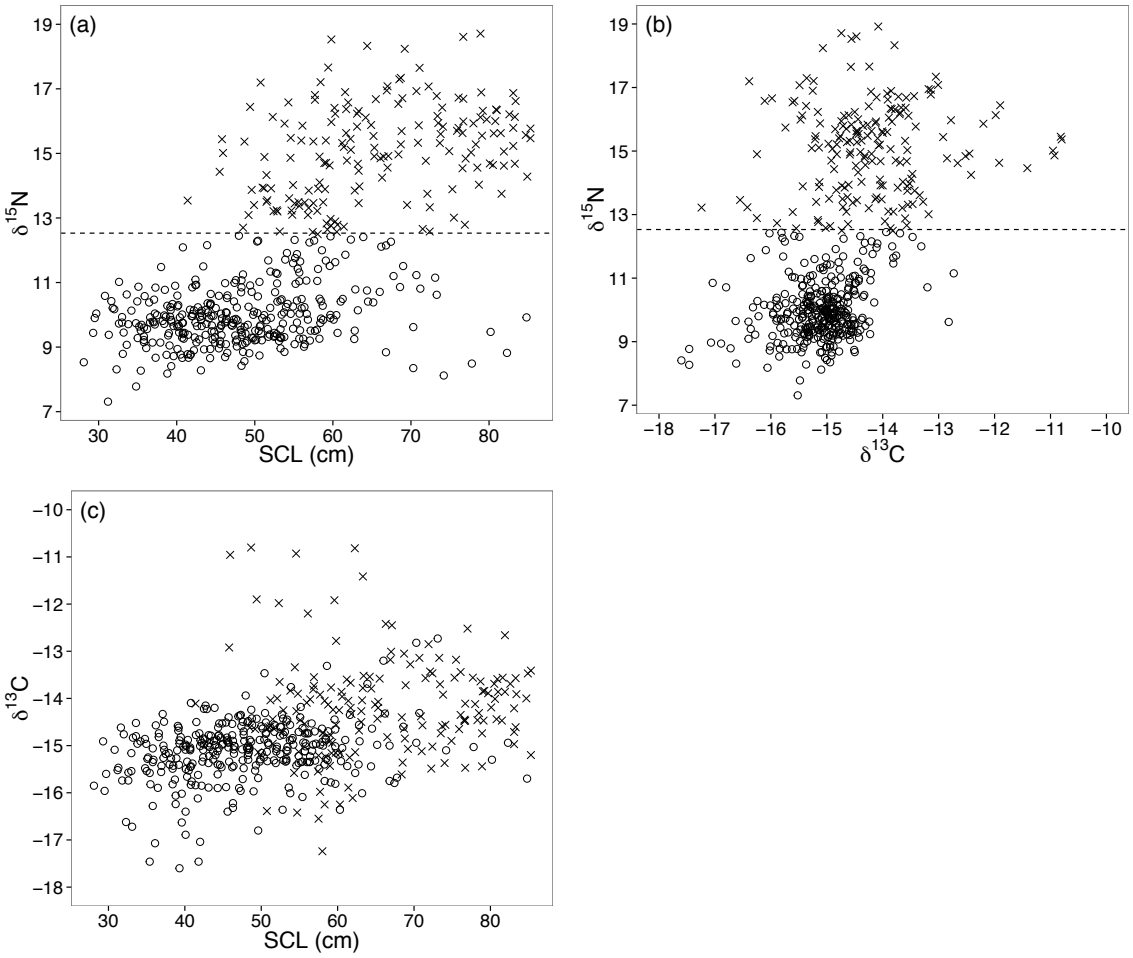

Figure 2.3. Comparison of (a) $\delta^{15}N$ and SCL, (b) $\delta^{15}N$ and $\delta^{13}C$, and (c) $\delta^{13}C$ and SCL of annual growth increments (n = 539) from juvenile loggerhead sea turtles (n = 84) Two clusters, based on $\delta^{15}N$ only, best fit the data. The depleted $\delta^{15}N$ cluster (o) and enriched $\delta^{15}N$ cluster (×) are separated at $\delta^{15}N$ = 12.47‰ (dashed horizontal line). SCL is the mean back-calculated straightline carapace length for the growth increment.

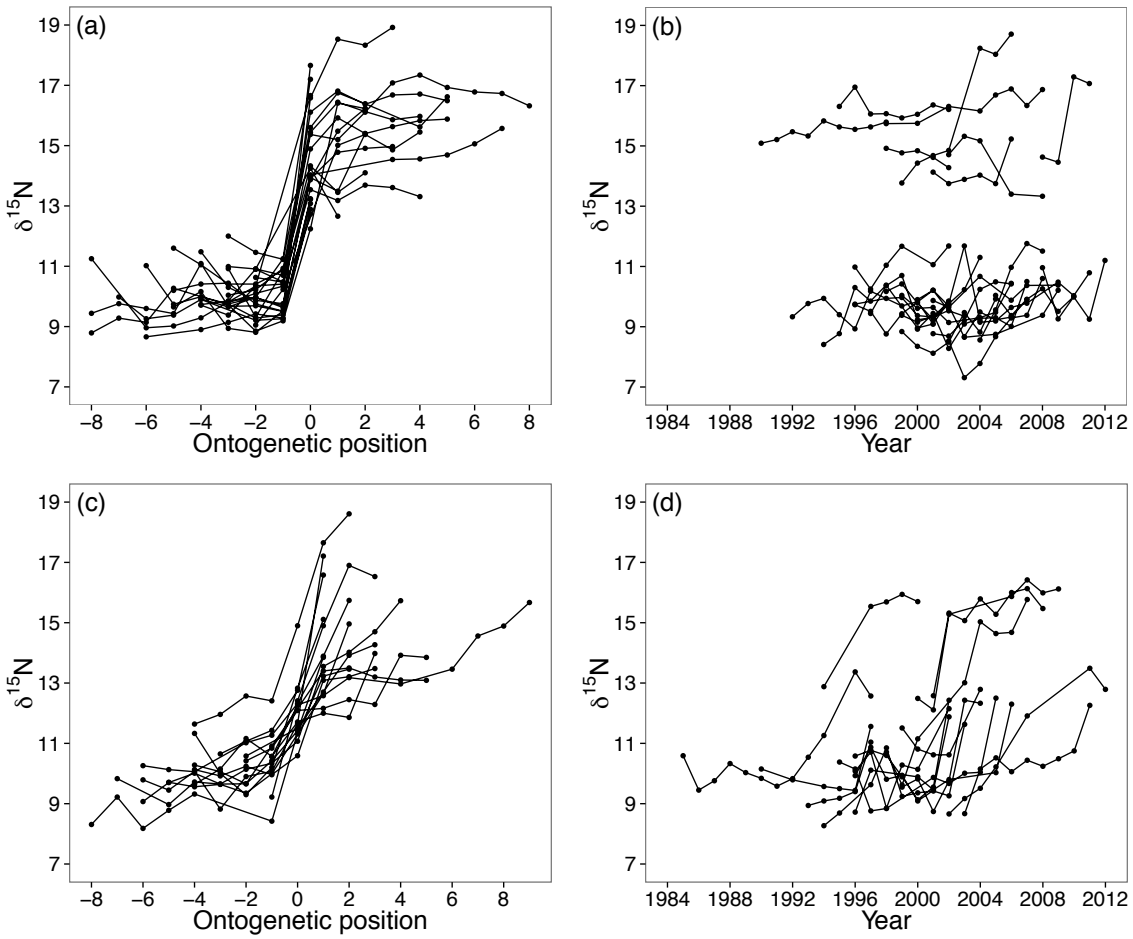

Figure 2.4. Nitrogen isotope ratio transects by life history pattern as determined by sequential isotopic analysis of successive annual growth increments. (a) *Discrete shifters* (*n* = 23), (b) *non-shifters* (*n* = 28), (c) *facultative shifters* (*n* = 16), and (d) *indeterminate shifters* (*n* = 17). Plots represent all sampled growth increments (points) within turtles (lines). (a,c) Ontogenetic position standardizes isotope transects across turtles with the LAG at the start of an ontogenetic shift assigned an integer value of 'zero'. All other values are years before and after the ontogenetic shift.

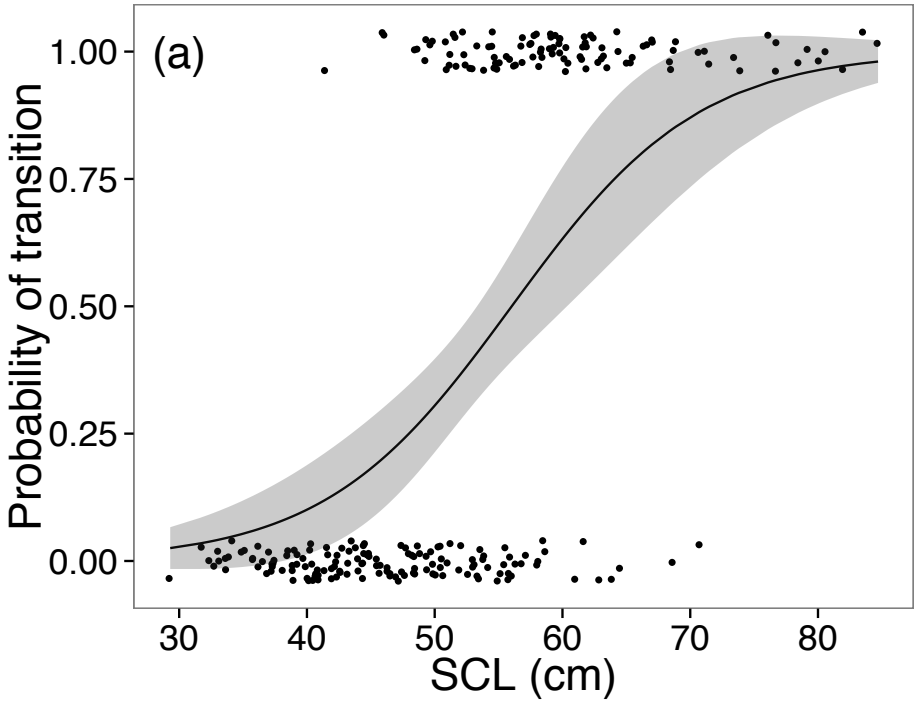

Figure 2.5. Probability of transition versus straightline carapace length (SCL) for juvenile loggerhead sea turtles. The black line is the predicted relationship from a logistic regression and is bounded by 95% confidence intervals. The model predicted transition from oceanic to neritic habitats (i.e., 50% probability) to occur at 56.0 cm SCL (95% CI: 52.8 to 59.2 cm SCL).

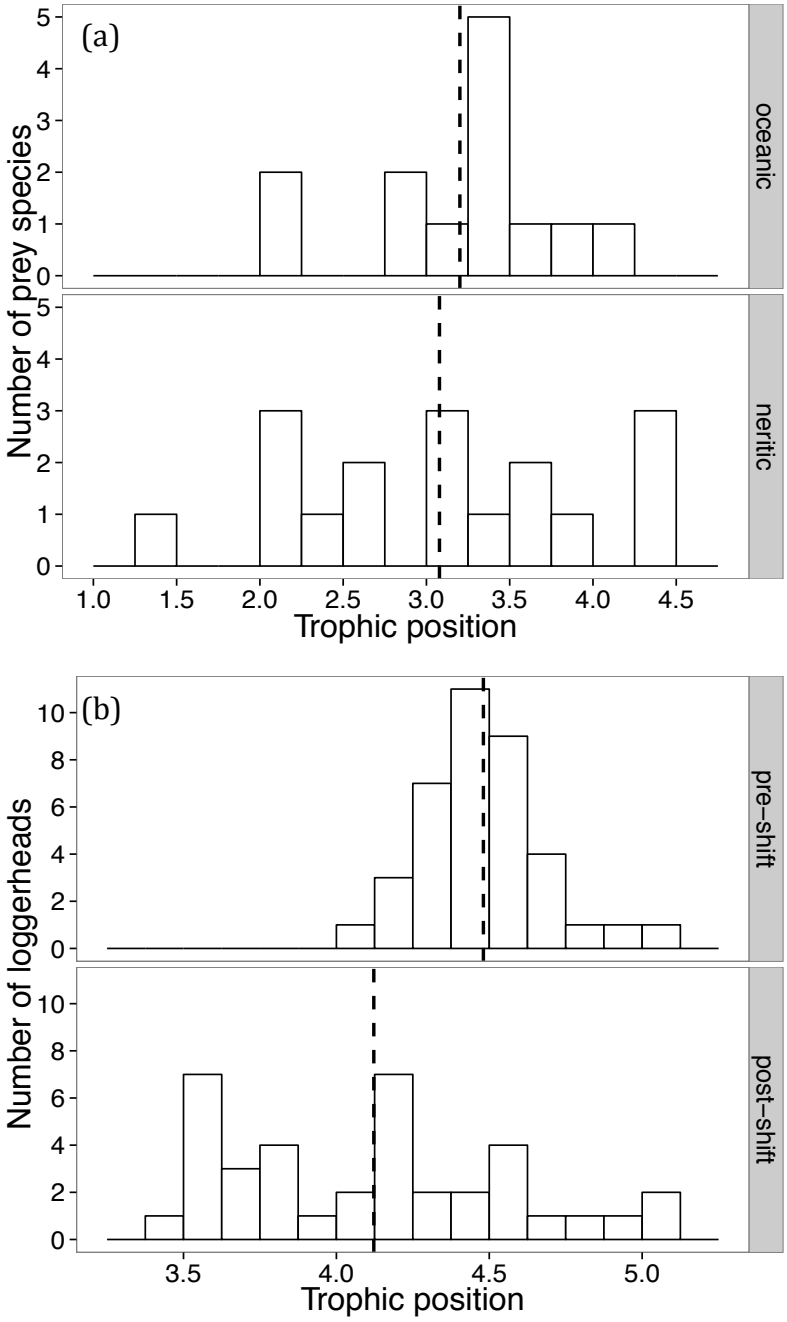

Figure 2.6. Estimated trophic positions of (a) oceanic and neritic prey and (b) *discrete shifters* and *facultative shifters* pre- and post-ontogenetic shift. The dashed vertical lines are means. Trophic position estimates were calculated as described in 'Materials and Methods.'

CHAPTER 3: GROWTH DYNAMICS OF LOGGERHEAD SEA TURTLES UNDERGOING ONTOGENETIC HABITAT SHIFTS

ABSTRACT

Somatic growth patterns may strongly influence behavior and population dynamics through effects on individual fitness and demographic parameters. Ontogenetic niche theory predicts that as individuals grow they will select habitats that allow for optimal growth and survival, where habitat shifts can infer a growth advantage. I combine skeletochronological and stable nitrogen isotope ($\delta^{15}N$) analyses of sea turtle humerus bones to characterize the ontogenetic growth dynamics of juvenile Northwest Atlantic loggerheads (*Caretta caretta*). The primary objective of this study was to determine if an oceanic-to-neritic habitat shift infers a growth advantage to loggerheads as predicted by ontogenetic niche theory and if this pattern is maintained in individuals exhibiting alternative life history patterns (i.e., *discrete shfters* vs. *facultative shifters*). Back-calculated growth rates peaked in the 50-59.9 cm straightline carapace length (SCL) size class, within the range of the known size at transition from oceanic-to-neritic habitats for this species. Examination of growth trajectories with respect to year to and from ontogenetic habitat shift (i.e. ontogenetic position) revealed annual growth rates generally peaked within one year of transition, providing support for an ontogenetic shift-associated growth advantage. However, there was considerable variation in the timing of observed maximal growth rate among turtles with some individuals exhibiting maximal growth prior to the habitat shift based on $\Delta\delta^{15}N$ (14/38).

Generalized additive mixed models of the potential influence of covariates on back-calculated growth rates showed significant effects of SCL $\delta^{15}N$, and ontogenetic position, with ontogenetic position the best predictor of juvenile growth. Growth variance was higher for *facultative shifters* when compared to *discrete shifters*, but size-at-age relationships and mean growth rates did not differ between shifter groups, likely limiting the influence of alternative life history patterns on time to maturity.

INTRODUCTION

Somatic growth is a strongly selected life history trait that can shape community and population dynamics through effects on population vital rates and individual fitness (Werner and Gilliam 1984, Stearns 1992, Dmitriew 2011). As many of the factors that influence growth rates vary spatially and temporally in the environment, life history theory predicts that individuals will choos habitats to meet their changing needs and reduce time to sexual maturity (Werner and Gilliam 1984). These size-specific habitat use decisions, or ontogenetic habitat shifts, often mark transitions between life stages where individuals seek to balance the benefits of optimal growth with risk of predation. Such trade-offs may ultimately result in the selection of habitats that minimize the ratio of mortality risk to growth rate (i.e., μ/g) and may lead to the use of potentially suboptimal growth habitats where predation risk is low until critical sizes are reached (Werner and Gilliam 1984, Dahlgren and Eggleston 2000, Snover 2008). When predation risk is similar among

habitats, individuals should select the habitat that allows for optimal growth (Werner and Gilliam 1984).

Empirical studies have shown that ontogenetic habitat shifts can infer a growth advantage in the new habitat (e.g., Werner and Hall 1988, Dahlgren and Eggleston 2000, Grol et al. 2011). Yet, despite the prevalence of ontogenetic habitat shifts among marine organisms relatively few studies have empirically tested ontogenetic niche theory in marine systems, and most are limited to coral reef fishes (Dahlgren and Eggleston 2000, Grol et al. 2011, Kimirei et al. 2013; but see Salvanes et al. 1994). Dahlgren and Eggleston (2000) coupled a caging experiment with a cost-benefit analysis to demonstrate that juvenile Nassau grouper selected habitats that minimized the ratio of mortality risk to growth rate dependent on body size (also see Grol et al. 2011, 2014, Kimirei et al. 2013). In one of the only quantitative assessments of this life-history theory in fish species from temperate regions, Salvanes et al. (1994 found that model predictions of the timing of Atlantic cod settlement to benthic habitats were largely consistent with field observations of changes in mortality and growth rate. Parallel studies in large marine vertebrates are lacking, undoubtedly due to difficulties associated with quantifying growth and morality rates in highly migratory species. Snover et al. (2010) provided initial support for this ontogenetic niche theory in sea turtles, where growth rates were higher for turtles immediately following an oceanic-to-neritic habitat shift. Though habitat-specific mortality estimates for sea turtles are lacking, predation risk can be assumed to scale with body size (Musick and Limpus 1997, Heithaus 2013) so that

the perceived ratio of mortality risk to growth rate for intermediate size classes is minimized in neritic habitats.

Loggerhead sea turtles (*Caretta caretta*) make transoceanic migration across the North Atlantic Ocean throughout their ontogeny (for review se Musick and Limpus 1997, Bolten 2003). After hatching, individuals enter the Gulf Stream and undergo an oceanic life stage that lasts roughly a decade (Bjorndal et al. 2000, Avens et al. 2013). Then, at critical sizes, individuals recruit from oceanic to neritic habitats where they were long thought to take up permanent residency (see Musick and Limpus 1997). However, this transition has been shown to be facultative, whereby individuals can take multiple years to fully transition to a neritic lifestyle (McClellan and Read 2007, Mansfield et al. 2009, Chapter 2). Growth rates and sources of mortality likely differ between oceanic and neritic habitats. Oceanic juvenile loggerheads are primarily "float-and-wait" predators that feed on epipelagic invertebrates clustered in floating sargassum (Bjorndal 1997, Bolten 2003). Because these foraging habitats are inherently patchy and stochastic, oceanic juveniles undergo bouts of food abundance and scarcity that likely reduce growth and contribute to their wide year-to-year growth variance (Bjorndal 1997, Bjorndal et al. 2003). Neritic juvenile loggerheads are presumably presented with more consistent foraging opportunities and more favorable growth conditions (Peckham et al. 2011). Turtles that undergo facultative ontogenetic shifts may therefore experience reduced growth during transitional years relative to conspecifics that

may extend life-stage duration and increase time to sexual maturity, important vital rates shaping population dynamics.

The hard body structures of animals can be used to study an individual's history of growth and foraging ecology (e.g., Walker and Macko 1999, Burton and Koch 1999, Snover et al. 2007). Skeletochronology, or the study of concentric bone growth marks, is used to estimate growth rates of reptiles and amphibians through back-calculation of body size estimates from successive growth mark measurements. The reconstruction of growth trajectories form skeletal growth marks assumes growth is periodic (e.g., daily, annual) and that there is some proportionality between the measurements of a skeletal feature and body size, both of which have been validated for juvenile Northwest Atlantic loggerhead sea turtles (Snover and Hohn 2004, Snover et al. 2007, Avens et al. 2013). The presence of an allometric relationship between humerus diameter and straightline carapace length (SCL) allows for the back-calculation of body size estimates, and thus growth rates, for each year of a turtles life, limited only by the amount of bone resorbed in the metabolically active core (Zug et al. 1986).

Information obtained through skeletochronology can be paired with sequential isotopic analyses of bone tissue to study how growth rates relate to changes in life history (Jones et al. 1983, Best and Schell 1996, Snover et al. 2010). Stable isotope ratios provide integrated information about a consumers diet and habitat use choices, with nitrogen (^{15}N:^{14}N; $\delta^{15}N$) and carbon (^{13}C:^{12}C; $\delta^{13}C$) isotopes typically used to study trophic relationships and migratory patterns, respectively

(see Peterson and Fry 1987, Hobson 2007). Numerous studies have demonstrated that these methods can be applied to the skeletal structures of marine organisms to detect ontogenetic changes in diet and habitat (e.g., Walker and Macko 1999, Estrada et al. 2006, Mendes et al. 2007). Snover et al. (2010) provided the first isotopic assessment of sea turtle bone tissue in light of individual growth trajectories. They showed that observed increases in growth rate within juvenile loggerhead sea turtles coincided with a diet and habitat shift, though their sampling method likely resulted in the collection of isotopic data from multiple growth years.

Here, I provide a detailed assessment of the ontogenetic growth dynamics of juvenile loggerhead sea turtles that completed an oceanic-to-neritic habitat shift. I sequentially analyzed sea turtle humerus bones for stable nitrogen isotopes ($\delta^{15}N$) to identify when turtles made this ontogenetic shift and to categorize individuals into alternative life history pattern groups. The primary objective of this study was to determine if ontogenetic niche theory was upheld in juvenile loggerhead sea turtles as suggested by Snover et al. (2010) when an individual's entire isotopic history is known. In addition, I investigated how growth patterns differed between sea turtles displaying alternative life history patterns (*discrete* vs. *facultative shifters*). This study provides one of the first detailed assessments of the interplay between growth variation, foraging ecology, and habitat use in sea turtles.

MATERIALS AND METHODS

Sample collection and skeletochronology

Humerus bones and carapace length measurements were obtained from 38 juvenile loggerhead sea turtles that stranded dead on beaches along the coasts of North Carolina (n = 26), Virginia (n = 7), Maryland (n = 4), and New Jersey (n = 1) from 1997 to 2012. For each stranded animal, body size, stranding location, and sex 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. For one turtle, only curved carapace length (CCL) was recorded, therefore SCL was calculated as described by Snover et al. (2010). SCL at stranding ranged from 54.1 to 88.4 cm.

This study utilized newly collected and previously processed humerus bones that were histologically prepared as described by Snover and Hohn (2004), Goshe et al. (2009), and Avens et al. (2012). Two sequential cross-sections (2 to 3 mm thick) were taken from each humerus bone, with one used for skeletochronology and the second for paired stable isotope analyses (see Chapter 2). Histological thin sections 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 mark (Avens et al. 2012).

Assuming annual LAG deposition (Bjorndal et al. 2003, Snover and Hohn 2004), a calendar year was assigned to each measureable skeletal growth mark 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 review of back-calculation method see Snover et al. 2007). A mean SCL was generated for each pair of successive LAGs that was used for all analyses. Age was estimated for each turtle following Parham and Zug (1997) and Avens et al. (2012). In summary, the number of LAGs lost to resorption in each turtle was estimated and added to the number of observed LAGs to give an initial age estimate for each turtle. This age estimate was used to back-assign an age estimate to each LAG. A final age estimate at stranding was determined by adjusting the initial age estimate to the nearest 0.25 years based on the mean hatch date for the population and individual stranding date (see Avens et al. 2013).

Life History Classification

Paired bone cross-sections were sequentially sampled for stable isotopes using a high-resolution micromilling system. Transparencies of the digital skeletochronology images were used to guide precision drilling and ensure milling of individual growth increments (see Chapter 2). In some cases composite samples of two narrow growth increments were collected due to my inability of individually sampling the narrowest growth increments. Nitrogen stable isotope data from 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 (Newsome et al. 2009, Avens et al. 2013). Only periosteal bone was sampled to eliminate the influence of reworked endosteal bone on results.

Approximately 1.6 mg of bone dust from each sample was packaged into sterilized tin cups and analyzed for $\delta^{15}N$ by a continuous-flow isotope-ratio mass spectrometer in the Stable Isotope Lab at Oregon State University, Corvallis, OR. The system consists of a Carlo Erba NA1500 elemental analyzer interfaced with a DeltaPlusXL isotope-ratio mass spectrometer (Finnigan MAT, Bremen, Germany). Stable isotope ratios of samples relative to the standard are presented in the standard delta (δ) notation as follows:

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

where X is ^{15}N and R is the ratio of heavy to light isotopes ($^{15}N/^{14}N$) in the sample and standard (IAEA 600 - caffeine), respectively. USGS 40-glutamic acid ($\delta^{15}N$ = -4.52‰) and IAEA N2 ammonium sulfate ($\delta^{15}N$ = +20.3‰) were used for calibration. Precision was 0.10‰ for $\delta^{15}N$. Nitrogen isotope ratios of bulk bone reflect that of bone collagen (*personal observation*), and are assumed to reflect the $\delta^{15}N$ values of loggerhead prey (DeNiro and Epstein 1981). When sampling skeletal tissue to reconstruct habitat shifts carbon isotope ratios of isolated bone collagen are typically measured as they reflect diet-based carbon sources (Schoeninger and DeNiro 1984, Lee-Thorp et al. 1989). However, high overlap in $\delta^{13}C$ values between

neritic and oceanic loggerhead prey species limited my ability to infer migratory patterns based on $\delta^{13}C$ values (see Figure 2.1, Table B1).

As described in Chapter 2, turtles were assigned to one of two life history pattern groups (*discrete shifter, facultative shifter* using the nitrogen isotope ratio transects generated for each turtle and a pre-defined $\Delta\delta^{15}N$ threshold (i.e., +3.0‰, see 'Appendix A'). Duration of ontogenetic shift was quantified for each turtle based on number of years it took the $\delta^{15}N$ values to surpass the $\Delta\delta^{15}N$ threshold. Turtles with shift durations of one year were classified as *discrete shifters* and turtles with shift durations greater than one year were classified as *facultative shifters*.

Growth Rates

Annual growth rates were calculated by taking the difference between mean back-calculated SCL estimates of successive LAGs. Growth rates were assigned to the year of the innermost LAG of the pair and binned into 10 cm size classes based on the estimated mean SCL of the LAG pair. A Kruskal-Wallis test was used to determine whether growth rates differed between size classes within groups of turtles, and Dunn's tests were used to determine which size classes had different growth rates. Mann-Whitney U tests were used to compare mean size-class specific growth rates between *shifter* groups. To further assess differences in growth patterns between *shifter* groups we quantified the magnitude of change in growth rate (i.e., |Δ growth rate|) between successive growth increments for all turtles and used a Mann-Whitney U test to compare them between *shifter* groups. Size (SCL)-at-

age and relationships between annual growth rates and estimated SCL, age, and year were modeled using nonparametric smoothing splines with the *mgcv* package in R (version 3.0.2; Wood 2006, R Core Team 2013). In order to characterize growth dynamics relative to sea turtle ontogenetic shifts, growth rates were averaged by ontogenetic position (i.e., years before and after the ontogenetic shift based on $\delta^{15}N$, OP; see Chapter 2, Figure 2.4) and compared qualitatively.

To evaluate the influence of SCL, Age, $\delta^{15}N$, and OP on growth rate, growth data were modeled using generalized additive mixed models (GAMMs) that included turtle-specific random effects (Chaloupka and Musick 1997, Wood 2006). Sex was not included as a covariate in analyses due to the limited number of positive identifications (male: $n = 6$, female: $n = 13$, unknown: $n = 19$). In addition, early model runs did not find year to be a significant predictor of growth; therefore, year was excluded from analyses. The remaining variables (SCL, age, $\delta^{15}N$, OP) were modeled separately as they displayed high collinearity, which can lead to concurvity within additive models and confound statistical inference (Ramsay et al. 2003, Wood 2006). Pairwise correlation coefficients and variance inflation factor (VIF) values exceeded collinearity diagnostic thresholds (0.7 and 3.0, respectively; see Zuur et al. 2010, Dormann et al. 2013). GAMM models included a log link, a quasi-likelihood error function, an autoregressive order 1 correlation structure for growth increments within turtles, and cubic regression smoothing splines to characterize the non-linear relationship between covariates and growth rate. Models were implemented in R using the *mgcv* and *nlme* packages (Wood 2006, Pinheiro et al.

2014). The contribution of covariates to each model was evaluated using *F*-ratio tests, and overall model fit was assessed using Akaike's information criterion and adjusted r^2 values.

RESULTS

Life History Classification

A total of 310 samples were collected and analyzed for stable isotopes from 38 turtles (*n* = 4 – 12 per turtle; median = 8 per turtle). Of these, 298 samples were growth increment-specific while 14 were composites of two growth increments (see 'Appendix A'). Nitrogen isotope ratios ranged from 8.18 to 18.92‰ (mean = 12.12‰). Based on the pattern of their $\delta^{15}N$ transect, 23 turtles were classified as *discrete shifters* while 15 were classified as *facultative shifters* (see Figure 2.4). *Discrete shifters* were assumed to be turtles following the traditional life history of a one-way, single-year transition from oceanic to neritic habitats and prey while *facultative shifters* were assumed to be turtles displaying the alternative life history pattern of a prolonged transition to fully neritic habitats and diets (see Chapter 2). In two cases, composite samples influenced life history pattern classification; both turtles were conservatively classified as *discrete shifters.* Mean growth increment-specific $\delta^{15}N$ values the year prior to and year of completion of ontogenetic shifts were 10.28 ± 0.78‰ and 13.92 ± 1.89‰, respectively, with a mean increase in $\delta^{15}N$ of 4.23 ± 1.22‰.

Growth Analyses

Back-calculated annual growth rates ranged between 0.1 and 9.0 cm/year with a mean of 2.9 cm/year for all measureable growth increments from all turtle humerus bones. Growth rates exhibited high variability relative to SCL, age, and calendar year (Figure B2). Mean annual growth rates were weakly different among size classes ($p = 0.056$, Kruskal Wallis test) and were the highest in the 50 to 59.9 cm SCL size class and lowest in the smallest (20 – 29.9, 30 – 39.9) and largest (80-89.9; Table 3.1) size classes. Mean growth rates of *discrete shifters* and *facultative shifters* were statistically different in three size classes ($p < 0.05$, Mann-Whitney U test), though this may have been due to small sample sizes (Table 3.1). Smoothing splines fit to back-calculated SCL-at-age data from all turtles (Figure 3.1a) and to *discrete* and *facultative shifters* separately (Figure 3.1b), revealed no difference in the SCL-at-age relationship between turtles exhibiting these alternative life history patterns.

Variance in annual growth within and among individuals was high (Figure 3.2), with the mean magnitude of change in growth rates (i.e., within-turtle variance in growth) higher for *facultative shifters* (mean |Δ growth rate| = 1.61) than *discrete shifters* (mean |Δ growth rate| = 1.22; $p < 0.05$, Mann-Whitney U test). Changes in $\delta^{15}N$ were not broadly correlated with changes in growth rate (Figure 3.3). In general, turtle-specific annual growth rates and mean annual growth rates were highest within one year of an ontogenetic shif (Figure 3.2, 3.5a) for both *discrete shifters* (Figure 3.2a) and *facultative shifters* (Figure 3.2b), but spanned years before

and after the shift year as detected by a change in nitrogen stable isotope values (Δ $\delta^{15}N$). Observed turtle-specific maximal growth rates were attained at ontogenetic positions (OP) of -2, -1, 0, 1, and 3 for three, eleven, twelve, eight, and one turtle(s), respectively (see Figure 3.2). In fact, 14 of 35 turtles exhibited maximal growth rates prior to an ontogenetic shift, though 31 turtles exhibited maximal growth rates within one year of transition. Maximal growth rates were unknown for three turtles that had missing growth rate information the year of an ontogenetic shift.

To determine how variance in observed maximal growth affected my interpretation of sea turtle growth dynamics, growth trajectories were re-centered on the year of maximal growth rate. Growth rates were then averaged by year to and from observed maximal growth rate, which revealed mean growth rates were similar across years before and after the year of maximal growth (Figure 3.5b). Mean annual growth rates by ontogenetic position and maximal growth rate were generally similar between *discrete shifters* and *facultative shifters* (Figure B3), though were slightly higher for *discrete shifters* one and two years prior to the ontogenetic shift (OP = -1, -2) and the year prior to the maximal growth rate.

According to the GAMM results, straightline carapace length (SCL), ontogenetic position (OP), and nitrogen stable isotope ratios ($\delta^{15}N$) were significant predictors of the growth response, with ontogenetic position explaining the most growth variance of all tested covariates (adjusted $r^2 = 15.8$). However, overall explanatory power of the GAMMs was low (Table 3.2, Figure 3.4). The GAMM based on $\delta^{15}N$ values displayed a similar pattern to that presented by Avens et al. (2013)

(Figure 3.4d). Age was not a significant predictor of growth response, suggesting that growth rates and ontogenetic shifts are driven by size rather than age.

DISCUSSION

My assessments of juvenile loggerhead sea turtle growth dynamics with respect to an oceanic-to-neritic habitat shift show that there is a growth advantage to making this habitat shift. Annual growth rates generally peaked within one year of transition between habitats, but one third of turtles exhibited maximal growth rates prior to this transition and thus may deviate from what is predicted by ontogenetic niche theory (Figure 3.2; Werner and Gilliam 1984). This individual variation in the timing of maximal growth rate strongly influenced the perceived ontogenetic growth dynamics of juvenile loggerheads (Figure 3.5), and demonstrated the role individual effects may play in understanding sea turtle growth. Growth variance was higher fo *facultative shifters* when compared to *discrete shifters* (Figure 3.2), but size-at-age relationships and mean growth rates did not substantially differ between shifter groups (Figure 3.1, B3), likely limiting the influence of alternative life history patterns on size and time to sexual maturity. Excluding the year of observed maximal growth (± 1 OP), I found no evidenc for habitat-specific growth rates and no broad relationship between growth rate and foraging ecology metrics (e.g., Δgrowth rate, $\Delta\delta^{15}$N; Table 3.1, Figure 3.2-3.4), which suggests that the type or trophic level of prey may not be a good predictor of sea turtle growth.

Growth rates and size-at-age relationships observed in this study were comparable to those from other studies for juvenile loggerhead turtles in the Northwest Atlantic (Bjorndal et al. 2003, Braun-McNeill et al. 2008, Avens et al. 2013). I found that body size (SCL), ontogenetic position (OP), and growth increment-specific nitrogen isotope values ($\delta^{15}N$) were significant predictors of body size and best explained the growth response function in the GAMM models (Table 3.2, Figure 3.4). All response functions were distinctly nonlinear. For SCL, the growth response peaked at ~57 cm SCL, which falls within the range of body sizes typical of the known oceanic-to-neritic habitat shift (Bjorndal et al. 2000, Avens et al. 2013, Chapter 2) and is consistent with the observation of highest mean growth rates in the 50-59.9 cm SCL size class (Table 3.1). Ontogenetic position was the best predictor of sea turtle growth, with the inflection of the growth response occurring at the time of ontogenetic habitat shift (OP = 0). The shape of the response function suggested that juvenile growth rates may increase and then subsequently decrease over multiple years before and after an ontogenetic habitat shift (Figure 3.4c). However, examination of individual growth trajectories revealed this pattern was driven by individual variation in the timing of observed maximal growth rate (Figure 3.5). In fact, within turtles there was generally only a single year of high relative growth rate, which most commonly fell within one year (before or after) the ontogenetic habitat shift. (31/35 turtles). The relationship between the growth response and $\delta^{15}N$ was weakly significant, with the lowest growth rates at intermediate $\delta^{15}N$ values consistent with the transition between oceanic and neritic

habitats (Figure 3.4d, Chapter 2). Avens et al. (2013) suggested this pattern of decreasing growth response in oceanic $\delta^{15}N$ values (<12.0‰) and increasing growth response in neritic $\delta^{15}N$ values (>12.0‰) may indicate that growth limitations signal this habitat shift (Bolten 2003, Avens et al. 2013). However, because a pre-ontogenetic shift decline in growth was not evident in the individual growth trajectories of these turtles (Figure 3.2, 3.5), it is unlikely that growth limitations are mediating this transition.

Ontogenetic niche theory predicts individuals should select habitats that allow for maximal growth dependent on habitat-specific mortality rate. When size-specific predation risk is similar between two habitats ontogenetic habitat shifts should occur to maximize growth rates, whereas when mortality risk differs between habitats individuals should seek to minimize the ratio of mortality risk to growth rate (Werner and Gilliam 1984). Unfortunately, habitat-specific mortality rates and predation risks are not well understood for sea turtles. Predation risk scales with body size so that once individuals reach sexual maturity they have escaped in size from most natural predators (Musick and Limpus 1997, Heithaus 2013). However, human induced mortality through interactions with fisheries, recreational boats, and debris is a persistent threat globally to sea turtles and can disproportionally impact turtles in certain habitats and stage classes (Heppell et al. 2002, Lewison et al. 2014). As both of these classes of stressors likely vary between habitats (e.g., oceanic vs. neritic; Bolten et al. 2011), habitat-specific estimates of natural and anthropogenic mortality are needed before studies can robustly assess

how such factors may influence growth and habitat shifts (National Research Council 2010).

Even in the absence of information on habitat-specific mortality rates foraging in neritic habitats might be expected to infer a growth advantage. Peckham et al. (2011) qualitatively demonstrated that the energy density of Pacific juvenile loggerhead turtle diets is higher in neritic versus oceanic habitats. This apparent advantage was further enhanced by the fact that these turtles traveled at slower speeds, which likely reduced foraging energy expenditure, and occupied habitats with higher temperatures, which can enhance energy assimilation (Bjorndal 1980, Dunham et al. 1989), than their oceanic conspecific (Peckham et al. 2011). Their hypothesis was supported by Snover et al. (2010), which found that juvenile growth was higher in neritic habitats following an ontogenetic shift relative to growth rates in oceanic habitats. In the present study, however, I found that this growth advantage to be short-term and that growth rates returned to pre-ontogenetic shift levels within two years after transition (Figure 3.2, 3.5). This contrast is likely driven by greater resolution of the timing of transition between habitats and larger sample sizes in the present study, particularly for growth rates in neritic habitats (OP > 0; Figure 3.5).

Additionally, growth rates did not peak the year of transition for all turtles. A large proportion of turtles (23/35) experienced observed maximal growth rates a year or more before or after the ontogenetic habitat shift (defined as $\Delta\delta^{15}N \geq 3.0‰$; see 'Appendix A'). A delay in growth response might be expected since turtles must

adjust their foraging strategies once they move into neritic habitats. However, increased growth prior to this ontogenetic shift was unexpected (14/35 turtles). This may be due in part to sampling, measurement, or classification error coupled with a dearth of knowledge on the time scale over which nitrogen isotopes are deposited in bone tissue. Still, there is likely a suite of biological and environmental factors independent of this habitat shift that strongly influences juvenile growth (Snover 2008). First, turtles may occupy habitats with disparate resources and conditions. Resource patches in oceanic habitats (e.g., sargassum mats) are inherently patchy and oceanic juveniles are known to select habitats that provide a thermal benefit and refuge that may enhance growth (Mansfield et al. 2014). Differential patch use under different levels of predation risk may also allow some turtles to achieve high growth prior to an ontogenetic shift. Second, individual metabolic rates may vary, which may lead turtles to respond differently to their environment. Turtles with relatively high metabolic rates would require disproportionately more food resources to maintain growth rates as compared to conspecifics with lower metabolic rates. Therefore, movement into thermally optimal or resource abundant patches may infer a growth advantage on individuals with lower metabolic requirements. Lastly, much like the timing of seasonal migrations in birds and large mammals, juvenile sea turtles may cue in to physiological, geophysical, or oceanographic information to guide these habitat use decisions. Loggerhead sea turtles possess a geomagnetic 'map' that is used to

circumnavigate the North Atlantic (Lohmann et al. 2007), which may be used to guide movements between oceanic and neritic habitats.

Sequential analysis of annual humerus bone growth increments allowed for the comparison of growth patterns between individuals displaying alternative life history patterns (*discrete shifters* vs. *facultative shifters*). Previous studies have suggested that alternative life history patterns may affect individual and population growth (Hatase et al. 2010, Peckham et al. 2011, Chapter 2). However, results herein are mixed. Size-at-age relationships and size-class specific growth rates were similar between life history patterns (Table 3.1, Figure 3.1b), with differences in size-class specific growth rates most likely driven by small sample size. Turtles exhibiting alternative life history patterns also displayed similar peaks and ranges in growth rate (Figure 3.2). Within turtles growth variance was higher for *facultative shifters* as compared to *discrete shifters*, which indicates these turtles experience more boom-and-bust periods in growth. In addition, growth rates differed slightly by ontogenetic position, with growth rates for *facultative shifters* being lower on average than those of *discrete shifters* one to two years prior to the ontogenetic habitat shift (Figure B3). Differences in growth variance and growth rates by ontogenetic position may ultimately be driven by the interaction of multiple environment factors, such as prey availability, patch use, predation risk, and temperature. More fine scale life history characterizations through trace element or additional stable isotope methods may aid in our understanding of what contributes to this growth variation. Though there are some differences in the growth

trajectories between these life history groups, their cumulative affect on population dynamics, specifically time to sexual maturity, may be minimal as size-at-age relationships and growth dynamics are similar and there is no difference in timing of ontogenetic habitat shift (Chapter 2, Table 2.1).

I demonstrate here the value of combining skeletochronological and growth increment-specific isotope analyses to understand sea turtle growth variation and life history. My results suggest that growth patterns are similar between individuals with alternative life histories. Growth is a key factor in determining time to sexual maturity; thus, it is unlikely that the presence of these alternative life history patterns strongly influence this life history parameter. However, mortality risk is an important factor that can guide individual behavior (Werner and Gilliam 1984), and these alternative life history patterns could be associated with different survival probabilities. Therefore, data on size- and habitat-specific predation risk and mortality rates would aid in understanding the factors that drive these habitat shifts. It is critical that we gain a full understanding of the mechanisms driving ontogenetic shifts and growth variance in loggerheads so that we can properly manage and conserve this species into the future.

TABLES AND FIGURES

Table 3.1. Mean annual growth rates by size class and life history pattern. Growth rates are back-calculated usin skeletochronology. SCL = straightline carapace length, All = all turtles (*n* = 38), Discrete = *discrete shifters* (*n* = 23), Facultative = *facultative shifters* (*n* = 15), * = size classes where growth rates statistically differed betwee *discrete* and *facultative shifters*. Significance level for differences among size classes and life history patterns was $P < 0.05$.

Size class (SCL in cm)	SCL growth rates (Mean ± SD [*sample size*])			Significantly different from size classes		
	All	Discrete	Facultative	All	Discrete	Facultative
20 (20-29.9)	2.5 ± 1.5 (*4*)	4.2 (*1*)	2.0 ± 1.2 (*3*)	-	-	70
30 (30-39.9)*	2.5 ± 1.2 (*65*)	2.1 ± 1.0 (*40*)	3.0 ± 1.3 (*25*)	50	40, 50, 60	60, 70
40 (40-49.9)	2.8 ± 1.5 (*117*)	2.8 ± 1.3 (*69*)	2.7 ± 1.7 (*48*)	50	30, 50, 60	60, 70
50 (50-59.9)	3.5 ± 1.9 (*98*)	3.8 ± 2.0 (*53*)	3.1 ± 1.7 (*45*)	30, 40, 50, 70	30, 40, 70	60, 70
60 (60-69.9)*	2.8 ± 1.5 (*42*)	3.3 ± 1.3 (*26*)	2.0 ± 1.4 (*16*)	50	30, 40, 70	30, 40, 50, 70
70 (70-79.9)*	2.7 ± 1.6 (*19*)	2.2 ± 1.1 (*16*)	5.5 ± 1.2 (*3*)	50	50, 60	20, 30, 40, 50, 60
80 (80-89.9)	2.5 ± 1.1 (*6*)	2.5 ± 1.2 (*5*)	2.5 (*1*)	-	-	-

Table 3.2. Statistical output from generalized additive mixed models (GAMMs) used to analyze the influence of covariates on growth response for all back-calculated growth increments. *n* = sample size, AIC = Akaike's information criterion, SCL = straightline carapace length, OP = Ontogenetic Position, Edf = estimated degrees of freedom.

Model	Adjusted r^2	AIC	Variable	Edf	*F*	Prob(F)
$GAMM_{SCL}$ (*n* = 350)	5.1	525.9	SCL (cm)	3.02	4.43	**0.004**
$GAMM_{Age}$ (*n* = 350)	1.9	533.9	Age (yr)	2.34	1.94	0.136
$GAMM_{\delta 15N}$ (*n* = 280)	3.0	357.6	δ^{15}N (‰)	3.13	4.57	**0.003**
$GAMM_{OP}$ (*n* = 350)	15.8	499.8	OP	5.00	9.28	**<0.001**

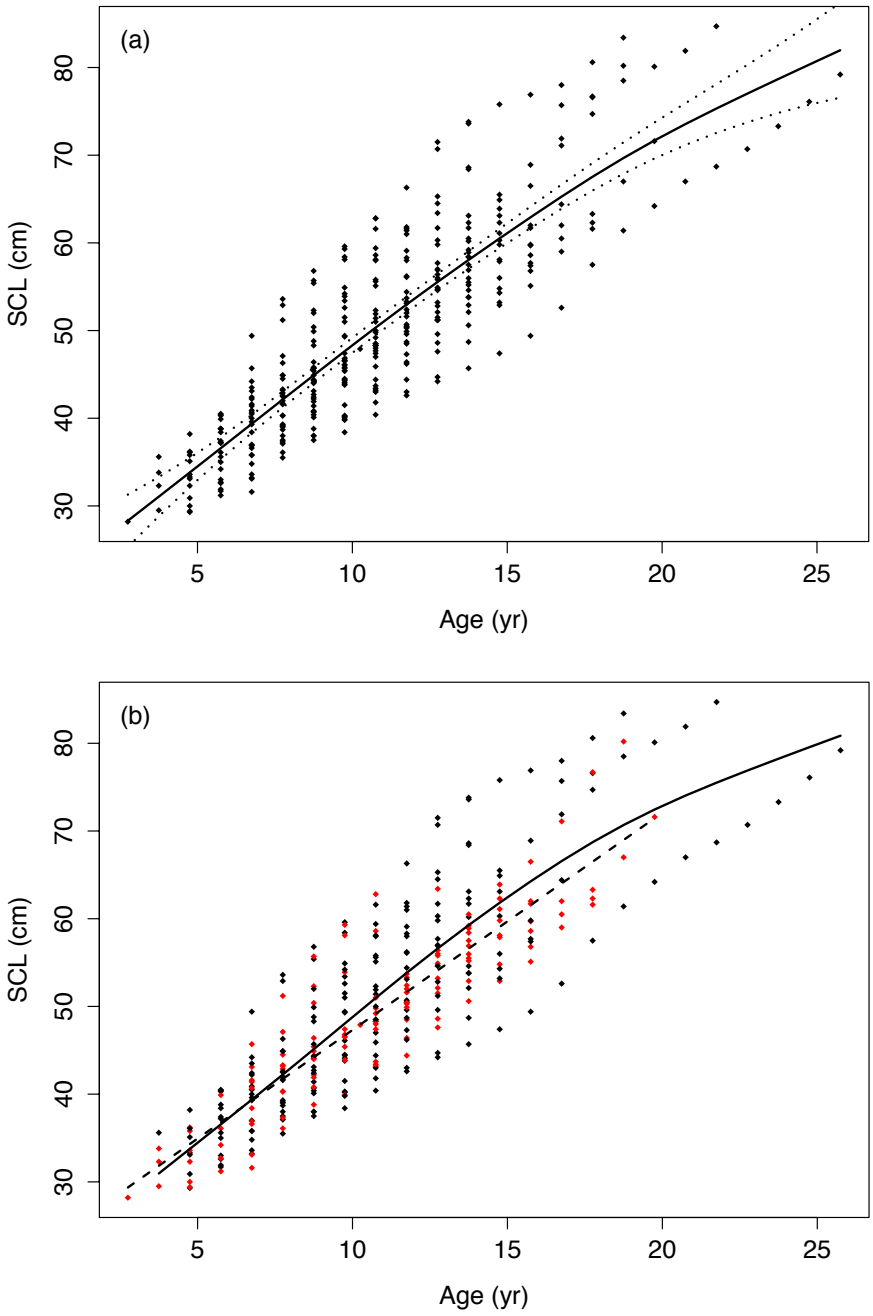

Figure 3.1. Smoothing spline models fit to (a) all back-calculated SCL-at-age data (*n* = 350) and (b) life history pattern-specific back-calculated SCL-at-age data (*Discrete shifters*, black points, solid line, *n* = 210; *facultative shifters*, red points, dashed line, *n* = 140). Dotted lines (a) denote 95% confidence interval.

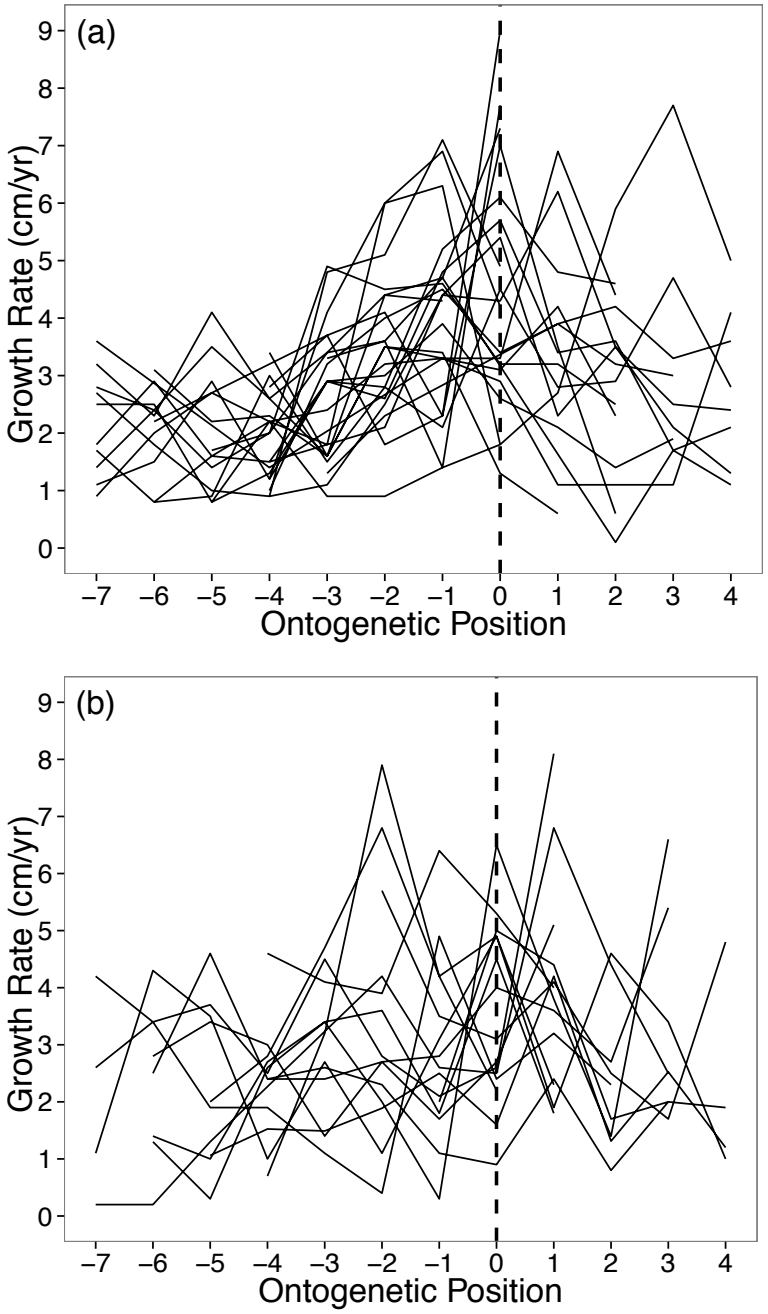

Figure 3.2. Individual loggerhead sea turtle growth trajectories (solid lines) by life history pattern centered on year of ontogenetic shift (ontogenetic position = 0). (a) *Discrete shifters* (*n* = 23). (b) *Facultative shifters* (*n* = 15). Vertical dashed lines designate the year of an ontogenetic shift based on $\Delta\delta^{15}N$ values.

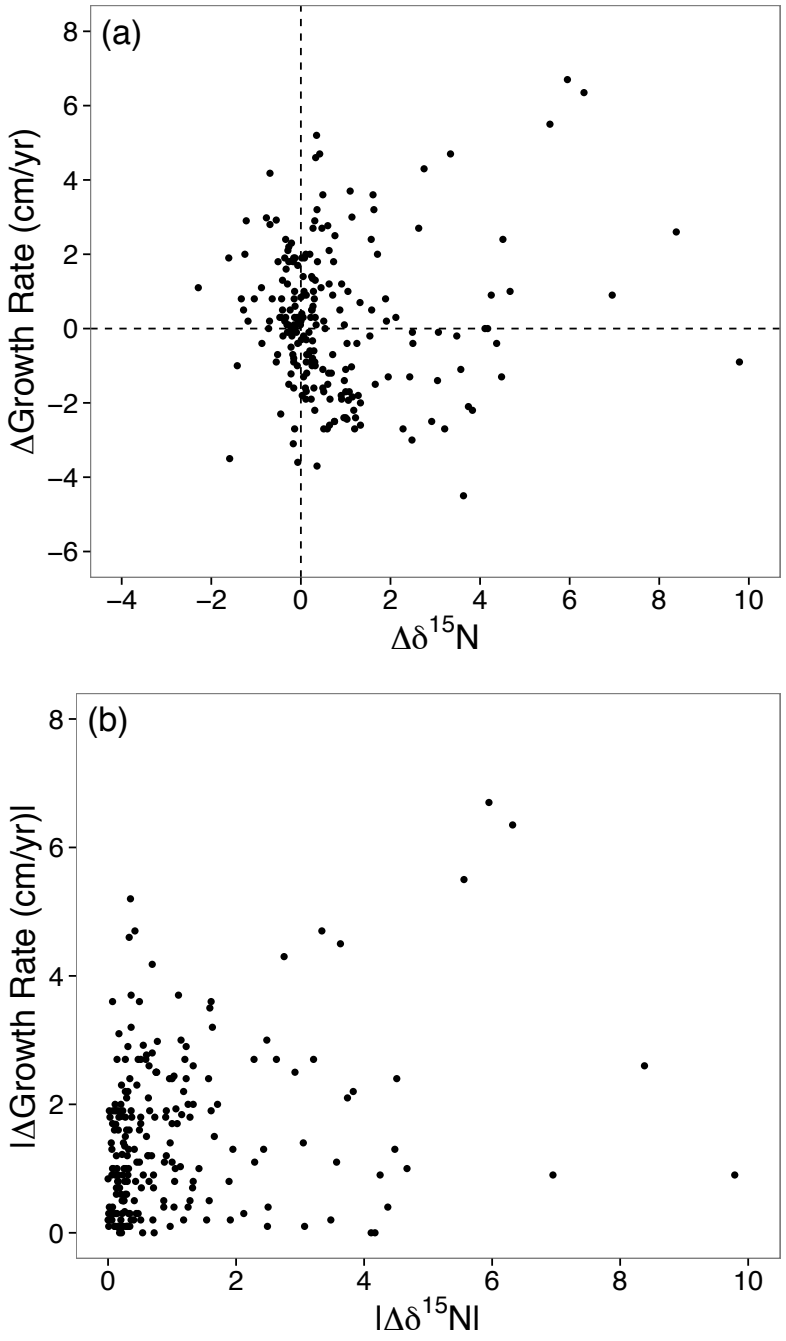

Figure 3.3. Comparison of $\Delta\delta^{15}N$ and Δgrowth rate of annual growth increments (*n* = 350) from juvenile loggerhead sea turtles (*n* = 38). (a) Real change. (b) Absolute change. Dashed lines represent no change.

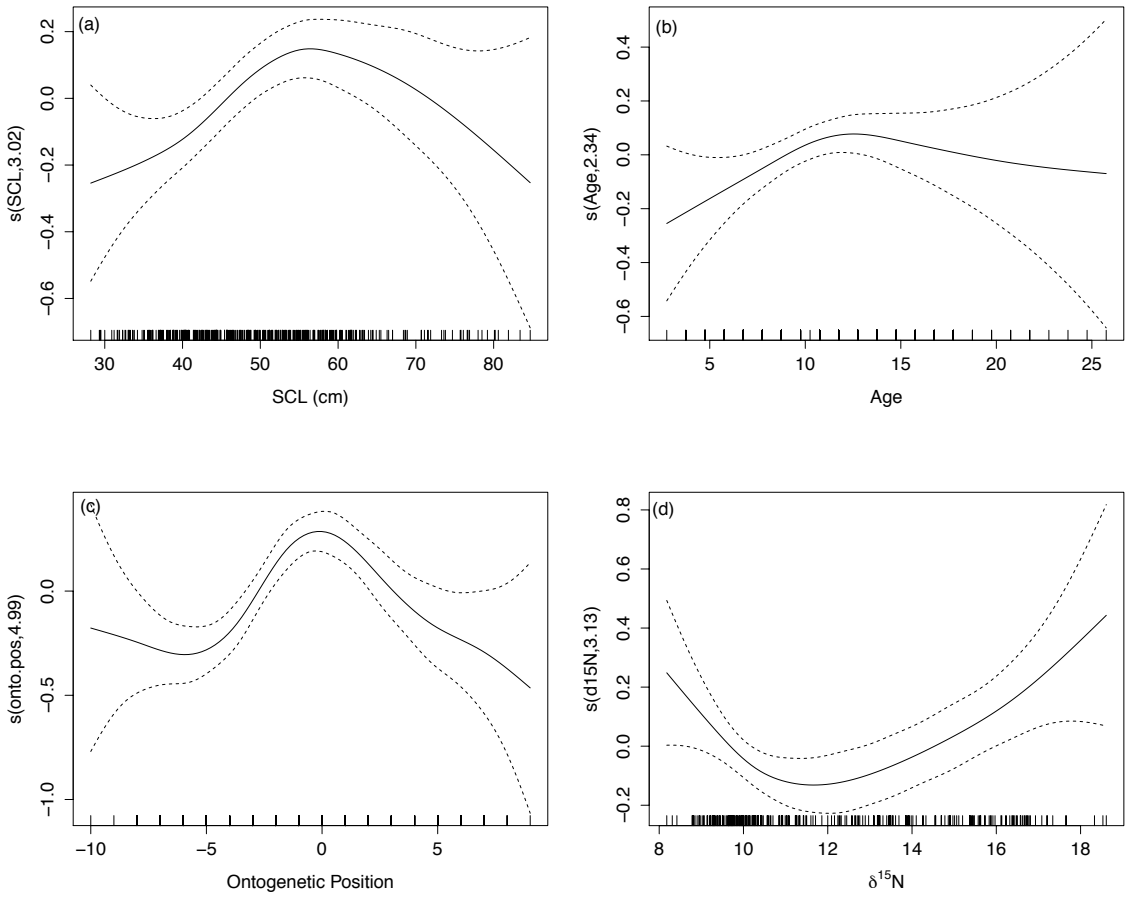

Figure 3.4. Estimated smoothing curves for the generalized additive mixed models (GAMMs) summarized in Table 3.2. (a) $GAMM_{SCL}$, (b) $GAMM_{Age}$ (c) $GAMM_{OP}$, (d $GAMM_{\delta 15N}$. Models include (a-c) all back-calculated growth rates (n = 350) or (d back-calculated growth rates for which growth increment-specific nitrogen stable isotope ($\delta^{15}N$) data were available (n = 280). Solid lines are the cubic smoothing spline fits for each covariate and dashed lines are 95% confidence intervals. SCL = mean straightline carapace length, Age = age estimated through skeletochronology, Ontogenetic Position = year before and after ontogenetic shift.

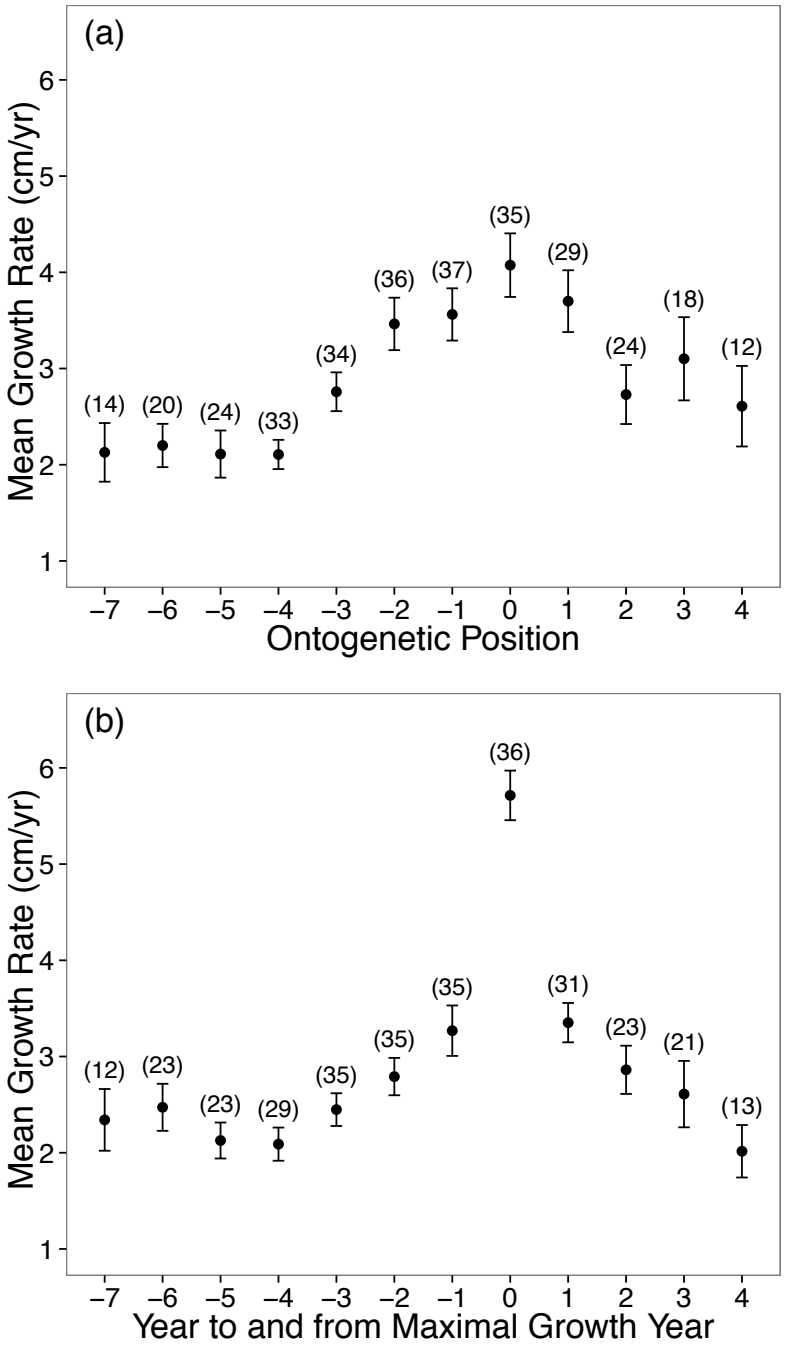

Figure 3.5. Mean annual growth rates with standard error bars for all turtles by year to and from (a) ontogenetic shift (i.e., ontogenetic position) and (b) turtle-specific maximal growth rate. Numbers in parentheses are sample sizes.

CHAPTER 4: GENERAL CONCLUSION

My research investigated the application of complementary skeletochronological and stable isotope analyses to the characterization of alternative life history patterns and ontogenetic growth dynamics of juvenile loggerhead sea turtles (*Caretta caretta*). My results demonstrated the utility of these methods to quantifying the timing, duration, and prevalence of alternative life history patterns in sea turtles, and exemplified the value of stranded marine organisms in life history parameter estimation. Additionally, these results provided novel insights into the role among-individual variation in growth may play in shaping our understanding of sea turtle growth rates.

Sequential isotopic analysis of loggerhead humerus bones allowed for the detection of an oceanic-to-neritic habitat shift, suggested by a marked increase in nitrogen isotope ratios ($\delta^{15}N$) within the bone tissue. Results of this study indicate that the observed $\delta^{15}N$ patterns within turtles were most likely driven by isotopic baseline differences (versus differences in turtle foraging trophic level), which are conserved up food webs and ultimately vary as function of the biological processes moving nitrogen through a system (Cherel and Hobson 2007, Montoya 2007). Areas that are highly productive (e.g., estuaries, salt marshes) and where ^{15}N is discriminated against through denitrification processes (e.g., continental shelf sediments) are generally enriched in baseline $\delta^{15}N$ values (Fennel et al. 2006, Montoya 2007). Meanwhile, areas of high N_2-fixation, such as the oligotrophic Sargasso Sea and tropical Atlantic (Montoya et al. 2002, Mompean et al. 2013), tend

to have low baseline $\delta^{15}N$ values. Loggerhead sea turtle undoubtedly move between habitats with these alternative oceanographic regimes throughout their transoceanic migrations that drive the $\delta^{15}N$ patterns of turtles, prey, and zooplankton observed in this study. Nitrogen isotope ratios are classically assumed to reflect trophic relationships. However, these findings highlight the potential value of $\delta^{15}N$ values to also study movement patterns for organisms in the Northwest Atlantic Ocean. Future research is needed to address the isotopic assumptions made in this study. Bone tissue is known to have turnover rates on the order of years, but I assume increment-specific isotope values reflect diet and habitat use within the same growth year due to the annual nature of bone deposition for loggerheads. Feeding studies in aquaria may provide a means of testing this assumption and those related to diet-tissue discrimination in sea turtles.

I presented some of the first estimates of the duration and prevalence of facultative ontogenetic shifts in loggerhead sea turtles. Previous studies found facultative shift duration of up to three years, though assessments were largely hindered by satellite tag failure and loss (McClellan and Read 2007, Mansfield et al. 2009). Estimated shift durations observed in this study were largely consistent with previous estimates as most turtles completed ontogenetic shifts in three years or less, though three turtles took between four and eight years, to complete this transition. Prevalence estimates of facultative ontogenetic shifts herein were also largely consistent with previous studies. Because current methods for studying sea turtle habitat shifts rely on capture (dead or alive) in nearshore waters, I propose

these estimates may be biased low. Turtles spending extended periods of time offshore (e.g., months, years) and only temporarily returning to nearshore habitats would be observed and tagged less often than turtles resident in nearshore habitats. In addition, turtles satellite tagged upon completion of an extended transition would exhibit migrations indistinguishable from turtles that did not undergo a facultative ontogenetic shift. In studies relying on stranded turtles, such as here, sampling is also biased towards turtles that die in nearshore waters and strand on beaches. It is not well understood how long dead turtles float in the ocean before sinking. Still, it is unlikely that the bodies of turtles that die in oceanic habitats ultimately return to nearshore waters. Satellite telemetry studies and collection of dead turtles in oceanic habitats would aid in exploring these hypotheses and biases associated with working with stranded turtle data.

Facultative ontogenetic shifts may have large implications for the successful management and conservation of this species. If turtles return to offshore habitats following an initial transition to nearshore habitats, length-frequency analyses in nearshore habitats may not be accurate predictors of population size of certain size classes. Altered survival probabilities associated with extended habitat transitions could also influence population growth. Historic population declines of primary loggerhead prey in neritic habitats (e.g., horseshoe crabs, blue crabs) have resulted in increased utilization of fishery bycatch discards as a dietary resource (Seney and Musick 2007). 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,

with two displaying the highest growth increment-specific $\delta^{15}N$ values of all turtles (18.61‰, 18.92‰). Increased interaction with fisheries in search of fish discard or catch could result in lowered survival probabilities overall for turtles i nearshore versus offshore habitats. This may then lead to the increased contribution of turtles making facultative ontogenetic shifts to population growth over time.

Growth rates and ontogenetic growth patterns observed in this study are consistent with those reported in the literature and indicated there is a short-term peak in growth around the time of the oceanic-to-neritic habitat shift in juvenile loggerheads (see Avens et al. 2013 for review of growth rates, Snover et al. 2010 for growth patterns). That there can be a growth advantage to making an ontogenetic habitat shift has been demonstrated previously in other studies and is broadly predicted by ontogenetic niche theory (Werner and Gilliam 1984, Werner and Hall 1988, Dahlgren and Eggleston 2000). Individual variation in the timing of this increased growth relative to the ontogenetic shift adds a new facet to our understanding of the growth dynamics of this species. Physiological, environmental, and ecological factors may ultimately interact to influence growth and estimates of size and age at transition to nearshore habitats in sea turtles.

Sampling and measurement error inherent to this study are important considerations when interpreting my results. Although best efforts were made to sample individual growth increments for stable isotopes, there was undoubtedly some sampling and isotopic measurement error (0.10‰ for $\delta^{15}N$) that may have impacted life history pattern classification and the designation of year of transition

between habitats, particularly for *facultative shifters*. This coupled with error in the growth increment diameter to back-calculated SCL estimate relationship (0.2 – 0.3 cm; Snover et al. 2007), may in turn affect estimates of size at and duration of facultative habitat shifts, and the ontogenetic growth dynamics of this species. Still, if I assume measurement error is similar across turtles it is unlikely that my analyses were inherently biased. Furthermore, the use of the raw data to guide the selection of the $\Delta\delta^{15}N$ threshold for life history classification allowed for the objective assignment of individuals to life history pattern groups. Ultimately, this classification would have been similar had I used another reasonable neighboring threshold (Figure B1).

The methods employed in this study allowed for the collection of a long-term data series from individuals that would have been difficult, if not impossible, to obtain via traditional sea turtle tracking methods (e.g., satellite telemetry, stable isotope analyses of soft tissues). While these methods ultimately tradeoff high spatial accuracy and direct tissue-habitat linkages with large sample sizes and assumptions related to isotopic turnover and diet-tissue linkages, they potentially allow for the rapid assessment of broad ontogenetic changes in life history that can be used to directly inform population models, management, and conservation. This may prove particularly critical in the study of cryptic species and life stages, as some may never be logistically feasible to track directly, such as the oceanic-to-neritic transition in loggerhead sea turtles.

BIBLIOGRAPHY

Angerbjörn, A., P. Börjesson, and K. Brandberg. 2006. Stable isotope analysis of harbour porpoises and their prey from the Baltic and Kattegat/Skagerrak Seas. Marine Biology Research 2:411–419.

Avens, L., J. Braun-McNeill, S. Epperly, and K. J. Lohmann. 2003. Site fidelity and homing behavior in juvenile loggerhead sea turtles (*Caretta caretta*). Marine Biology 143:211–220.

Avens, L., L. Goshe, M. Pajuelo, K. Bjorndal, B. MacDonald, G. Lemons, A. Bolten, and J. Seminoff. 2013. Complementary skeletochronology and stable isotop analyses offer new insight into juvenile loggerhead sea turtle oceanic stage duration and growth dynamics. Marine Ecology Progress Series 491:235–251.

Avens, L., L. R. Goshe, C. A. Harms, E. T. Anderson, A. G. Hall, W. M. Cluse, M. H. Godfrey, J. Braun-McNeill, B. Stacy, R. Bailey, and M. M. Lamont. 2012. Population characteristics, age structure, and growth dynamics of neritic juvenile green turtles in the northeastern Gulf of Mexico. Marine Ecolog Progress Series 458:213–229.

Best, P. B., and D. M. Schell. 1996. Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. Marine Biology 124:483–494.

Bjorndal, K. A. 1980. Nutrition and grazing behavior of the green turtle *Chelonia mydas*. Marine Biology 56:147–154.

Bjorndal, K. A. 1997. Foraging ecology and nutrition of sea turtles. Pages 199–231 *in* P. L. Lutz and J. A. Musick, editors. The biology of sea turtles. CRC Press, Boca Raton, FL.

Bjorndal, K. A., A. B. Bolten, T. Dellinger, C. Delgado, and H. R. Martins. 2003. Compensatory growth in oceanic loggerhead sea turtles: response to a stochastic environment. Ecology 84:1237–1249.

Bjorndal, K. A., A. B. Bolten, and H. R. Martins. 2000. Somatic growth model of juvenile loggerhead sea turtles *Caretta caretta*: duration of pelagic stage. Marine Ecology Progress Series 202:265–272.

Bolnick, D. I., R. Svanbäck, J. A. Fordyce, L. H. Yang, J. M. Davis, C. D. Hulsey, and M. L. Forister. 2003. The ecology of individuals: incidence and implications of individual specialization. The American Naturalist 161:1–28.

Bolten, A. B. 2003. Active swimmers–passive drifters: the oceanic juvenile stage of loggerheads in the Atlantic system. Pages 63–78 *in* A. B. Bolten and B. E. Witherington, editors. Loggerhead Sea Turtles. Smithsonian Books, Washington, D.C.

Bolten, A. B., L. B. Crowder, M. G. Dodd, S. L. MacPherson, J. A. Musick, B. A. Schroeder, B. E. Witherington, K. J. Long, and M. L. Snover. 2011. Quantifying multiple threats to endangered species: an example from loggerhead sea turtles. Frontiers in Ecology and the Environment 9:295–301.

Braun-McNeill, J., S. P. Epperly, L. Avens, M. L. Snover, and J. C. Taylor. 2008. Growth rates of loggerhead sea turtles (*Caretta caretta*) from the western North Atlantic. Herpetological Conservation and Biology 3:273–281.

Broderick, A. C., M. S. Coyne, W. J. Fuller, F. Glen, and B. J. Godley. 2007. Fidelity and over-wintering of sea turtles. Proceedings of the Royal Society B: Biological Sciences 274:1533–1539.

Bucci, J. P., W. J. Showers, S. Rebach, D. DeMaster, and B. Genna. 2007. Stable isotope analyses ($\delta^{15}N$ and $\delta^{13}C$) of the trophic relationships of *Callinectes sapidus* in two North Carolina estuaries. Estuaries and Coasts 30:1049–1059.

Buchheister, A., and R. J. Latour. 2011. Trophic ecology of Summer Flounder in lower Chesapeake Bay inferred from stomach content and stable isotope analyses. Transactions of the American Fisheries Society 140:1240–1254.

Burton, R. K., and P. L. Koch. 1999. Isotopic tracking of foraging and long-distance migration in northeastern Pacific pinnipeds. Oecologia 119:578–585.

Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings o the National Academy of Sciences of the United States of America 93:10844–10847.

Casale, P., G. Abbate, D. Freggi, N. Conte, M. Oliverio, and R. Argano. 2008. Foraging ecology of loggerhead sea turtles *Caretta caretta* in the central Mediterranean Sea: evidence for a relaxed life history model. Marine Ecology Progress Series 372:265–276.

Ceriani, S. A., J. D. Roth, C. R. Sasso, C. M. McClellan, M. C. James, R. E. Haas, R. J. Smolowitz, D. R. Evans, D. S. Addison, D. A. Bagley, L. M. Ehrhart, and J. F. Weishampel. 2014. Modeling and mapping isotopic patterns in the Northwest Atlantic derived from loggerhead sea turtles. Ecosphere 5:1-24

Chaloupka, M., and J. A. Musick. 1997. Age, growth, and population dynamics. Pages 233–376 *in* P. L. Lutz and J. A. Musick, editors. The biology of sea turtles. CRC Press, Boca Raton, FL.

Cherel, Y., and K. A. Hobson. 2007. Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. Marine Ecology Progress Series 329:281–287.

Crouse, D. T. 1999. The consequences of delayed maturity in a human-dominated world. Pages 195–202 *in* J. A. Musick, editor. Life in the slow lane: ecology and conservation of long-lived marine animals. American Fisheries Society Symposia 23, Bethesda, MD.

Crowder, L. B., D. T. Crouse, S. S. Heppell, and T. H. Martin. 1994. Predicting the impact of turtle excluder devices on Loggerhead sea turtle populations. Ecological Applications 4:437–445.

Dahlgren, C. P., and D. B. Eggleston. 2000. Ecological processes underlying ontogenetic habitat shifts in a coral reef fish. Ecology 81:2227–2240.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45:341–351.

Dittel, A. I., C. E. Epifanio, and M. L. Fogel. 2006. Trophic relationships of juvenile blue crabs (*Callinectes sapidus*) in estuarine habitats. Hydrobiologia 568:379–390.

Dmitriew, C. M. 2011. The evolution of growth trajectories: what limits growth rate? Biological Reviews 86:97–116.

Dodge, K. L., J. M. Logan, and M. E. Lutcavage. 2011. Foraging ecology of leatherback sea turtles in the Western North Atlantic determined through multi-tissue stable isotope analyses. Marine Biology 158:2813–2824.

Dormann, C. F., J. Elith, S. Bacher, C. Buchmann, G. Carl, G. Carré, J. R. G. Marquéz, B. Gruber, B. Lafourcade, P. J. Leitão, T. Münkemüller, C. McClean, P. E. Osborne, B. Reineking, B. Schröder, A. K. Skidmore, D. Zurell, and S. Lautenbach. 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography 36:27–46.

Dunham, A. E., B. W. Grant, and K. L. Overall. 1989. Interfaces between biophysical and physiological ecology and the population ecology of terrestrial vertebrate ectotherms. Physiological Zoology 62:335–355.

Eggleston, D. B. 1995. Recruitment in Nassau grouper *Epinephelus striatus*: post-settlement abundance, microhabitat features, and ontogenetic habitat shifts. Marine Ecology Progress Series 124:9–22.

Epperly, S. P., J. Braun-McNeill, and P. M. Richards. 2007. Trends in catch rates of sea turtles in North Carolina, USA. Endangered Species Research 3:283–293.

Estrada, J. A., A. N. Rice, M. E. Lutcavage, and G. B. Skomal. 2003. Predicting trophic position in sharks of the north-west Atlantic Ocean using stable isotope analysis. Journal of the Marine Biological Association of the UK 83:1347–1350.

Estrada, J. A., A. N. Rice, L. J. Natanson, and G. B. Skomal. 2006. Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. Ecology 87:829–834.

Fantle, M. S., A. I. Dittel, S. M. Schwalm, C. E. Epifanio, and M. L. Fogel. 1999. A food web analysis of the juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. Oecologia 120:416–426.

Fennel, K., J. Wilkin, J. Levin, J. Moisan, J. O'Reilly, and D. Haidvogel. 2006. Nitrogen cycling in the Middle Atlantic Bight: Results from a three-dimensional model and implications for the North Atlantic nitrogen budget. Global Biogeochemical Cycles 20:GB3007.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnology and Oceanography 33:1182–1190.

Fry, B., and R. B. Quinones. 1994. Biomass spectra and stable isotope indicators of trophic level in zooplankton of the northwest Atlantic. Marine Ecology Progress Series 112:201–204.

Goshe, L. R., L. Avens, J. Bybee, and A. A. Hohn. 2009. An evaluation of histological techniques used in skeletochronological age estimation of sea turtles. Chelonian Conservation and Biology 8:217–222.

Grol, M. G. G., I. Nagelkerken, A. L. Rypel, and C. A. Layman. 2011. Simple ecological trade-offs give rise to emergent cross-ecosystem distributions of a coral reef fish. Oecologia 165:79–88.

Grol, M. G. G., A. L. Rypel, and I. Nagelkerken. 2014. Growth potential and predation risk drive ontogenetic shifts among nursery habitats in a coral reef fish. Marine Ecology Progress Series 502:229–244.

Haramis, G. M., W. A. Link, P. C. Osenton, D. B. Carter, R. G. Weber, N. A. Clark, M. A. Teece, and D. S. Mizrahi. 2007. Stable isotope and pen feeding trial studies confirm the value of horseshoe crab *Limulus polyphemus* eggs to sprin migrant shorebirds in Delaware Bay. Journal of Avian Biology 38:367–376.

Hatase, H., K. Omuta, and K. Tsukamoto. 2010. Oceanic residents, neritic migrants: a possible mechanism underlying foraging dichotomy in adult female loggerhead turtles (*Caretta caretta*). Marine Biology 157:1337–1342.

Hatase, H., K. Sato, M. Yamaguchi, K. Takahashi, and K. Tsukamoto. 2006. Individual variation in feeding habitat use by adult female green sea turtles (*Chelonia mydas*): are they obligately neritic herbivores? Oecologia 149:52–64.

Hatase, H., N. Takai, Y. Matsuzawa, W. Sakamoto, K. Omuta, K. Goto, N. Arai, and T. Fujiwara. 2002. Size-related differences in feeding habitat use of adult female loggerhead turtles *Caretta caretta* around Japan determined by stable isotope analyses and satellite telemetry. Marine Ecology Progress Series 233:273–281.

Hawkes, L. A., A. C. Broderick, M. S. Coyne, M. H. Godfrey, L.-F. Lopez-Jurado, P. Lopez-Suarez, S. E. Merino, N. Varo-Cruz, and B. J. Godley. 2006. Phenotypically linked dichotomy in sea turtle foraging requires multiple conservation approaches. Current Biology 16:990–995.

Heithaus, M. R. 2013. Predators, prey, and the ecological roles of sea turtles. Pages 249–284 *in* J. Wyneken, K. J. Lohmann, and J. A. Musick, editors. The biology of sea turtles. CRC Press, Boca Raton, FL.

Heppell, S. S., L. B. Crowder, D. T. Crouse, S. P. Epperly, and N. B. Frazer. 2002. Population models for Atlantic loggerheads: past, present and future. Pages 255–273 *in* A. B. Bolten and B. E. Witherington, editors. Loggerhead Sea Turtles. Smithsonian Institution Press, Washington, D.C.

Hobson, K. A. 2007. Isotopic tracking of migrant wildlife. Pages 155–175 *in* R. H. Michener and K. Lajtha, editors. Stable isotopes in ecology and environmental science. Blackwell, Malden, MA.

Jones, D. S., D. F. Williams, and M. A. Arthur. 1983. Growth history and ecology of the Atlantic surf clam, *Spisula solidissima* (Dillwyn), as revealed by stable isotopes and annual shell increments. Journal of Experimental Marine Biology and Ecology 73:225–242.

Kaufman, L., and P. J. Rousseeuw. 1990. Finding groups in data: an introduction to cluster analysis. Wiley, New York, NY.

Kimirei, I. A., I. Nagelkerken, M. Trommelen, P. Blankers, N. van Hoytema, D. Hoeijmakers, C. M. Huijbers, Y. D. Mgaya, and A. L. Rypel. 2013. What drives ontogenetic niche shifts of fishes in coral reef ecosystems? Ecosystems 16:783–796.

Knoff, A. J., S. A. Macko, and R. M. Erwin. 2001. Diets of nesting laughing gulls (*Larus atricilla*) at the Virginia Coast Reserve: observations from stable isotope analysis. Isotopes in environmental and health studies 37:67–88.

Lee-Thorp, J. A., J. C. Sealy, and N. J. van der Merwe. 1989. Stable carbon isotope ratio differences between bone collagen and bone apatite, and their relationship to diet. Journal of Archaeological Science 16:585–599.

Lewison, R. L., L. B. Crowder, B. P. Wallace, J. E. Moore, T. Cox, R. Zydelis, S. McDonald, A. DiMatteo, D. C. Dunn, C. Y. Kot, R. Bjorkland, S. Kelez, C. Soykan, K. R. Stewart, M. Sims, A. Boustany, A. J. Read, P. Halpin, W. J. Nichols, and C. Safina. 2014. Global patterns of marine mammal, seabird, and sea turtle bycatch reveal taxa-specific and cumulative megafauna hotspots. Proceedings of the National Academy of Sciences 111:5271–5276.

Logan, J. 2009. Tracking diet and movement of Atlantic bluefin tuna (*Thunnus thynnus*) using carbon and nitrogen stable isotopes. University of New Hampshire.

Lohmann, K. J., C. M. F. Lohman, and N. F. Putman. 2007. Magnetic maps in animals: nature's GPS. Journal of Experimental Biology 210:3697–3705.

Lutcavage, M., and J. A. Musick. 1985. Aspects of the biology of sea turtles in Virginia. Copeia 1985:449–456.

Maechler, M., P. Rousseeuw, A. Struyf, M. Hubert, and K. Hornik. 2013. cluster: cluster analysis basics and extensions. R Package verison 1.14.4.

Mansfield, K. L., V. S. Saba, J. A. Keinath, and J. A. Musick. 2009. Satellite tracking reveals a dichotomy in migration strategies among juvenile loggerhead turtles in the Northwest Atlantic. Marine Biology 156:2555–2570.

Mansfield, K. L., J. Wyneken, W. P. Porter, and J. Luo. 2014. First satellite tracks of neonate sea turtles redefine the "lost years" oceanic niche. Proceedings of the Royal Society B: Biological Sciences 281:20133039–20133039.

McClellan, C. M., J. Braun-McNeill, L. Avens, B. P. Wallace, and A. J. Read. 2010. Stable isotopes confirm a foraging dichotomy in juvenile loggerhead sea turtles. Journal of Experimental Marine Biology and Ecology 387:44–51.

McClellan, C. M., and A. J. Read. 2007. Complexity and variation in loggerhead sea turtle life history. Biology Letters 3:592–594.

McCormick, M. I. 1999. Delayed metamorphosis of a tropical reef fish (*Acanthurus triostegus*): a field experiment. Marine Ecology Progress Series 176:25-38.

McKinney, R. A., A. J. Oczkowski, J. Prezioso, and K. J. W. Hyde. 2010. Spatial variability of nitrogen isotope ratios of particulate material from Northwest Atlantic continental shelf waters. Estuarine, Coastal and Shelf Science 89:287–293.

McMahon, K. W., L. Ling Hamady, and S. R. Thorrold. 2013. A review of ecogeochemistry approaches to estimating movements of marine animals. Limnology and Oceanography 58:697–714.

Mendes, S., J. Newton, R. J. Reid, A. F. Zuur, and G. J. Pierce. 2007. Stable carbon and nitrogen isotope ratio profiling of sperm whale teeth reveals ontogenetic movements and trophic ecology. Oecologia 151:605–615.

Michener, R. H., and D. M. Schell. 1994. Stable isotope ratios as tracers in marine aquatic food webs. Pages 138–157 *in* K. Lajtha and R. H. Michener, editors. Stable isotopes in ecology and environmental science. Blackwell, Oxford.

Miller, S. E. 1993. Larval period and its influence on post-larval life history: comparison of lecithotrophy and facultative planktotrophy in the aeolid nudibranch *Phestilla sibogae*. Marine Biology 117:635-645.

Moksnes, P. O., L. Pihl, and J. Van Montfrans. 1998. Predation on postlarvae and juveniles of the shore crab *Carcinus maenas*: importance of shelter, size and cannibalism. Marine Ecology Progress Series 166:211–225.

Mompean, C., A. Bode, V. M. Benitez-Barrios, J. F. Dominguez-Yanes, J. Escanez, and E. Fraile-Nuez. 2013. Spatial patterns of plankton biomass and stable isotopes reflect the influence of the nitrogen-fixer *Trichodesmium* along the subtropical North Atlantic. Journal of Plankton Research 35:513–525.

Montoya, J. P. 2007. Natural abundacnes of 15N in the marine environment. Pages 176–201 *in* R. H. Michener and K. Lajtha, editors. Stable isotopes in ecolog and environmental science. Blackwell Publishing, Malden, MA.

Montoya, J. P., E. J. Carpenter, and D. G. Capone. 2002. Nitrogen fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic. Limnology and Oceanography 47:1617–1628.

Musick, J. A., and C. J. Limpus. 1997. Habitat utilization and migration in juvenile sea turtles. Pages 137–164 *in* P. L. Lutz and J. A. Musick, editors. The biology of sea turtles. CRC Press, Boca Raton, FL.

National Research Council. 2010. Assessment of sea-turtle status and trends: integrating demography and abundance. The National Academies Press, Washington, D.C.

Newman, R. A. 1992. Adaptive plasticity in amphibian metamorphosis. Bioscience 42:671-678.

Newsome, S., M. Etnier, D. Monson, and M. Fogel. 2009. Retrospective characterization of ontogenetic shifts in killer whale diets via δ13C and δ15N analysis of teeth. Marine Ecology Progress Series 374:229–242.

Ohman, M. D., G. H. Rau, and P. M. Hull. 2012. Multi-decadal variations in stable N isotopes of California Current zooplankton. Deep Sea Research Part I: Oceanographic Research Papers 60:46–55.

Olson, D. B. 2001. Biophysical dynamics of western transition zones: a preliminary synthesis. Fisheries Oceanography 10:133–150.

Parham, J.F., and G.R. Zug. 1997. Age and growth of loggerhead sea turtles (*Caretta caretta*) of coastal Georgia: an assessment of skeletochronological age-estimates. Bulletin of Marine Science 61:287-304.

Pechenik, J. A. 2006. Larval experience and latent effects – metamorphosis is not a new beginning. Integrative and Comparative Biology 46:323-333.

Peckham, S., D. Maldonado Diaz, Y. Tremblay, R. Ochoa, J. Polovina, G. Balazs, P. Dutton, and W. Nichols. 2011. Demographic implications of alternative foraging strategies in juvenile loggerhead turtles *Caretta caretta* of the North Pacific Ocean. Marine Ecology Progress Series 425:269–280.

Peterson, B. J., and B. Fry. 1987. Stable Isotopes in Ecosystem Studies. Annual Review of Ecology and Systematics 18:293–320.

Peterson, B. J., and R. W. Howarth. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. Limnology and oceanography 32:1195–1213.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2014. nlme: linear and nonlinera mixed effects models. R Package verison 3.1-117.

Plotkin, P. T. 2003. Adult migrations and habitat use. Pages 225–241 The biology of sea turtles. CRC Press, Boca Raton, FL.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718.

Post, D. M. 2003. Individual variation in the timing of ontogenetic niche shifts in largemouth bass. Ecology 84:1298–1310.

Ramsay, T. O., R. T. Burnett, and D. Krewski. 2003. The effect of concurvity in generalized additive models linking mortality to ambient particulate matter. Epidemiology 14:18–23.

Rau, G. H., R. E. Sweeney, and I. R. Kaplan. 1982. Plankton ^{13}C:^{12}C ratio changes with latitude: differences between northern and southern oceans. Deep Sea Research Part A. Oceanographic Research Papers 29:1035–1039.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reich, K. J., K. A. Bjorndal, M. G. Frick, B. E. Witherington, C. Johnson, and A. B. Bolten. 2010. Polymodal foraging in adult female loggerheads (*Caretta caretta*) Marine Biology 157:113–121.

Salvanes, A. G. V., J. Giske, and J. T. Nordeide. 1994. Life history approach to habitat shifts for coastal cod. Aquaculture and Fisheries Management 25:215–228.

Schmitz, O. J., A. P. Beckerman, and K. M. O'Brien. 1997. Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. Ecology 78:1388–1399.

Schoeninger, M. J., and M. J. DeNiro. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochimica et Cosmochimica Acta 48:625–639.

Seney, E. E., and J. A. Musick. 2007. Historical diet analysis of loggerhead sea turtles (*Caretta Caretta*) in Virginia. Copeia 2007:478–489.

Skelly, D. K., and E. E. Werner. 1990. Behavioral and life-historical responses of larval American toads to an odonate predator. Ecology 71:2313–2322.

Skulason, S., and T. B. Smith. 1995. Resource polymorphisms in vertebrates. Trends in Ecology & Evolution 10:366–370.

Smith, R. J., K. A. Hobson, H. N. Koopman, and D. M. Lavigne. 1996. Distinguishing between populations of fresh- and salt-water harbour seals (*Phoca vitulina* using stable-isotope ratios and fatty acid profiles. Canadian Journal of Fisheries and Aquatic Sciences 53:272–279.

Snover, M., A. Hohn, L. Crowder, and S. Macko. 2010. Combining stable isotopes and skeletal growth marks to detect habitat shifts in juvenile loggerhead sea turtles *Caretta caretta*. Endangered Species Research 13:25–31.

Snover, M. L. 2008. Ontogenetic habitat shifts in marine organisms: influencing factors and the impact of climate variability. Bulletin of Marine Science 83:53–67.

Snover, M. L., L. Avens, and A. A. Hohn. 2007. Back-calculating length from skeletal growth marks in loggerhead sea turtles *Caretta caretta*. Endangered Specie Research 3:95–104.

Snover, M. L., and A. A. Hohn. 2004. Validation and interpretation of annual skeletal marks in loggerhead (*Caretta caretta*) and Kemp's ridley (*Lepidochelys kempii*) sea turtles. Fisheries Bulletin 102:682–692.

Sponaugle, S., and R. K. Cowen. 1997. Early life history traits and recruitment patterns of Caribbean Wrasses (Labridae). Ecological Monographs 67:177-202.

Stearns, S. C. 1992. The Evolution of Life Histories. Oxford University Press, Oxford, U.K.

Szczebak, J. T., and D. L. Taylor. 2011. Ontogenetic patterns in bluefish (Pomatomus saltatrix) feeding ecology and the effect on mercury biomagnification. Environmental Toxicology and Chemistry 30:1447–1458.

Vander Zanden, H. B., K. A. Bjorndal, K. J. Reich, and A. B. Bolten. 2010. Individual specialists in a generalist population: results from a long-term stable isotope series. Biology Letters 6:711–714.

Vander Zanden, M. J., G. Cabana, and J. B. Rasmussen. 1997. Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (δ15N) and literature dietary data. Canadian Journal of Fisheries and Aquatic Sciences 54:1142–1158.

Walker, J. L., and S. A. Macko. 1999. Dietary studies of marine mammals using stable carbon and nitrogen isotopic ratios of teeth. Marine Mammal Science 15:314–334.

Wallace, B. P., L. Avens, J. Braun-McNeill, and C. M. McClellan. 2009. The diet composition of immature loggerheads: Insights on trophic niche, growth rates, and fisheries interactions. Journal of Experimental Marine Biology and Ecology 373:50–57.

Werner, E. E., and J. F. Gilliam. 1984. The ontogenetic niche and species interactions in size-structured populations. Annual Review of Ecology and Systematics 15:393–425.

Werner, E. E., and D. J. Hall. 1988. Ontogenetic habitat shifts in bluegill: the foraging rate-predation risk trade-off. Ecology 69:1352–1366.

Witzell, W. N. 1999. Distribution and relative abundance of sea turtles caught incidentally by the US pelagic longline fleet in the western North Atlantic Ocean, 1992-1995. Fishery Bulletin 97:200–211.

Witzell, W. N. 2002. Immature Atlantic loggerhead turtles (*Caretta caretta*) suggested changes to the life history model. Herpetological Review 33:266–269.

Woodland, R. J., D. H. Secor, and M. E. Wedge. 2011. Trophic resource overlap between small elasmobranchs and sympatric teleosts in Mid-Atlantic Bight nearshore habitats. Estuaries and Coasts 34:391–404.

Wood, S. N. 2006. Generalized Additive Models: An Introduction with R. Chapman and Hall.

Xu, X., M. C. Newman, M. C. Fabrizio, and L. Liang. 2013. An ecologically framed mercury survey of finfish of the lower Chesapeake bay. Archives of Environmental Contamination and Toxicology 65:510–520.

Zug, G. R., A. H. Wynn, and C. Ruckdeschel. 1986. Age determination of loggerhead sea turtles, *Caretta caretta*, by incremental growth marks in the skeleton. Smithsonian Contributions to Zoology 427:1–44.

Zuur, A. F., E. N. Ieno, and C. S. Elphick. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1:3–14.

APPENDICES

APPENDIX A: METHODS AND RESULTS

Determination of Δ $\delta^{15}N$ threshold values

Counts of turtle classifications were examined against threshold values to identify where concordance in classification counts was reached (Figure B1). This threshold value was used in all further analyses. This iterative method was chosen over classification based on mean prey isotope values to avoid uncertainty and biases associated with turtle diet specializations, turtle-diet isotopic discrimination, variance in turtle/diet isotopic fractionation and turnover, and heterogeneity in prey isotope signatures.

Iterative classification of turtles into life-history patterns based on a series of $\Delta\delta^{15}N$ thresholds resulted in multiple classification estimates per pattern (Figure B1). Counts of *discrete shifters*, *facultative shifters*, and *non-shifters* were between 7 and 23, 9 and 14, and 17 and 31 turtles, respectively. Counts of *ontogenetic shifters* and *non-shifters* between thresholds varied by 1 or less up until a threshold of +3.00‰, after which counts increased or decreased by 2 or more turtles with each increase in $\Delta\delta^{15}N$ threshold. Variance in counts above the +3.00‰ threshold was attributed to turtles classified as either *discrete shifters* or *facultative shifters* at lower thresholds being reclassified as *non-shifters* due to lack of additional data points (i.e., turtles died one or two years into/after an ontogenetic shifts). Visual inspection of the $\delta^{15}N$ transects for these reclassified turtles revealed patterns more similar to those of either *discrete shifters* or *facultative shifters* than *non-shifters*. Therefore, in order to avoid biases associated with timing of death and to use the

most conservative threshold for classification, a $\Delta\delta^{15}N$ threshold of +3.00‰ was used for final assignment of individual turtles to life-history pattern groups (Figure 2.4).

Composite growth increments

In seven turtles composite samples of two narrow growth increments were taken at points critical to life history pattern classification. For two of these turtles (both *facultative shifters*), classification was unaffected by the presence of the composite sample, while three other turtle were classified as an *indeterminate shifter* due to our inability to accurately assign an alternative life-history pattern. The remaining two turtles were conservatively classified as *discrete shifters* based on the composite $\delta^{15}N$ values measured, though may have been classified as *facultative shifters* had both growth increments been wide enough to sample individually.

APPENDIX B: TABLES AND FIGURES

Table B1. Stabl isotop ratios (δ^{15}N δ^{13}C) and estimated trophic positions (TP of zooplankton and potential prey items of juvenile loggerhead sea turtles summarized by species and habitat. Values are means SD *n* = sample size, NA = not available.

Species	*n*	δ^{15} (‰)	δ^{13} (‰)	TP[a]	Source(s)[b]
Neritic Prey					
Zooplankton	25	7.92 ± 1.40	-20.65 ± 2.11	2.0	2, 3, 6, 7
Bivalves					
Blue mussel *Mytilus edulis*	10	8.43 ± 0.78	-19.85 ± 2.03	2.2	4, 7, 9
Ribbed mussel *Geukensia demissa*	11	7.95 ± 0.35	-17.85 ± 0.78	2.0	5, 7, 13
Gastropod					
Moon snail *Neverita duplicata*	1	11.80	NA	3.2	18
Whelk *Busycon* spp.	11	9.06 ± 0.50	-16.26 ± 1.07	2.3	1, 11
Common periwinkle *Littorina littorea*	2	10.30	NA	2.7	7
Crustacean					
Blue crab *Callinectes sapidus*	145	10.13 0.97	-16.70 ± 2.48	2.7	1, 4, 5, 7, 8, 11, 14
Spider crab *Libinia emarginata*	16	11.63 1.38	-17.46 ± 0.65	3.1	11, 14
Mantis shrimp *Squilla empusa*	10	12.97 0.49	-18.53 ± 0.11	3.5	18
Mysid shrimp *Neomysis americana*	3	12.52 1.86	-20.16 ± 1.36	3.4	15, 18
Sand shrimp *Crangon septemspinosa*	17	13.18 1.27	-18.90 ± 0.49	3.6	18
Chelicerate					
Horseshoe crab *Limulus polyphemus*	20	11.74 1.50	-15.72 ± 2.19	3.2	5, 11, 14
Bony Fish (bycatch)					
Atlantic croaker *Micropogonias undulatus*	69	15.39 0.16	-19.66 ± 0.65	4.3	18, 20
Spot *Leiostomus xanthurus croaker*	76	14.35 1.84	-17.49 ± 3.14	3.9	1, 10, 16
Bay anchovy *Anchoa mitchilli*	37	15.81 1.43	-19.27 ± 1.17	4.4	18, 20
Bluefish *Pomatomus saltatrix*	154	15.53 0.44	-17.11 ± 0.52	4.3	5, 11, 18, 20
Miscellaneous					
Cannonball jellyfish *Stomolophus meleagris*	12	8.61 ± 0,60	-19.39 ± 0.91	2.2	11, 17
Spartina *Spartina* spp.	20	5.76 ± 1.44	-13.83 ± 1.35	1.4	1, 4, 7, 14
Oceanic Prey					
Zooplankton	64	1.93 ± 1.17	-19.37 ± 0.98	2.0	3, 19
Bivalves					
Barnacle *Lepas* spp.	1	7.60	-20.00	3.7	14
Gastropod					
Nudibranch *Scyllaea pelagica*	1	6.70	NA	3.4	14
Crustacean					
Sargassum crab *Planes minutes*	1	6.30	NA	3.3	14

Table B1. (Continued)

Sargassum crab *Portunus sayi*	11	6.45 ± 1.92	-16.57 1.45	3.4	12, 14
Brown grass shrimp *Leander tenuicornis*	10	6.44 ± 3.34	-16.83 ± 0.24	3.4	12, 14
Larval Fish					
Filefish *Stephanolepis hispidus*	11	6.61 ± 0.98	-17.63 ± 1.37	3.4	12, 14
Atlantic blue marlin *Makaira nigricans*	46	2.20 ± 0.70	-19.00 ± 1.00	2.1	12
Miscellaneous					
Mauve stinger jellyfish *Pelagia noctiluca*	8	4.61 ± 0.68	-17.95 ± 0.51	2.8	12
Cannonball jellyfish *Stomolohus meleasgris*	1	8.40	-19.20	4.0	14
Moon jellyfish *Aurelia aurita*	5	8.52 ± 0.55	-19.50 ± 0.58	4.0	12
Se nettle jellyfish *Chrysaora quinquecirrha*	6	5.19 ± 0.25	-17.20 ± 0.68	3.0	12
Lion's mane jellyfish *Cyanea capillata*	1	5.29	-17.46	3.0	12
Sargassum *Sargassum* spp.	27	2.21 ± 1.84	-16.86 ± 0.67	2.1	12, 14

[a]Calculated as described in Chapter 2

[b](1) Peterson & Howarth 1987, (2) Fry 1988, (3) Fry Quinones 1994, (4) Fantle et al. 1999, (5) Knoff et al. 2001, (6) Estrada et al. 2003, (7) Dittel et al. 2006, (8) Bucci et al. 2007, (9) Haramis et al. 2007, (10) Logan 2009, (11) Wallace et al. 2009, (12) McClellan et al. 2010, (13) McKinney et al. 2010, (14) Snover et al. 2010, (15) Woodland et al. 2011, (16) Szczebak & Taylor 2011, (17) Dodge et al. 2011, (18) Buchheister & Latour 2011, (19) Mompean et al. 2013, (20) Xu et al. 2013

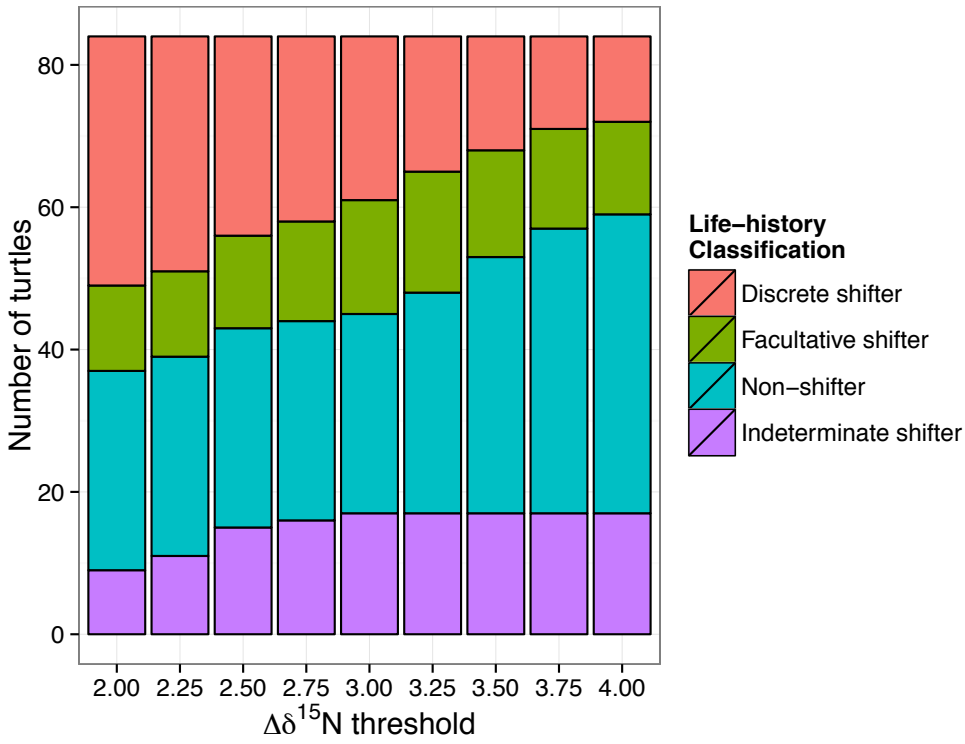

Figure B1. Iterative classification of turtles into life history pattern groups based on a series of $\delta^{15}N$ thresholds. A $\Delta\delta^{15}N$ threshold of +3.00‰ was used for final assignment of individual turtles to life-history pattern groups.

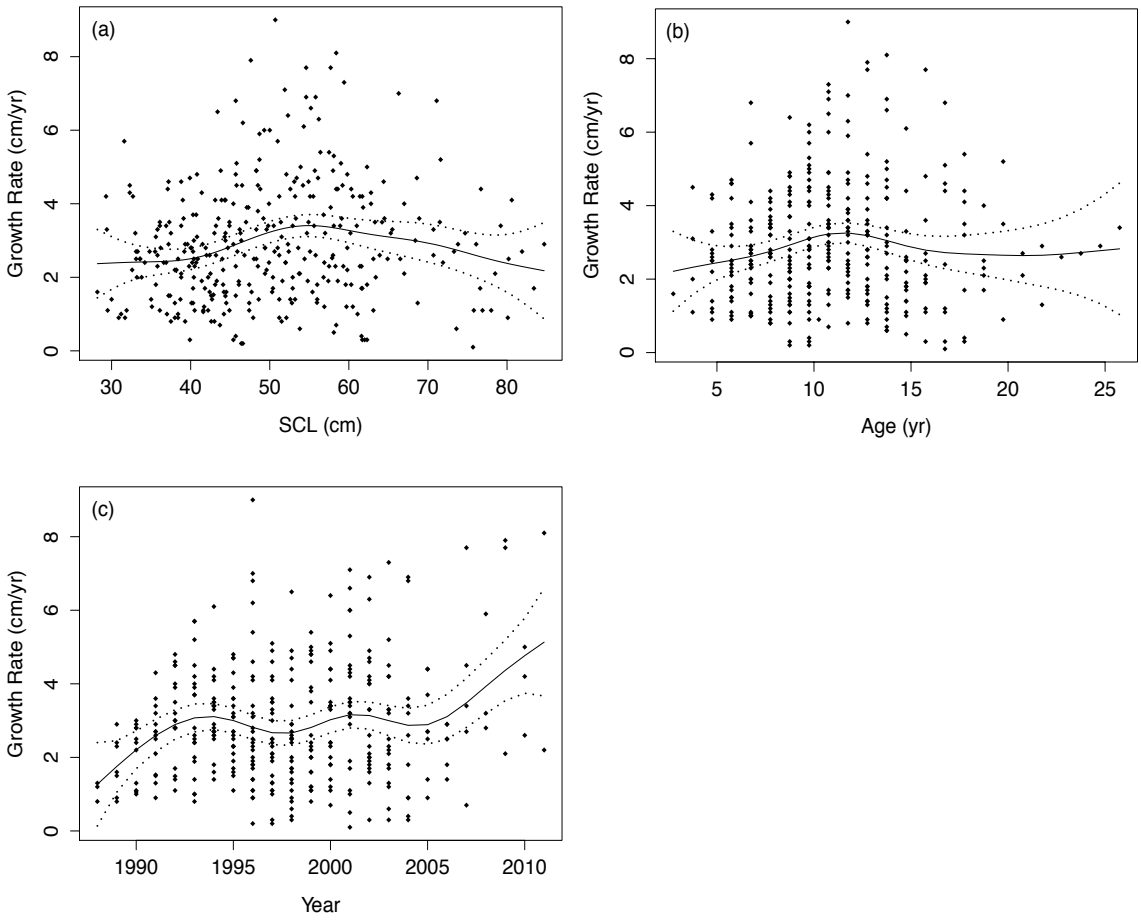

Figure. B2. Smoothing splines fit to size, age, and year-specific growth data for all back-calculated growth increments (n = 350). Dashed lines denote 95% confidence intervals**.**

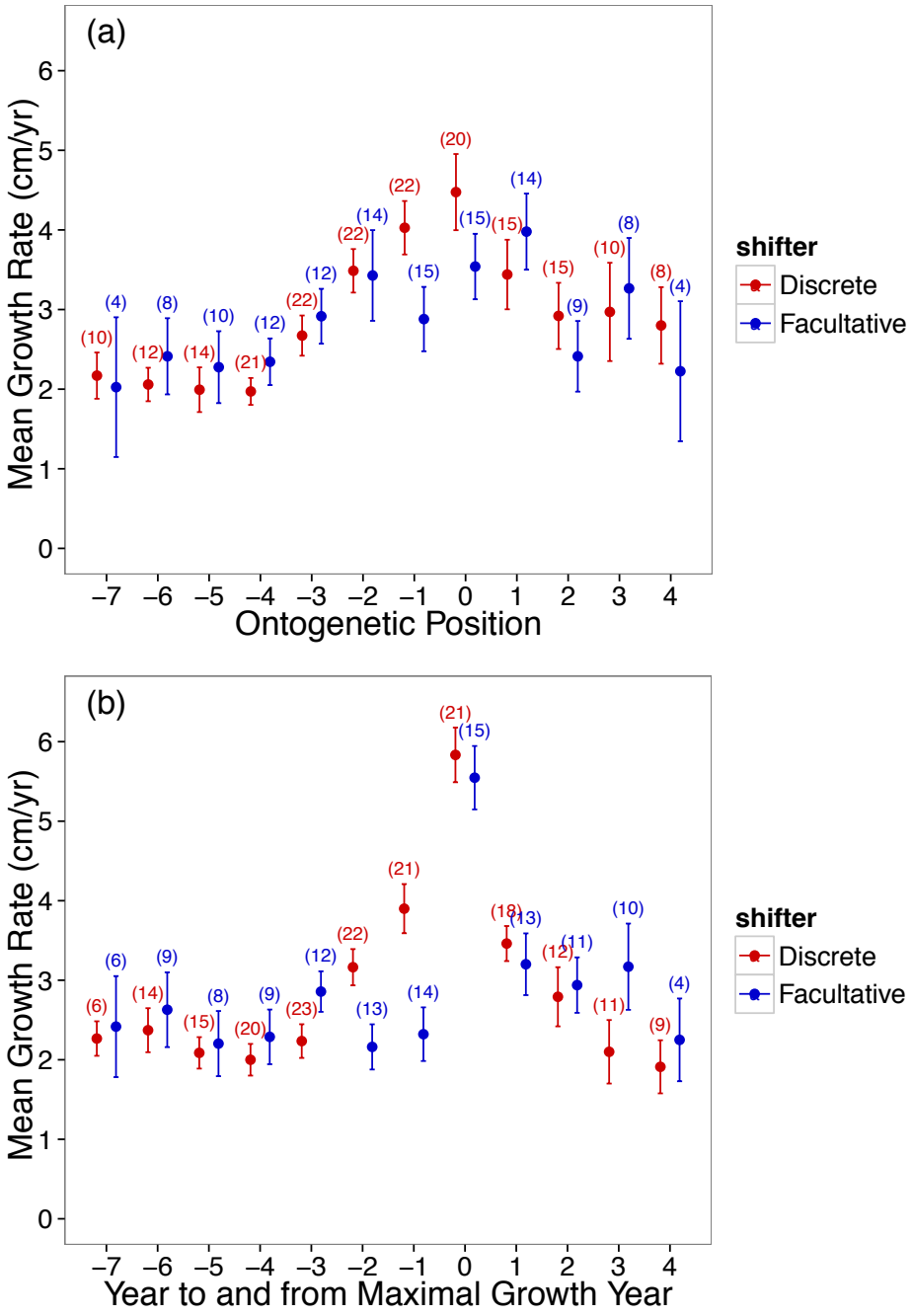

Figure B3. Mean annual growth rates by life history pattern with standard error bars for all turtles by (a) ontogenetic position and (b) year to and from turtle-specific maximal growth year. Numbers in parentheses are sample sizes.