

Complementary skeletochronology and stable isotope analyses offer new insight into juvenile loggerhead sea turtle oceanic stage duration and growth dynamics

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ABSTRACT: Stage durations are integral to wildlife population models that can inform management, as they influence age at maturation and stage-specific survival rates. To refine oceanic stage duration estimates for western North Atlantic loggerhead sea turtles *Caretta caretta*, skeletochronological analysis was conducted on humeri collected in the Azores islands and along the US Atlantic coast. Complementary skeletal growth increment-specific stable isotope analysis was also performed for a sub-set of the humeri, to identify the skeletal growth mark associated with the shift from oceanic to neritic habitat through stable nitrogen isotope ($\delta^{15}\text{N}$) values and the presence of turtles in inshore waters. Although the transitional growth mark in this sub-sample corresponded to a range of sizes similar to those described in previous studies, mean size at recruitment (55.3 cm straightline carapace length [SCL]) for these turtles was larger than previously estimated. Similarly, while the range of ages at recruitment—corresponding both with the transitional growth mark and those yielded by fitting smoothing splines to SCL-at-age data—overlapped almost fully with earlier estimates, the mean age estimate (12.4 yr) differed from previous studies. Validated back-calculation of somatic growth rates from skeletal growth marks yielded means and ranges that encompassed those of previous loggerhead growth studies in this geographic area. Generalized additive models and generalized additive mixed models used to assess the potential influence of discrete and continuous covariates on back-calculated growth rates spanning 1984 to 2009 indicated significant effects of age, SCL, calendar year, and $\delta^{15}\text{N}$, but none for sex or location.

KEY WORDS: *Caretta caretta* · Age · Skeletal growth marks · Life history · Ontogenetic habitat shift

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INTRODUCTION

Effective management of threatened and endangered sea turtle populations is often constrained by the lack of data needed to accurately parameterize

models used to assess population status and predict the outcomes of management decisions (Heppell et al. 2003a, National Research Council 2010). For example, even for the comparatively well-studied loggerhead sea turtle *Caretta caretta* populations in

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the western North Atlantic (reviews in Bolten & Witherington 2003), additional characterization of demographic and life-history parameters is required to increase confidence in the results of modeling efforts (Heppell et al. 2003a, National Research Council 2010). Despite the clear need for this information, collecting it is difficult, as loggerheads display a complex life history typical of most sea turtles, involving multiple ontogenetic habitat shifts and highly migratory behavior (Musick & Limpus 1997, Bolten 2003, McClellan & Read 2007). As a result, apart from females on nesting beaches, sea turtles are difficult to access in the marine environment for study, particularly in an unbiased manner (Heppell et al. 2003b, National Research Council 2010).

Juvenile stage durations are essential components of population models, as they directly influence age at maturation and also offer insight into length of exposure to stage-specific threats that impact survival probability (Bjørndal et al. 2000, Snover 2008). After leaving western North Atlantic beaches as hatchlings, small juvenile loggerheads spend a protracted period of time in an oceanic developmental stage prior to recruiting to neritic foraging areas (Musick & Limpus 1997). Defining the length of this oceanic stage using traditional mark-recapture methods has been challenging, due to low recapture rates (Bjørndal et al. 2000). Length–frequency analysis of oceanic juvenile loggerheads captured near the Azores islands yielded stage duration estimates of 6.5, 8.2, and 11.5 yr at neritic recruitment sizes of 46, 53, and 64 cm curved carapace length (CCL), respectively (Bjørndal et al. 2000). Similarly, growth analyses incorporating estimates of post-hatchling growth rates updated using Lagrangian drifter data and size at arrival in oceanic foraging areas also estimated age at 53 cm CCL as approximately 8 yr (confidence interval 8 to 9 yr) (Scott et al. 2012).

Skeletochronology, which is the analysis of growth marks in bones, can allow researchers to estimate ages and stage durations through growth mark counts and calculate growth rates through conversion of successive growth mark measures to estimates of body size (Avens & Snover 2013). However, the success of the technique is contingent on meeting assumptions regarding annual deposition of growth marks and ontogenetic consistency in the proportional relationship between bone measures and relevant somatic measures (Avens & Snover 2013). Snover (2002) back-calculated growth trajectories in humerus bones from stranded neritic juvenile loggerheads and estimated mean (\pm SD) oceanic stage duration as 14.8 ± 3 yr (range 9 to 24 yr) for turtles

recruiting to neritic habitat at 48.5 to 51.1 cm straight carapace length (SCL). In contrast, Bjørndal et al. (2003) conducted a skeletochronological analysis of humeri from oceanic juvenile loggerheads and estimated oceanic stage duration at 7 yr for loggerheads recruiting to neritic habitat at 46 cm CCL. These differing estimates may be due in part to the inability to account for the allometric relationship between humerus and somatic (carapace) measures (subsequently described in Snover et al. 2007) and the discreteness of the sample sets for the 2 studies (i.e. entirely neritic vs. entirely oceanic).

Whereas skeletochronology can provide information about past growth rates and size-at-age relationships, analysis of stable isotope ratios in animal tissues can offer insight into past trophic ecology, as an organism's incorporated isotope ratios are derived from habitat and prey items. For example, stable nitrogen isotope ratios ($^{15}\text{N}/^{14}\text{N}$, $\delta^{15}\text{N}$) can allow discernment of trophic relationships within a community, as selective excretion of lighter nitrogen isotopes in consumers often results in predictable $\delta^{15}\text{N}$ enrichment with each trophic level that can allow characterization of trophic position (DeNiro & Epstein 1981, Michener & Kaufman 2007). An important caveat of this approach is that $\delta^{15}\text{N}$ values in consumer tissues are driven not only by trophic position, but are also strongly influenced by baseline $\delta^{15}\text{N}$ (i.e. that of primary producers), which has been shown to have a strong geographic variation (Seminoff et al. 2012, Vander Zanden et al. 2013). Thus, ecological inferences drawn from $\delta^{15}\text{N}$ values in consumer tissues depend on a sound knowledge of stable isotope values at the base of the food chain. Within the marine environment, carbon isotope ratios ($\delta^{13}\text{C}$) vary latitudinally, as well as between nearshore and offshore foraging environments (Hobson 2007, Michener & Kaufman 2007), facilitating characterization of past foraging locations. The time frame over which trophic history can be inferred using isotopic signatures depends on the isotopic replenishment rate (i.e. turnover rate) of the tissue being analyzed (Hobson 2007); as bone remains relatively inert after it is formed, it can retain a long-term (i.e. many years) record of isotopic exposure, limited only by the extent of bone reconstruction at the core (Snover et al. 2010).

Here, we present a study incorporating complementary skeletochronological and growth increment-specific stable isotope analyses of humeri from oceanic and neritic loggerheads in the western North Atlantic to refine estimates of oceanic stage duration for the species in this region. In addition, we charac-

terize growth patterns over several decades, as inferred through back-calculation incorporating ontogenetic allometry in the humerus–carapace length relationship, and assess the potential influence of age, size, year, sex, location, and $\delta^{15}\text{N}$ on growth rates.

MATERIALS AND METHODS

Sample collection

Oceanic stage loggerhead *Caretta caretta* samples were obtained from turtles either stranded dead or by-caught in fisheries in the Azores islands in the eastern North Atlantic Ocean (Fig. 1). The National Sea Turtle Stranding and Salvage Network collected samples from neritic turtles that were either stranded dead or were debilitated and stranded alive, but later died. For each turtle, a front flipper was collected for subsequent extraction of the humerus bone for skeletochronological and stable isotope analyses (Avens & Snover 2013). In addition, stranding location and

carapace length (SCL and/or CCL from nuchal notch to posterior tip) were recorded and, when possible, a necropsy was conducted to determine sex through examination of the gonads. As only SCL was used for analysis in the present study, for one oceanic juvenile for which only CCL was available, SCL was estimated using the following equation based on the CCL:SCL relationship for the remaining oceanic juveniles in the sample:

$$\text{SCL} = 0.931 \times \text{CCL} - 0.568 \quad (r^2 = 0.995) \quad (1)$$

For neritic juveniles, when necessary, CCL was converted to SCL using the conversion from Snover et al. (2010).

Skeletochronology

The sample for skeletochronological analysis incorporated newly collected humeri, as well as a number of bone samples processed for previous skeletochronology studies (e.g. Snover 2002, Snover et al. 2007, Goshe et al. 2009). For the newly collected humeri, 2 sequential cross-sections were taken from each humerus (Avens & Snover 2013; see Supplement 1 at www.int-res.com/articles/suppl/m491p235_supp.pdf). The first section was 1 to 2 mm thick and intended for stable isotope analysis, while the second was 2 to 3 mm thick and used for skeletochronological analysis.

Each 'new' skeletochronology section was histologically prepared according to methods described in Avens et al. (2012) and humerus sections from previous studies were histologically processed using the methods outlined in Snover & Hohn (2004) and Goshe et al. (2009) (Supplement 1). Calibrated, digital images of entire humerus sections at 4× magnification were obtained (e.g. Fig. 2a) and analyzed according to the methods of Avens et al. (2012) (Supplement 1). For a subset of the calibrated, digital skeletochronology images, the contour of each line of arrested growth (LAG) that delimits the outer edge of a skeletal growth mark (Fig. 2a) was traced and the image printed onto a transparency to guide stable isotope sampling (see following section).

Stable isotope analysis

Humerus sections used for stable isotope analysis were mounted onto microscope slides

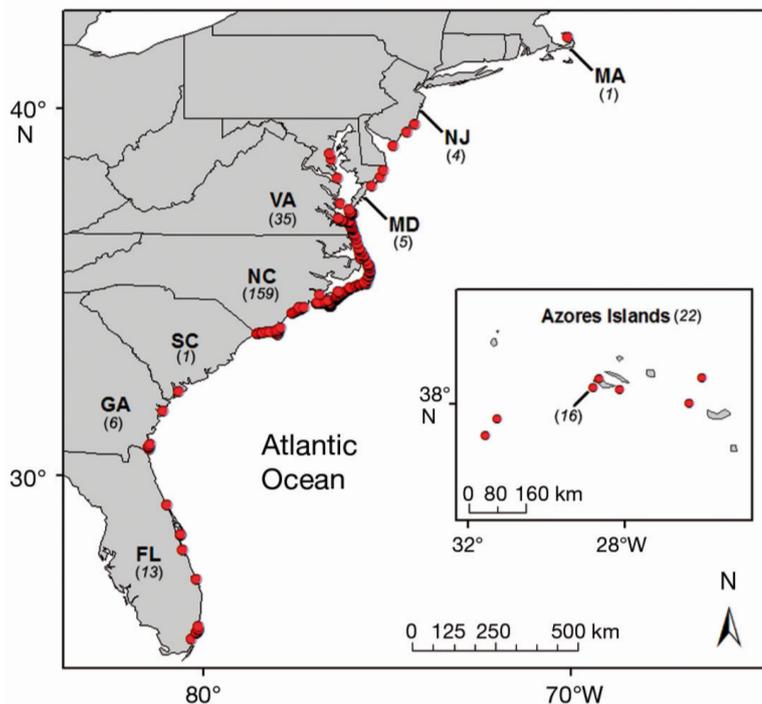


Fig. 1. *Caretta caretta*. Map of loggerhead sea turtle humerus sample collection sites. Each red dot represents an individual collection location and numbers in parentheses represent sample size by location. US Atlantic Coast state abbreviations are: MA = Massachusetts, NJ = New Jersey, MD = Maryland, VA = Virginia, NC = North Carolina, SC = South Carolina, GA = Georgia, and FL = Florida

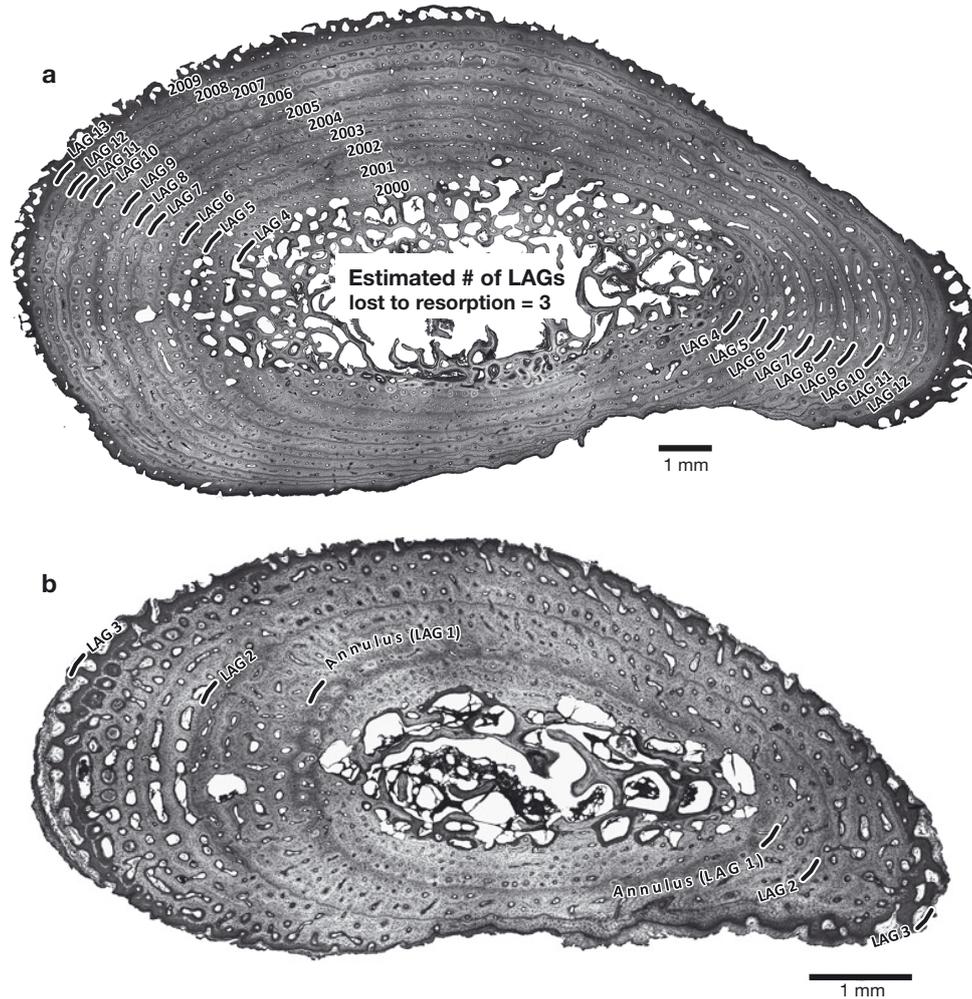


Fig. 2. *Caretta caretta*. Images of histologically processed humerus cross-sections. (a) Neritic juvenile loggerhead 51.3 cm straightline carapace length (SCL) stranded in 2009, with estimated age of 13 yr. Lines of arrested growth (LAGs) that delimit the outer margins of skeletal growth marks are labeled, with most recent growth at the outer margin and earliest LAGs at core lost to resorption. (b) Oceanic juvenile loggerhead 23.3 cm SCL. Assigned age is 3 yr, based on presence of a diffuse first-year mark, or 'annulus', at core

with the side originally proximal to the skeletochronology section facing upward. Each slide was secured to a bracket mounted beneath an Olympus SZX10 microscope that could be oriented along x, y, and z axes using a MC-4SA low power micro motor system (National Aperture). A 64 megapixel SPOT Flex microscope camera was used in conjunction with Carpenter Microsystems CM-2 software to yield a calibrated field of view onto which a transparency of the skeletochronology image for each humerus was overlaid to serve as a guideline for LAG placement and curvature. This allowed programming of automated pathways for drilling from each growth increment using a NSK Volvere Max precision drill. Pathways were placed between LAG pairs to ensure that drilled material from each line only represented a single increment (annual layer). Furthermore, sam-

ples were collected only from increments within periosteal bone to eliminate the potential influence of recent stable isotope signatures incorporated during reconstruction of endosteal bone on results obtained from early growth marks located near the resorption core (Klevezal 1996). Approximately 0.6 mg of bone dust resulted from each increment and samples were immediately packed into sterilized tin capsules, and then analyzed by a continuous-flow isotope-ratio mass spectrometer in the Stable Isotope Laboratory at the University of Florida, Gainesville, USA (Supplement 1).

Stable isotope analysis of well-preserved bone collagen is perhaps the most direct approach for a general assessment of animal diet, as collagen carbon reflects the carbon sources from an animal's bulk diet (Schoeninger & DeNiro 1984, Lee-Thorp et al. 1989).

However, low bone-dust yield from the smallest of growth increments precluded our ability to isolate the bone collagen fraction from hydroxyapatite (the mineral component of bone) via decalcification (Ambrose & Norr 1993). We therefore focused our analyses on stable nitrogen, for which $\delta^{15}\text{N}$ values of unprocessed bone dust are thought to reflect the $\delta^{15}\text{N}$ values of loggerhead prey. Based on previous stable isotopic studies of loggerhead turtles along the US East Coast (Wallace et al. 2009, McClellan et al. 2010), loggerheads foraging on surface-dwelling and mid-water prey in the oceanic zone were anticipated to deposit growth increments with significantly lower $\delta^{15}\text{N}$ ratios than those foraging on higher-trophic-order benthic prey in neritic zones.

Age estimation

Validation of annual LAG deposition was conducted following the methods of Snover et al. (2007), by analyzing humeri from 12 neritic juveniles in our sample that had been captured, measured, tagged, and released prior to stranding. We used the body proportional hypothesis (BPH)-corrected allometric equation and values for mean hatchling humerus diameter (1.9 mm) and SCL (4.6 cm) for loggerhead turtles in the western North Atlantic from Snover et al. (2007) to convert the diameter of the LAG thought to have been deposited closest to the time of tagging to an estimate of SCL. This relationship was also applied to estimate SCL from humerus section diameter for 1 oceanic juvenile for which neither a SCL nor CCL measurement was available. Estimated SCL at tagging was compared with SCL measured at tagging using a Wilcoxon signed rank test. Concurrence between estimated and measured values would not only support annual LAG deposition, but also partially validate the use of this approach for calculating somatic growth rates by converting successive LAG diameters to estimates of SCL (see following section).

Age was assigned according to total LAG count for those humeri retaining a diffuse LAG toward the center (e.g. Fig. 2b) whose appearance was consistent with that of the annulus that marks the end of the first year of growth in Kemp's ridleys *Lepidochelys kempii* (Snover & Hohn 2004). However, as sea turtles age and grow, early growth marks towards the center of the bone are often destroyed as the proportion of cancellous bone at the core increases—a process called resorption. As a result, we developed correction factors based on the relationship between LAG number and LAG diameter to estimate the num-

ber of LAGs lost in each humerus (Parham & Zug 1997, Avens et al. 2012). The estimated number of resorbed LAGs was added to the number of observed LAGs (Fig. 2a) and this number was adjusted to the nearest 0.25 yr according to mean hatch date for the population and individual stranding date to yield a final age estimate (Supplement 1).

Size (SCL)-at-age was modeled using a non-parametric smoothing spline (Avens et al. 2012) implemented using the mgcv package in the statistical program R (Wood 2006). Spline fits were used to predict means and ranges of ages at different SCLs potentially corresponding with the initial oceanic to neritic transition for loggerheads in the western North Atlantic as identified both in previous studies (Bjørndal et al. 2000, 2003 [with CCL values converted to SCL according to Bjørndal et al. 2000], Snover 2002) and from growth increment-specific stable isotope values generated by the present study (see 'Stable isotopes' in 'Results').

Growth rates

The feasibility of back-calculating somatic growth rates from LAG measures was first assessed through (1) characterization of the positive, allometric relationship between humerus diameter and SCL and (2) comparison of back-calculated and measured SCLs for tagged loggerheads (see previous section). However, to provide additional support for this approach, the association between bone growth and somatic growth was also evaluated by modeling relationships between estimated age and SCL, and estimated age and humerus section diameter (Goshe et al. 2010, Avens et al. 2012). Residuals from the best-fitting models (as determined through comparison of r^2 values) were then plotted to determine whether they exhibited a significant, positive relationship.

Every measurable LAG in each humerus was assigned a calendar year, counting backward from the most recent LAG at the outer edge of the bone (Fig. 2a), and converted to an estimate of SCL (see previous section). Annual growth rates were calculated by taking the difference between successive SCL estimates and were assigned to (1) the calendar year of the innermost LAG of the pair and (2) the 10 cm size class corresponding with the estimated mean SCL of the LAG pair (Chaloupka & Musick 1997). A Kruskal-Wallis test with post hoc multiple comparison was applied to determine whether growth rates differed significantly among size classes. Size-class-specific growth rates relative to calendar year were

modeled using non-parametric smoothing splines (see previous section) to qualitatively assess temporal trends.

Growth data were modeled using both generalized additive models (GAMs) and generalized additive mixed models (GAMMs) to evaluate the influence of both continuous and discrete variables on growth (Avens et al. 2012). When only data associated with the final growth increment for each turtle were analyzed, a GAM was used (Hastie & Tibshirani 1990, Chaloupka & Musick 1997), whereas when multiple but varying numbers of growth increments from each individual turtle were analyzed, a GAMM was implemented to account for turtle-specific, random effects (Chaloupka & Musick 1997, Wood 2006). In addition to dividing growth data according to associated covariates, the factors SCL and age were separated into different models because they exhibited a high degree of concurvity, which can confound statistical inference (Hastie & Tibshirani 1990, Avens et al. 2012). Models GAM_{SCL} and GAM_{Age} incorporated SCL or Age, stranding location (Location), calendar year (Year), and Sex, and models $GAMM_{SCL}$ and $GAMM_{Age}$ included SCL or Age, Year, and Sex. Two additional GAMMs were applied to the sub-set of the growth data for which associated $\delta^{15}N$ values were available. During initial runs of these 2 models, calendar year was not a significant factor; therefore, model $GAMM_{\delta^{15}N_{SCL}}$ incorporated only the covariates SCL and $\delta^{15}N$, while $GAMM_{\delta^{15}N_{Age}}$ included Age and $\delta^{15}N$.

Both the GAM and GAMM approaches incorporated an identity link, a robust quasi-likelihood error function, and cubic smoothing splines to characterize the non-linear relationship between the continuous variables and the response (growth rate) (Wood 2006). Models were implemented using the *mgcv* (GAM and GAMM) and *nlme* (GAMM) packages in the statistical program R (Wood 2006). Significance of GAM and GAMM model factors was determined by *t*-ratio statistical inference (non-parametric covariates) and non-parametric *F*-ratio test (continuous covariates) and overall model fit was assessed using Akaike's information criterion and adjusted R^2 values.

RESULTS

A total of 246 humeri from oceanic ($n = 22$) and neritic ($n = 224$) loggerheads ranging from 7.8 to 88.6 cm SCL (mean \pm SD = 55.8 \pm 15.8 cm SCL) and collected between 1996 and 2010 was processed and analyzed.

Of the total sample, 67 were females, 33 were males, and 146 were of unknown sex. Oceanic turtles ranged from 8.2 to 63.3 cm SCL (mean = 23.3 \pm 17.7 cm SCL) and neritic turtles ranged from 7.8 to 88.6 cm SCL (mean = 59.0 \pm 11.4 cm SCL), with the inclusion of a 7.8 cm SCL post-hatchling and an unusually small 23.1 cm SCL juvenile. Early analyses demonstrated that setting maximum turtle size for the samples at 65 cm SCL (i.e. presumed maximum size at initial neritic transition) artificially truncated the spline fit to the SCL-at-age data (see 'Age' below) and did not allow estimation of mean and range of ages at the largest sizes at recruitment. As a result, it was necessary to extend the size range for the samples well beyond the presumed maximum size at the oceanic to neritic shift, to also include data from large neritic juveniles well past the transition stage. Although neritic samples from the US Atlantic coast were collected in each state from Massachusetts to Florida, the majority (71 %) were obtained from North Carolina strandings (Table S1 in Supplement 1).

Stable isotopes

A sub-set of 15 humeri from neritic juveniles ranging from 49.0 to 72.7 cm SCL (mean \pm SD = 60.7 \pm 6.7 cm SCL) stranded in inshore waters in North Carolina and Virginia from 1997 to 2008 (median of 2004) was sub-sampled for stable isotope values corresponding with measurable growth increments, yielding 109 total samples. Of the 15 humeri, 8 collected from turtles 55.1 to 72.7 cm SCL at stranding (mean = 62.9 \pm 6.3 cm SCL) exhibited a single, pronounced increase in $\delta^{15}N$ (from a mean of 9.65 \pm 1.00 to a mean of 12.12 \pm 1.39; Table 1), consistent with a transition from oceanic to neritic habitat (Snover et al. 2010). As a result, for further analyses of size, age, and growth relative to the habitat shift (see 'Age' and 'Growth' below), transition was assigned to the LAG immediately preceding the elevated $\delta^{15}N$ value. Seven of the humeri collected from juveniles 49 to 67.2 cm SCL at stranding (mean = 58.2 \pm 6.6 cm SCL) did not display an increase in $\delta^{15}N$, with mean (\pm SD) terminal values (9.43 \pm 0.74) not differing significantly from mean pre-shift $\delta^{15}N$ values (9.65 \pm 1.00) for the group of 8 turtles for which a marked change in $\delta^{15}N$ was observed ($p = 0.65$, Mann-Whitney test; Table 1). However, the presence of these 7 turtles in inshore waters indicated that they had recently shifted to neritic habitat; therefore, size and age at transition were presumed to correspond with final values at stranding. Overall, SCLs at stranding of tur-

Table 1. *Caretta caretta*. Skeletal growth increment-specific nitrogen stable isotope ($\delta^{15}\text{N}$) values for 15 neritic juvenile loggerheads stranded in inshore waters, with 8 (left-hand side) exhibiting a pronounced increase in $\delta^{15}\text{N}$ consistent with the transition from oceanic to neritic foraging

Turtle ID	Pre-shift	Post-shift	Difference	Turtle ID	Terminal (no shift)
Cc AHP031009-01	10.08	11.72	1.64	Cc EH040114-01	10.06
Cc CR990814-01	10.52	12.49	1.97	Cc JBM971106-01	9.36
Cc KXR041221-01	8.82	11.79	2.97	Cc JD040724-01	9.41
Cc LJP041108-01	9.41	11.27	1.86	Cc JMA080107-19	9.49
Cc LRG050712-01	7.82	10.19	2.37	Cc LW070621	9.97
Cc ML050103-01	9.30	12.70	3.40	Cc ML971118-06	9.86
Cc RB980607-01	10.44	15.00	4.56	Cc RMM040514-01	7.89
Cc WMC020909-01	10.77	11.81	1.04		
Mean \pm SD	9.65 \pm 1.00	12.12 \pm 1.39	2.48 \pm 1.12		9.43 \pm 0.74

tles whose humeri displayed the $\delta^{15}\text{N}$ shift tended to have greater SCLs than those that did not, although this difference was not statistically significant ($p = 0.06$, Mann-Whitney test). Likewise, back-calculated (see 'Age' and 'Growth' below) mean SCL at transition for the turtles exhibiting the change in $\delta^{15}\text{N}$ (53.9 ± 4.2 cm) tended to be less than mean SCL of stranded turtles that did not exhibit the $\delta^{15}\text{N}$ shift, yet this difference was not statistically significant ($p = 0.22$, Mann-Whitney test).

Age

For the 12 neritic juvenile loggerheads tagged prior to stranding, no significant difference was found between SCL estimated from LAG diameter and SCL measured at tagging (Table 2; $p = 0.81$, Wilcoxon signed rank test) and mean absolute difference was 1.1 cm SCL. Determining which LAG was deposited closest to the time of tagging was based on the premise of annual LAG deposition and therefore the lack of significant difference supports this assumption. Furthermore, the result also partially validates the approach of annual somatic growth rate back-calculation through conversion of sequential LAG diameters to estimates of SCL (see the following section).

A diffuse annulus was at least partially visible in 10 humeri (5 oceanic and 5 neritic) (e.g. Fig. 2b) and these were designated as 'Group 1'. Age based on direct LAG count for turtles in Group 1 ranged from 2.25 to 12.0 yr (mean \pm SD = 5.5 ± 3.9 yr) for turtles

17.8 to 57.8 cm SCL (mean = 33.8 ± 14.7 cm). Total annulus diameters could be measured in 5 of those humeri and corresponded with SCL estimates of 13.3, 13.5, 16.3, 17.1, and 18.3 cm (mean = 15.7 ± 2.2 cm SCL) for 0.75 yr old loggerheads. Humeri for turtles in Group 1 retained a total of 34 LAGs and a linear regression described the relationship between LAG number and LAG diameter well:

$$\text{LAG diameter (mm)} = 1.805 \times \text{LAG number} + 3.9989 \quad (r^2 = 0.920) \quad (2)$$

The regression was used as a first order correction factor (Fig. S1a in Supplement 1) to estimate the number of LAGs lost to resorption for those humeri whose resorption core diameters did not exceed maximum LAG diameters incorporated into the regression (i.e. Group 2; $n = 191$). LAG numbers and LAG diameters for Groups 1 and 2 ($n = 225$ humeri, 2031

Table 2. *Caretta caretta*. Comparison of straightline carapace lengths (SCL) measured at initial tagging and back-calculated through skeletochronological analysis for 12 neritic juvenile loggerhead sea turtles. Mean difference (absolute value) = 1.1. Dates given as mo/d/yr

Turtle ID	Stranding date	Tagging date	SCL at tagging Measured (cm)	SCL at tagging Estimated (cm)	Difference (cm)
Cc SC970523-01	5/23/1997	10/11/1995	50.7	48.1	2.6
Cc JBM040723-01	7/23/2004	6/24/2003	55.7	56.8	-1.1
Cc KAR070306-01	3/6/2007	6/29/2006	63.3	62.5	0.8
Cc BD040602-01	6/2/2004	20/10/2003	63.5	63.0	0.5
Cc LIA040610-01	6/10/2004	9/30/2003	61.7	62.3	-0.6
Cc KMS020428-01	4/28/2002	10/8/1998	46.9	46.6	0.3
Cc KLC080523-03	5/23/2008	8/6/2007	56.1	54.0	2.1
Cc SGB 040523-01	5/23/2004	5/28/2001	59.1	57.9	1.2
Cc LRB030630-01	6/30/2003	6/28/2002	63.5	63.2	0.3
Cc KB030103-01	1/3/2003	7/12/2002	62.8	62.5	0.3
Cc JRC040121-04	1/21/2004	6/27/2003	61.4	61.3	0.1
Cc DT060528-01	5/28/2006	5/20/2004	61.8	58.6	3.2

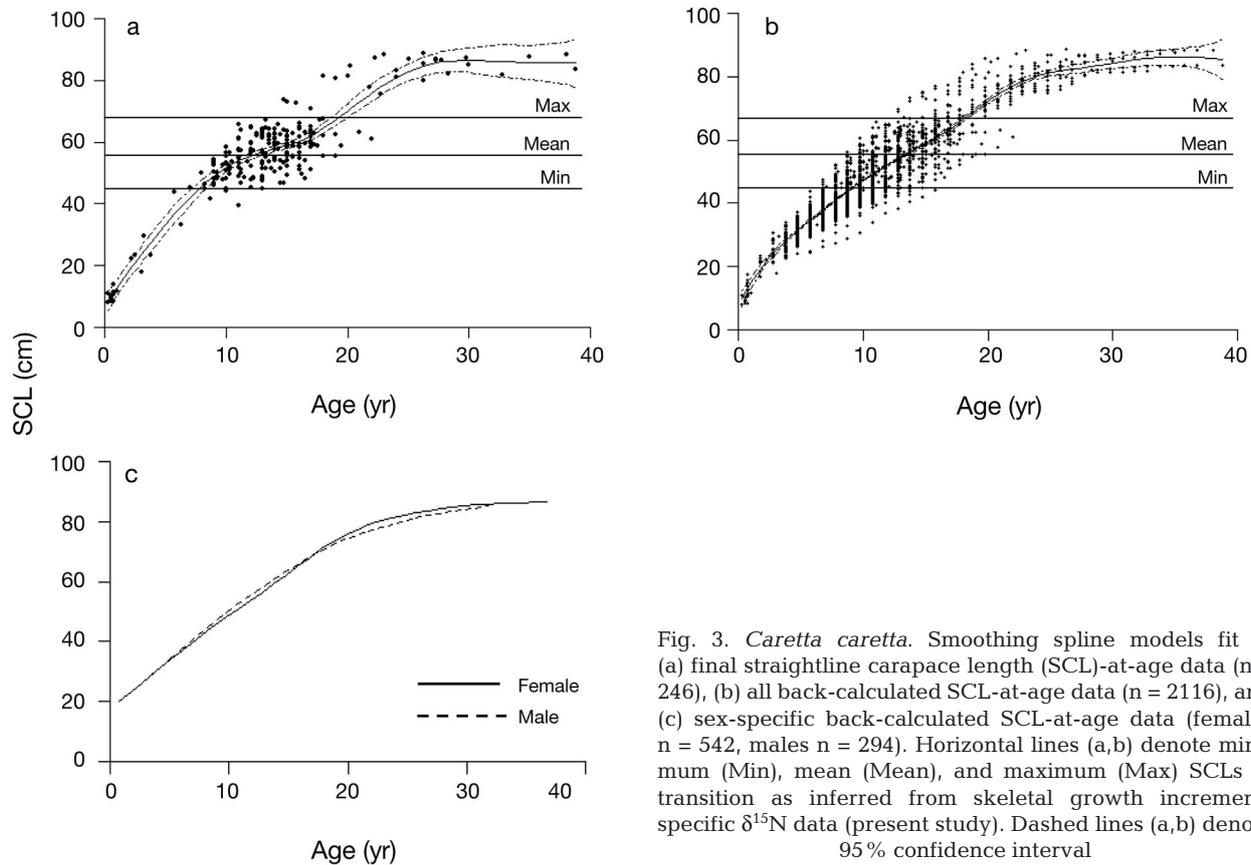


Fig. 3. *Caretta caretta*. Smoothing spline models fit to (a) final straightline carapace length (SCL)-at-age data ($n = 246$), (b) all back-calculated SCL-at-age data ($n = 2116$), and (c) sex-specific back-calculated SCL-at-age data (females $n = 542$, males $n = 294$). Horizontal lines (a,b) denote minimum (Min), mean (Mean), and maximum (Max) SCLs at transition as inferred from skeletal growth increment-specific $\delta^{15}\text{N}$ data (present study). Dashed lines (a,b) denote 95% confidence interval

LAGs) were combined and a smoothing spline was fit to these data to yield a second order correction factor (Fig. S1b in Supplement 1) to estimate the number of resorbed LAGs for the remainder of the humeri ($n = 21$), for which resorption core diameters exceeded the maximum diameter allowed for the first order correction factor. For the entire sample, the estimated number of resorbed LAGs ranged from 0 to 24, with a mean \pm SD of 5 ± 4 LAGs and median of 4 LAGs. Estimated number of resorbed LAGs was 0 for turtles up to 30 cm SCL and ≤ 9 for all turtles 30 to 80 cm SCL, but ranged from 7 to 24 for turtles 80 to 90 cm SCL, due to greater variability in resorption core size for the largest turtles.

Age estimates for the total sample ranged from 0.25 to 38.75 yr (mean \pm SD = 14 ± 6 yr) and revealed overlap between oceanic and neritic turtles. Whereas ages for oceanic turtles ranged from 0.25 to 21 yr (mean = 4.43 ± 17.66 yr), ages for neritic turtles (excluding 2 anomalous strandings: a 7.8 cm SCL post-hatchling and a 23.1 cm SCL juvenile) ranged from 5.75 to 38.75 yr (mean = 14.67 ± 5.17 yr) and SCLs from 33.3 to 88.6 cm (mean = 59.4 ± 10.7 cm). For the sub-set of stable isotope analysis turtles,

mean size at transition \pm SD was 55.3 ± 5.6 cm SCL (range 43.9 to 67.2 cm SCL) and mean age \pm SD was 12.4 ± 2 yr (range 9.75 to 15.75 yr) for years spanning 1992 to 2006 (median = 2002).

A smoothing spline was fit to the SCL and age estimate data corresponding with stranding (i.e. 'final' SCL-at-age, $n = 246$) (Fig. 3a). However, to fill in data-sparse regions in the terminal SCL-at-age relationship, SCL was also back-calculated for every measurable LAG in each humerus to which an age had been assigned and a smoothing spline was also fit to these data for comparison (i.e. 'all' SCL-at-age, $n = 2116$; Fig. 3b). Although inflections in both splines were indicative of polyphasic growth (as opposed to monotonic, declining), increasing the sample size decreased the prominence of the inflection. Spline fits were used to predict means and ranges of ages associated with the size at transition from oceanic to neritic habitat for loggerheads in the western North Atlantic at both SCLs from earlier studies and those indicated by the $\delta^{15}\text{N}$ results from the present study (Table 3). Smoothing splines fit to the back-calculated male and female SCL-at-age data revealed no sex-specific differences (Fig. 3c).

Table 3. *Caretta caretta*. Estimated ages for juvenile loggerhead sea turtles at different straightline carapace lengths (SCLs) associated with the transition from oceanic to neritic habitat in the western North Atlantic. Numbers in parentheses denote minimum and maximum values for each age range unless prefaced by 'CI', which denotes confidence interval

SCL at transition (cm)	Estimated age at oceanic-to-neritic transition (yr)					
	Length-frequency	Skeleto-chronology	Lagrangian drift + neritic mark-recapture growth data ^a	Present study (skeletalochronology and $\delta^{15}\text{N}$ data ^b)	Spline – final data ^c	Spline – all back-calculated data ^d
Present study^b						
Mean	55.3	–	–	12.4	12 (9–19)	13 (9–20)
Min	43.9	–	–	9.75	8 (6–12)	9 (6–16)
Max	67.2	–	–	15.75	19 (13–21)	18 (13–20)
Bjorndal et al. (2000)						
Mean	49.0 ^e	8.2	–	8.0 (CI 8.0–9.0)	–	9 (8–16)
Min	42.4 ^e	6.5	–	–	–	7 (6–9)
Max	59.5 ^e	11.5	–	–	–	15 (10–20)
Snover (2002)						
Min	48.5	–	14.8±3 (9–24)	–	–	9 (8–16)
Max	51.1	–	14.8±3 (9–24)	–	–	10 (9–17)
Bjorndal et al. (2003)						
Min	42.4 ^e	–	7	–	–	7 (6–9)
Snover et al. (2010)						
Mean	45.5	–	–	–	–	8 (7–13)
Min	43.6	–	–	–	–	8 (6–11)
Max	47.4	–	–	–	–	9 (8–16)

^aScott et al. (2012) using size at transition from Bjorndal et al. (2000)
^bAs inferred from growth increment-specific nitrogen stable isotope ($\delta^{15}\text{N}$) values (Table 1)
^cFig. 3a
^dFig. 3b
^eConverted from curved carapace length using Eq. 1 in Bjorndal et al. (2000)

Growth

Both the relationship between estimated age (Age) and SCL and that for Age and humerus section diameter (HSD) were best described by third order polynomials. The relationship between the residuals from these 2 models was positive and best characterized by a linear regression ($p < 0.001$), validating back-calculation of somatic growth rates from bone growth increments. The relationship between SCL and HSD was allometric, with $b = 3.109$ and $c = 0.936$.

Back-calculated annual growth rates for every measurable growth increment in all humeri revealed a high degree of variability in somatic growth relative to SCL, age, and calendar year (Fig. S2 in Supplement 1). However, mean growth rates were highest for the smallest size classes (hatchling to 19.9 cm SCL), with a slight increase again in the 50 to 59.9 cm SCL size class (Table 4), and overall ranged from 0.0 to 13.7 cm yr^{-1} . Growth rates were significantly different among size classes ($p < 0.001$, Kruskal Wallis test with post hoc multiple comparison; Table 4). On the whole, the ranges and means of the back-calculated growth rates encompassed those yielded

Table 4. *Caretta caretta*. Size-class (straightline carapace length, SCL, in cm)-specific growth rates back-calculated using skeletochronology in the present study. Significance level for differences among size-class-specific growth rates was $p < 0.05$ in all cases

Size class (SCL in cm)	n	SCL growth rates (cm yr^{-1})		Significantly different from size classes
		Mean ± SD	Range	
5 (4.6–9.9)	2	8.8±0.1	8.7–8.9	80
10 (10–19.9)	10	6.2±4.7	1.3–13.7	60, 70, 80
20 (20–29.9)	99	2.6±1.1	0.7–4.7	80
30 (30–39.9)	490	2.7±1.4	0.1–7.3	60, 80
40 (40–49.9)	614	2.8±1.7	0.1–9.8	60, 70, 80
50 (50–59.9)	363	3.1±2.0	0.1–11.7	60, 70, 80
60 (60–69.9)	123	2.1±1.6	0.1–7.0	10, 30, 40, 50, 80
70 (70–79.9)	94	2.1±1.5	0.0–6.8	10, 40, 50, 80
80 (80–89.9)	82	1.1±1.1	0.0–4.4	All

by previous mark-recapture studies of loggerhead populations in geographic areas overlapping with the scope of the present study (Table S2 in Supplement 1). The long time frame over which samples were collected and the number of LAGs retained in the humeri made it possible to estimate size-class-

Table 5. *Caretta caretta*. Summary of statistical output from the generalized additive models (GAMs) applied to analyze the influence of different potential covariates on growth response during the year before stranding and from the generalized additive mixed models (GAMMs) for all back-calculated growth increments. Edf = estimated degrees of freedom; SCL = straight-line carapace length; AIC = Akaike's information criterion. US Atlantic Coast state abbreviations are: MA = Massachusetts, NJ = New Jersey, MD = Maryland, VA = Virginia, NC = North Carolina, SC = South Carolina, GA = Georgia, and FL = Florida. **Bold** indicates significance ($p < 0.05$)

Model	Deviance explained (%)	AIC	Smooth terms				Parametric coefficients				
			Variable	Edf	F	Prob(F)	Variable	Estimate	SE	t	Pr> t
GAM _{Age} (n = 233)	26.5	990	Age (yr)	4.607	9.41	<0.001	Sex _{male}	0.306	0.445	0.688	0.492
			Year	1	0.001	0.975	Location _{MA}	0.673	0.907	0.742	0.459
							Location _{NJ}	0.230	1.104	0.208	0.835
							Location _{MD}	-1.040	2.062	-0.504	0.614
							Location _{VA}	4.019	1.160	3.466	<0.001
							Location _{NC}	1.225	0.744	1.647	0.101
							Location _{SC}	1.170	1.264	0.926	0.356
							Location _{GA}	1.641	2.146	0.765	0.445
							Location _{FL}	1.310	0.445	0.688	0.492
GAM _{SCL} (n = 233)	26.8	1006	SCL (cm)	6.309	4.364	<0.001	Sex _{male}	0.444	0.456	0.974	0.331
			Year	7.664	1.625	0.116	Location _{MA}	0.714	0.976	0.731	0.466
							Location _{NJ}	0.049	1.374	0.035	0.972
							Location _{MD}	-1.797	2.330	-0.771	0.441
							Location _{VA}	4.382	1.237	3.541	<0.001
							Location _{NC}	1.261	0.831	1.517	0.131
							Location _{SC}	1.051	1.363	0.771	0.442
							Location _{GA}	1.691	2.395	0.706	0.481
							Location _{FL}	1.502	0.872	1.722	0.087
GAMM _{Age} (n = 1877)	14.7	7120	Age (yr)	8.346	23.123	<0.001	Sex _{male}	-0.011	0.158	-0.067	0.946
			Year	6.068	9.786	<0.001	Sex _{unk}	-0.161	0.114	-1.412	0.158
GAMM _{SCL} (n = 233)	13.1	7078	SCL (cm)	7.159	24.97	<0.001	Sex _{male}	-0.089	0.181	-0.493	0.622
			Year	5.683	4.17	<0.001	Sex _{unk}	-0.147	0.130	-1.129	0.259
GAMM _{δ¹⁵N_{Age}} (n = 109)	19.4	448	Age (yr)	1.496	3.601	0.012	-	-	-	-	
			δ ₁₅ N	3.457	4.089	0.030	-	-	-	-	
GAMM _{δ¹⁵N_{SCL}} (n = 109)	19.4	439	SCL (cm)	2.046	6.452	0.002	-	-	-	-	
			δ ₁₅ N	3.613	4.353	0.003	-	-	-	-	

specific growth rates back as early as 1984; however, sample sizes from 1984 to 1988 were quite small (Table S3 in Supplement 1). Smoothing splines fit to the size-class-specific growth data revealed diverse temporal growth patterns (Fig. S3 in Supplement 1).

For both the GAMs and GAMMs, Age and SCL were significant predictors of growth response (Table 5 and Fig. 4a,c). Although no effect of calendar year (Year) on growth response was characterized in the GAMs (Table 5), influence of this covariate was significant for both GAMMs, with growth patterns displaying several fluctuations over the decades represented in the sample ($p < 0.001$ for both models; Table 5 and Fig. 4b,d). Effect of Sex was not significant in any of the models (Table 5) and Location (as inferred over the previous year from stranding location) was also not found to significantly influence growth response, with the exception of Virginia (Table 5). GAMMs incorporating the growth increment-specific isotopic data revealed a significant

association between growth response and $\delta^{15}\text{N}$, with growth initially decreasing and then abruptly increasing at $\delta^{15}\text{N}$ values associated with the shift to neritic foraging (Table 5 and Fig. 4e,f). Despite the significant result for some of the available covariates on growth response in these GAM and GAMM models, overall explanatory power remained low (Table 5).

DISCUSSION

Oceanic stage duration

In the present study, mean oceanic stage duration and size at neritic recruitment for loggerhead sea turtles in the western North Atlantic was estimated at 12 to 13 yr and 55.3 cm SCL (mean minima 8 to 10 yr at 43.9 cm; mean maxima 16 to 19 yr at 67.2 cm) using SCL-at-age relationships and skeletal growth in-

crement-specific stable isotope analysis of $\delta^{15}\text{N}$ (Tables 1 & 3). The stage duration estimates presented herein are not directly comparable with previously published values that were calculated using

somewhat smaller estimates of SCL at transition (summarized in Table 3; Bjorndal et al. 2000, 2003, Snover 2002). However, application of SCL-at-age relationships from the present study to earlier SCL

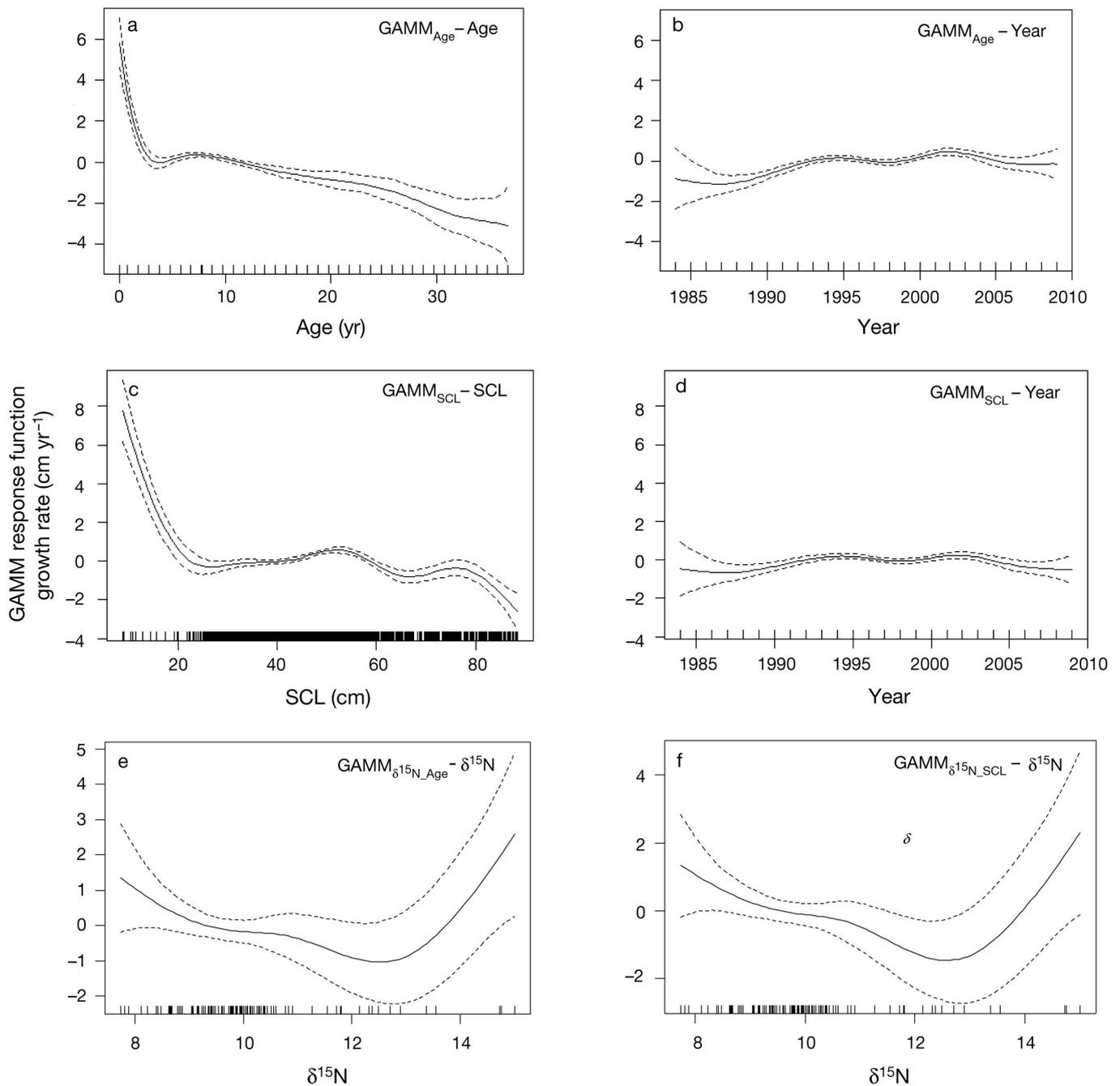


Fig. 4. *Caretta caretta*. Graphical summary of covariates exhibiting a significant influence on growth response in generalized additive mixed models (GAMMs) incorporating (a–d) all back-calculated growth rates ($n = 1877$) for juvenile loggerhead sea turtles in the North Atlantic and (e,f) back-calculated growth rates for which growth increment-specific nitrogen stable isotope ($\delta^{15}\text{N}$) data were available. Age is the age estimated through skeletochronology, SCL is the mean straightline carapace length for the growth increment, and Year is the calendar year corresponding with the growth increment. (a,b) GAMM_{Age}, (c,d) GAMM_{SCL}, (e) GAMM_{δ¹⁵N_{Age}}, (f) GAMM_{δ¹⁵N_{SCL}}. Solid line represents mean growth response for the covariate and dashed lines represent the 95% Bayesian credible interval. Short, vertical lines above the horizontal axes represent the numbers and distributions of covariate values

values that ranged from 42.4 to 59.5 cm SCL yielded mean stage duration estimates of 7 to 15 yr, which encompassed earlier estimates of 7 to 12 yr (Bjørndal et al. 2000, 2003) and 15 yr (Snover 2002). These discrepancies are likely to be at least partially due to incorporation for the first time of (1) the allometric relationship between humerus diameter and SCL (Snover et al. 2007) and (2) both oceanic and neritic loggerhead samples in the present skeletochronological analyses. Although the mean values differ to some extent, the range of possible oceanic stage durations yielded by the present approach overlaps almost fully with those yielded by earlier analyses (Table 3), highlighting the scope of variability potentially associated with this life-history parameter.

Periodic habitat shifts are common in marine organisms such as sea turtles, whose ontogeny involves changes in body size spanning several orders of magnitude (Snover 2008). As juvenile fitness is contingent on minimizing time to maturation and maximizing survival, time spent in any particular stage or habitat may vary depending on multiple, potentially interdependent factors, such as size and condition-dependent predation risk and habitat use, habitat-specific population densities and predator guilds, environmental variability, and individual genotypes and phenotypes (Werner & Hall 1988, Dahlgren & Eggleston 2000, Snover 2008). The complexity of these processes has implications for extrapolation of stage duration estimates beyond the time frames over which they were collected, as this parameter is likely to fluctuate over time (Werner & Hall 1988, Snover 2008). Nonetheless, characterization of potential changes in ontogenetic stage durations is essential for accurate interpretation of population trends (Dahlgren & Eggleston 2000, Snover 2008). For example, morphometric data collected from neritic loggerheads in North Carolina, USA, from 1995 to 2003 indicated an increase in mean size, with the dominant 5 cm size class for the population shifting from 55 to 59 cm SCL early in the study period to 60 to 64 cm SCL by its end (Epperly et al. 2007). Although such an increase might be attributed to a corresponding decrease in the number of small turtles recruiting to the population, perhaps suggesting higher mortality of oceanic stage turtles, the change could instead reflect an increase in the size at transition (Snover 2008). Comparison of the present results with loggerhead ontogenetic shift data presented in Snover et al. (2010) provides some support for the increase in size at transition interpretation. Whereas mean size at recruitment was estimated at 45.5 cm SCL for 23 neritic juvenile loggerheads

stranded during 1997 and 1998 (Snover et al. 2010), data presented herein indicate mean size at transition as 55.3 cm SCL for 15 loggerheads stranded in the same area during a time period with a median year of 2004 (range 1997 to 2008). This possibility merits further investigation through analysis of larger numbers of samples comprising more recent years.

Characterization of stage duration is further complicated by the fact that shifts can occur over a continuum, instead of being discrete (e.g. Childress & Herrnkind 2001, Casale et al. 2008). Organisms in transition may return to their original habitat after sampling a new habitat and perceiving high predation risk and/or inadequate foraging opportunities (Werner & Hall 1988). Neritic juvenile loggerheads in the western North Atlantic have occasionally been observed to revert to oceanic habitat for long periods of time (McClellan & Read 2007, Mansfield & Putman 2013), although the cause of this behavior has yet to be described. In the present study, growth increment-specific $\delta^{15}\text{N}$ values were not indicative of multiple transitions between oceanic and neritic habitat. However, the ability to detect the initial habitat shift through the complementary skeletochronology and stable isotope analyses presented herein (Table 1) and in Snover et al. (2010) highlights the utility of this approach for future investigations into the specific nature of ontogenetic habitat shifts not only for loggerheads, but other sea turtle species as well.

Use of $\delta^{15}\text{N}$ to infer habitat shifts

As isotopic compositions of consumer tissues incorporate information from foraging environments (DeNiro & Epstein 1981, Michener & Kaufman 2007), when an animal moves among spatially discrete food webs that are isotopically distinct, the stable isotope values of its tissues can provide information about its previous environment (Hobson 2007, Pajuelo et al. 2012a, Seminoff et al. 2012). Stable isotope values—particularly $\delta^{15}\text{N}$ —of the primary foods consumed by loggerhead turtles in the oceanic environment appear to differ substantially from those consumed in the neritic environment (e.g. Snover et al. 2010). In the present study, each growth increment-specific bone tissue sample was regarded as an integration of information over the entire growth year, thus reflecting the ‘mean’ dietary stable isotope value for that time interval (Lee-Thorp et al. 1989, Newsome et al. 2010). Thus, analysis of bone tissue from sequential humerus growth increments should retrospectively

detect any potential shifts in $\delta^{15}\text{N}$ on an annual time frame.

The $\delta^{15}\text{N}$ shift reported herein (mean = 2.5) was consistent with a shift from oceanic to neritic habitat. However, both this absolute shift and the mean pre- and post-shift $\delta^{15}\text{N}$ values in the present study (9.65 and 12.12, respectively) were somewhat less than those reported by Snover et al. (2010) (mean shift = 3.1, mean pre-shift = 11.0, mean post-shift = 14.1). This discrepancy may have resulted from temporal variation in baseline $\delta^{15}\text{N}$ (e.g. Ohman et al. 2012) or a shift in foraging preference for turtles in this geographic area between the time frames encompassed by the 2 studies (e.g. Seney & Musick 2007). Furthermore, recent isotope studies of loggerheads by Ceriani et al. (2012) and Pajuelo et al. (2012a,b) show discrete differences in $\delta^{15}\text{N}$ in skin and red blood cells among loggerheads foraging in different neritic habitats in the Northwest Atlantic Ocean. As a result, variation in specific foraging locations between the turtles sampled by Snover et al. (2010) and those in the present study might also manifest as differences in bone $\delta^{15}\text{N}$ values.

Given these differences in $\delta^{15}\text{N}$ among neritic habitats, it is also possible that the shift observed in the present study might represent movement from one isotopically distinct neritic habitat to another. Although it is not possible to rule out a neritic–neritic shift based on the present results, we believe that when viewed in light of the corresponding growth rate shifts, the isotope values described herein reflect movements between 2 habitats that have disparate energetic resources, such as would be expected between oceanic vs. neritic habitats in the Northwest Atlantic. To establish a firmer context for such isotope flux, it will be instructive to include stable carbon analyses in future studies, as $\delta^{13}\text{C}$ values differ markedly between oceanic and neritic prey species.

Somatic growth rates

Correspondence between measured and estimated SCLs for the tagged, juvenile loggerheads in the sample provided additional validation for back-calculation of annual, somatic growth rates through conversion of every measurable LAG diameter to an estimate of SCL (Table 2; Snover et al. 2007). This validated back-calculation can significantly amplify sample sizes for age and growth studies by generating size-at-age data associated with every measurable LAG within each bone, thereby allowing characterization of individual age and growth histories over

long periods of time (e.g. Avens et al. 2012). The present analyses yielded extremely large sample sizes (1877 measurable growth increments spanning several decades), which should provide representation of broader, population-wide relationships while minimizing the influence of any anomalous, individual effects on growth patterns.

Back-calculated somatic growth rates for both oceanic and neritic loggerheads were highly variable, irrespective of turtle size (Fig. S2 in Supplement 1), consistent with results of previous studies (Bjorndal et al. 2003, Braun-McNeill et al. 2008), and this variability was also manifest in the range of SCL-at-age relationships determined for the sample (Fig. 3a,b). The particularly high variability indicated for the 10 to 19.9 cm size class (Table 4) may reflect the stochastic environmental conditions and foraging opportunities characteristic of oceanic habitat (Bjorndal et al. 2003, Mansfield & Putman 2013). Overall, mean growth rates were highest for the smallest size classes (to 19.9 cm SCL) and then decreased abruptly, followed by another slight increase through the 50 to 59.9 cm SCL size class and subsequent decrease through the 80 to 89.9 cm SCL size class (Table 4 and Fig. S2 in Supplement 1). Comparison of these growth rates with those measured through mark-recapture, skeletochronology, and length–frequency analyses in geographic areas that correspond with the scope of the present study showed that they fall within the range of what has previously been reported (Table S2 in Supplement 1).

Both age and SCL were found to significantly influence growth response in the GAM and GAMM models (Table 5 and Fig. 4a,c). For SCL, inflection in the growth response was most evident for all back-calculated growth data, with a peak spanning approximately 50 to 55 cm SCL (Fig. 4c). As recruitment to neritic habitat is thought to correspond with increased somatic growth (e.g. Snover et al. 2010), it is possible that this peak with a mean of ~52.5 cm SCL represents mean size at recruitment integrated over the entire study period spanning 1984 to 2009. This size is somewhat smaller than the 55.3 cm mean SCL at transition inferred through stable isotope analysis for a sub-set of samples in the present study and greater than the 45.5 cm estimated by Snover et al. (2010), each of which represents only a portion of the total study time period. Although these differences may be indicative of shifts in size at transition over time (see also ‘Oceanic stage duration’ above), consideration must be given to the discrepancy between sample sizes for these growth analyses and those of the stable isotope analyses in the present

study and in Snover et al. (2010). As a result, additional characterization of size at transition is recommended. Interestingly, a similar inflection was not found in growth response for all back-calculated growth data relative to age, suggesting that perhaps neritic recruitment during the study period occurred over a broader, more evenly distributed range of ages (Fig. 4a).

The relationship between growth response and $\delta^{15}\text{N}$ was also statistically significant (Table 5), with growth decreasing as $\delta^{15}\text{N}$ levels approached those associated with the transition from oceanic to neritic habitat (Snover et al. 2010) and subsequently becoming greater as $\delta^{15}\text{N}$ values increased further, reflecting a shift to neritic foraging (Fig. 4e,f). However, it is notable that growth response was also elevated at low $\delta^{15}\text{N}$ values associated with oceanic foraging early in life (Fig. 4e,f). These fluctuations, with minimum mean growth response corresponding with intermediate $\delta^{15}\text{N}$ values associated with transition, are consistent with the potential role of growth limitation as at least a partial impetus for a habitat shift (Bolten 2003). Nonetheless, we acknowledge that the mean pre-shift and post-shift isotope values remain within the 95% Bayesian credible intervals in Fig. 4e,f, making it difficult to interpret the true significance of the downward slope in mean growth response.

Calendar years for back-calculated growth rates spanned 1984 to 2009 (Fig. 4b,d and Table S3 in Supplement 1) and, similar to a previous study of oceanic juvenile loggerheads in the Northern Atlantic (Bjornrdal et al. 2003), the effect of calendar year in both the GAMM_{Age} and GAMM_{SCL} models was found to be significant. Although it was not possible to address the source of the observed variation in the present study, these fluctuations may be related to factors associated with source populations, cohorts, environmental factors, or most likely a combination of these (Heppell et al. 2003a). Examination of size-class-specific temporal growth response through smoothing spline fits (Fig. S3 in Supplement 1) revealed diverse patterns that were somewhat difficult to reconcile; however, this result is not unexpected given the overlapping size ranges for oceanic and neritic habitats where factors influencing growth are likely to differ. Qualitative examination of trends indicated growth response to be stable or even increasing from 1985 to 2010 for the 20 to 39.9 cm SCL juveniles likely to have inhabited the oceanic environment at the time those growth rates occurred. In contrast, growth response for the 50 to 59.9 and 60 to 69.9 cm size classes was stable or increasing through

2001 and 1997, respectively, followed by a steady decrease through 2009. Owing to the prevalence of neritic juveniles from North Carolina turtles in the sample, this trend may reflect factors specific to this region and merits future study, given the extensive use of this habitat by loggerhead sea turtles (Epperly et al. 2007). Although the 70 to 79.9 cm SCL size classes also exhibited a decrease in growth response from 1995 to 2000, this was followed by an increase through 2008 and 2009. Cumulatively, these results highlight the need for long-term, size-class-specific sea turtle growth studies with large sample sizes, to reduce the possibility that somatic growth rate estimates are extrapolated from anomalous individuals, seasons and/or years, or are focused on particular size classes, but instead are fully representative of study populations.

As indicated by the sex-specific SCL-at-age spline fits (Fig. 3c), growth response did not differ between females and males in the study population (Table 5). This result contrasts with those from some studies of other species, such as Australian hawksbill *Eretmochelys imbricata* (Chaloupka & Limpus 1997) and green sea turtles *Chelonia mydas* (Limpus & Chaloupka 1997), as well as green turtles in the Northwestern Atlantic (Goshe et al. 2010). However, because the size range for the present study was focused on immature individuals and not fully representative of adult sizes and ages, it is possible that sex-specific differences might manifest closer to the onset of, or subsequent to, reproductive maturity. For example, despite the differences observed between female and male green turtles spanning the full size range from hatchlings to adults in Goshe et al. (2010), no sex-specific differences in size-at-age and growth response were observed for neritic juvenile green turtles up to 78.5 cm SCL in the northeast Gulf of Mexico (Avens et al. 2012). Furthermore, no sex-specific differences were observed in immature green turtles <60 cm CCL in the Bahamas (Bolten et al. 1992) and Australia (Limpus & Chaloupka 1997).

Finally, in contrast to results observed for Australian loggerheads (Limpus & Limpus 2003), terminal ('final') growth response for western North Atlantic loggerheads generally did not vary relative to location (Table 5). However, because location was inferred from the stranding site, it was not possible to determine with certainty that turtles were foraging in the region prior to stranding. The only exception to this result was Virginia, where growth response conditioned on the other potential covariates was significantly greater than that observed for other locations (Table 5). Mean growth rates for loggerheads from

the Chesapeake Bay back-calculated using skeletochronology (Klinger & Musick 1995) are fairly high relative to the mean growth rates for the same size classes in other studies (Table S2 in Supplement 1). This area has long been characterized as an important foraging habitat for loggerhead sea turtles and it is possible that high productivity in this area and/or supplementation of diet with fishery discards (Seney & Musick 2007) might support relatively rapid growth. Nevertheless, the question of location effects cannot be addressed in detail without analyses of humerus samples and/or mark-recapture growth data based on annual intervals that are comparably partitioned among foraging areas and, considering growth variance among calendar years, consistent in temporal scope.

Future directions

The utility of combining analyses of growth marks in hard or calcified structures with complementary isotopic, microchemistry, contaminant, and/or genetic data to elucidate life history has long been recognized for other marine organisms such as fish (Campana 1999), marine mammals (Newsome et al. 2010), and invertebrates (Richardson 2001). Similarly, the results presented herein emphasize the value of combined skeletochronology and growth increment-specific $\delta^{15}\text{N}$ analyses for investigating sea turtle size-at-age, growth rates, and oceanic stage duration. While these results offer intriguing insights into the potential variability associated with size and age at transition from oceanic to neritic habitat for loggerheads in the Northwestern Atlantic, additional study is needed to fully characterize the scope and underlying causes. Subsequent efforts to refine estimates of mean and variance for these parameters would benefit from the information yielded by these complementary approaches.

Furthermore, despite the statistical significance of the influence of some of the available covariates on loggerhead growth response in the GAM and GAMM models in the present study, overall explanatory power remained low (Table 5). Future skeletochronological studies of sea turtle age and growth would be enhanced by inclusion of genetic, health (as assessed through necropsy), and contaminant data, to determine the potential influence of these factors. Expansion of the complementary approach described herein for sea turtles to also include other growth increment-specific isotopic (e.g. $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^{34}\text{S}$) and trace element data could yield additional

information about habitat use and foraging history (e.g. Campana 1999, Hobson 2007, Talavera-Saenz et al. 2007) and offer vital insights into the influence of these factors on somatic growth and size-at-age relationships.

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