Skeletochronological estimation of age and growth of loggerhead sea turtles (*Caretta caretta*) in the western South Atlantic Ocean

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Abstract   Age and growth are important parameters for better understanding of life history and population dynamics of animal species, as well as for formulating management strategies. However, these data are difficult to obtain for sea turtles because of overall slow growth, delayed maturation and highly migratory behaviour. The loggerhead sea turtle, *Caretta caretta*, is a widely distributed species, globally listed as endangered. Although the species has been well-studied in some regions, little is known about various aspects of its biology in other populations, such as those in the waters of the western South Atlantic Ocean, especially outside nesting areas. To address age and growth, loggerhead turtles found dead stranded on the northern coast of the state of Rio Grande do Sul during a period of 16 years (1994–2010) were utilized for estimation of age and growth rates using skeletochronology. The individuals analyzed were predominantly neritic juveniles, ranging from 53 to 101 cm curved carapace length (CCL; mean = 71 cm), with estimated ages between 10 and 29 years (mean = 15 years). Mean estimated annual growth rate was 2.1 cm CCL year^{-1} (1.9 cm SCL yr^{-1}), showing large variation among individuals and between successive years in the same individual. Generalized additive mixed models analysis indicated that growth response was influenced by age, CCL and year. The results demonstrated that the southern coast of Brazil is an important area for the development of neritic juveniles of this species, which appear to recruit to this region beginning at about 12 years of age and sizes greater than 55 cm CCL.

Key words: feeding ground, marine turtles, neritic juveniles, recruitment.

INTRODUCTION

Measuring the natural growth rates and age of the individuals in a population is fundamental to understand its demography, to evaluate habitat quality and develop management plans and adequate conservation measures for endangered species (Bjorndal & Bolten 1988). Although sea turtles have been more studied in comparison with other species of the marine fauna, management actions and their evaluation are still often hampered by the lack of key data on biology, human interactions, status of populations and threats (Hamann *et al.* 2010). An understanding of the growth and population dynamics of sea turtles is fundamental to determine if a stock is at risk and which management strategies are best suited for their

protection (Chaloupka & Musick 1997). Although growth and age can be obtained directly for many species, this is not feasible for most populations of sea turtles and another marine taxa due to their conservation status, long life and the logistic difficulties of making direct observations (Zug & Glor 1998). Since the mid 1990's, sclerochronology studies have been used in the investigation of temporally associated life history characteristics through analysis of hard or calcified structures in organisms (Avens & Snover 2013). This type of study includes analyses of bones (skeletochronology), scales, fish otoliths, mammalian teeth and other hard structures in many species (Hohn 2009; Avens & Snover 2013). In the case of long-lived and infrequently sighted animals, like marine mammals and turtles, dead stranded specimens can provide valuable data through this kind of analysis (Zug *et al.* 1986).

Study of the concentrically deposited skeletal growth marks in cortical bone, skeletochronology, is a powerful tool for providing information about age, growth

rates, age at sexual maturity, sexual dimorphism and inter-populational variation of current and extinct reptiles (Castanet 1994). Skeletochronology has been applied to numerous turtle species and has provided a better understanding of growth patterns and rates (Avens & Snover 2013). The pattern of annual deposition of growth marks in bones of loggerhead turtles has been validated in various studies, by tagging individuals with oxytetracycline and later recapture (Klinger & Musick 1992; Coles *et al.* 2001) and by analysis of samples from known-age (Snover & Hohn 2004) and tagged turtles (Snover *et al.* 2007; Avens *et al.* 2013).

In addition to age and size, various environmental parameters, such as food availability and temperature, can influence the growth rates of sea turtles (Heppell *et al.* 2003). Thus, growth rates obtained for a particular population/area or period cannot be extrapolated to another population or area, making it necessary to obtain specific estimates for different populations and even different foraging areas frequented by the same population (Casale *et al.* 2011). It has been demonstrated that skeletochronology can be utilized to back-calculate lengths in earlier ages and to obtain growth rates of loggerhead turtles (Snover *et al.* 2007; Avens *et al.* 2013). Growth rates of loggerhead turtles have been evaluated using skeletochronology in the Atlantic (Zug *et al.* 1986; Klinger & Musick 1995; Parham & Zug 1997; Bjorndal *et al.* 2003; Snover *et al.* 2010; Petitet *et al.* 2012; Avens *et al.* 2013), Pacific (Zug *et al.* 1995) and Mediterranean (Casale *et al.* 2011), and show substantial individual and populational variation. Casale *et al.* (2011) demonstrated that growth rates estimated using skeletochronology were comparable with those obtained through mark-recapture in loggerheads from the Mediterranean Sea.

The loggerhead turtle, *Caretta caretta* (Linnaeus, 1758), is circumglobally distributed and is the most abundant sea turtle along the Brazilian coast (Reis *et al.* 2010). The loggerhead is listed as endangered worldwide (Marine Turtle Specialist Group 1996) and in Brazil (BRASIL 2014). The principal foraging areas of loggerhead turtles in Brazil are thought to be located in waters of the southern region, and mature female turtles are expected to migrate seasonally to the north to lay their eggs on the beaches of Rio de Janeiro, Espirito Santo, Bahia and Sergipe (Marcovaldi & Chaloupka 2007).

As in other species of sea turtles, the life cycle of loggerhead turtles consists of stages of development that are spatially and temporally segregated, involving marked changes in habitat and diet (Musick & Limpus 1997; Reis *et al.* 2010). Soon after emerging from the nest, the turtles move immediately into the sea and swim actively to oceanic areas where they passively migrate with the oceanic gyres (Musick & Limpus 1997). After a period of a few years, they actively migrate to neritic development habitats in temperate and tropical areas (Musick & Limpus 1997). The recruitment from oceanic environment, where turtles are predominantly epipelagic, to neritic environment, where they are primarily benthic, may involve a transition period before individuals become completely neritic (Bolten 2003). The duration of the oceanic stage of loggerhead turtles is highly variable, with estimates ranging from 7 to 19 years in the North Atlantic (Bjorndal *et al.* 2000; Bjorndal *et al.* 2003; Avens *et al.* 2013) and from 8 to 19 years in southern Brazil (Petitet *et al.* 2012).

Although much knowledge has been obtained for South Atlantic loggerhead turtles from nesting areas in Brazil (Marcovaldi & Chaloupka 2007), feeding areas are still poorly studied (Di-Bernardo *et al.* 2003; Petitet *et al.* 2012). Dead loggerhead turtles are frequently found stranded in the extreme south of Brazil, about 1270 km south from the nearest nesting areas and may provide valuable samples to elucidate many aspects of biology for the species in the region. Information regarding age and growth rates of loggerhead turtles in this area is restricted to a single study based on specimens collected during 1 year (November 2008 to December 2009) in the oceanic and neritic environments from southern Rio Grande do Sul (Petitet *et al.* 2012). The current study aims to expand our knowledge on the age structure and growth rates of loggerhead turtles in the western South Atlantic based on specimens collected over a period of 16 years on the north coast of Rio Grande do Sul.

METHODS

Collection of samples

Samples were collected from dead-stranded sea turtles in the extreme south of Brazil, on the northern coast of the State of Rio Grande do Sul, along 270 km of sandy beaches between Lagoa do Peixe, Mostardas municipality (31°22' S, 51°02' W) and the Mampituba river, Torres municipality (29° 19' S, 49° 43' W) (Fig. 1). Sample collection was carried out through a partnership between the Universidade Federal do Rio Grande do Sul (UFRGS) and Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul (GEMARS) between 1994 and 2010. Dead-stranded sea turtles were identified to species, measured (curved carapace length and width – CCL and CCW, according to Bolten 1999) and subjected to necropsy *in situ*, where the humerus was dissected and collected. During the study, 294 specimens of loggerhead turtles were recorded, corresponding to 54% of the strandings of sea turtles on the northern coast of Rio Grande do Sul. A representative sample of 84 humeri was selected for skeletochronological analysis.
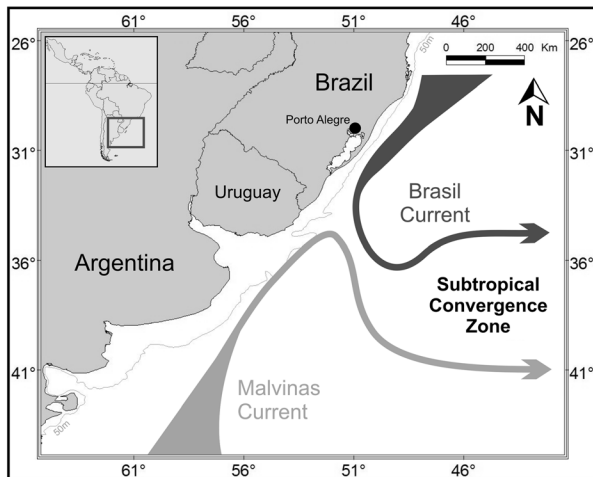
Fig. 1. General panorama of the study region, featuring the prevailing ocean currents. Humeri were obtained from loggerhead turtles on the southern shore of Brazil between 31°22' S, 51°02' W and 29°19' S, 49°43' W.

Humeri were macerated in water to remove soft tissues, dried and stored in the collection. Prior to histological preparation, 12 measurements of each humerus were taken, utilizing a pachymeter with a precision of 0.02 mm, in accordance with Zug *et al.* (1986). Values of CCL and diameters are presented here as mean ± standard deviation.

Skeletochronology

The methodology utilized for skeletochronology was based on Snover and Hohn (2004). Histological preparation began with removal of a transverse section of 3–5 mm of humerus in the portion distal to the deltopectoral crest, using a manual hacksaw. These sections were then decalcified in a commercial decalcifying solution (RDO, Apex Engineering Products Corporation). After decalcification, 25 μm thick sections were obtained with a cryostat (Leica CM 1850) at approximately −12°C. The sections were stained with Ehrlich's hematoxylin diluted with distilled water (1:1), and mounted on slides in 100% glycerin.

Identification, counting and measurements of lines of arrested growth (LAGs) were performed utilizing digital photographs, according to the method proposed by Goshe *et al.* (2010). Each section was photographed (Leica DFC 290HD camera, Leica Application Suite (LAS) version 3.4.1) in sequential portions under a light microscope (Leica DM750) or stereomicroscope (Leica S6D) with a magnification 20× or more. These sequential photographs were assembled using Adobe Photoshop (Adobe Systems Incorporated, San Jose, California, USA) to obtain high-resolution mosaics (Fig. 2) of the entire cross-section for each humerus.

Each photomontage was analyzed independently by two readers (AJL and LA) and the readings compared to obtain

a consensus on the position and number of LAGs that delimit the outer edge of individual skeletal growth marks. Next, the diameter of each LAG, as well as the whole section, was measured using the software ImageJ 1.46 (Research Services Branch, National Institute of Mental Health, Bethesda, Maryland). The interpretation of LAGs was performed according to Castanet and Smirina (1990) and Snover and Hohn (2004). Each LAG was examined around the entire circumference of the bone section for validation. When a LAG divided into two or more lines, each was counted as one independent skeletal growth mark (Snover & Hohn 2004).

Age estimation

In decalcified and stained humerus sections, regions of bone growth appear as wide zones with light coloration, followed by dark growth lines defined as LAGs (*lines of arrested growth*) (Goshe *et al.* 2010). Resorption and redeposition of growth lines is extensive in humeri of larger loggerheads because of a more than 10-fold increase in diameter from young up to adult age, in addition to the occurrence of alterations in the proportions and orientation of the longitudinal axis (Zug *et al.* 1986). Resorption leads to the loss of lines deposited earlier in life and requires an extrapolation from the mean diameter of LAGs in humeri of younger turtles that do not exhibit resorption, to estimate the number of LAGs lost in older individuals (Parham & Zug 1997).

Snover and Hohn (2004) recorded the deposition of a diffuse *annulus* in the first year of life of *Lepidochelys kempii* Garman, 1880 and well-defined growth lines starting at the end of the second year. LAGs consistent in appearance with annuli have been found in oceanic stage juvenile loggerheads
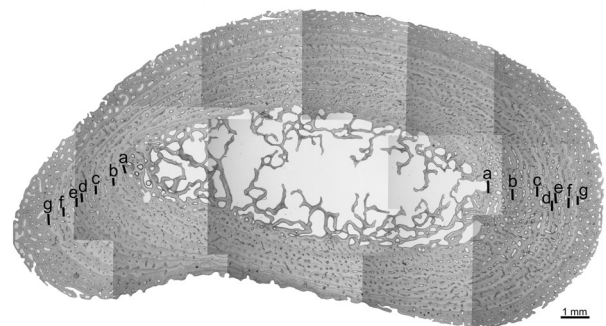

Fig. 2. Assembled mosaic from the sequential photographs (4× magnification) of a histologically processed loggerheads humerus cross-section. Lines of arrested growth are marked at their lateral edges with black lines. The diameter of each line of arrested growth was measured parallel to the major axis between the two marks corresponding to the ends of the same line. Bar = 1 mm.

as well (Petitet *et al.* 2012; Avens *et al.* 2013). In turtles that retain the *annulus*, age can be directly inferred. However, in individuals that show resorption of the first LAGs, it is necessary to apply a correction factor enabling the determination of the number of lines lost from measurements of LAGs of turtles that retain the *annulus* (Goshe *et al.* 2010; Avens *et al.* 2012).

In this study, no loggerhead turtle humeri analyzed retained a growth mark consistent in appearance with an *annulus*. To estimate number of resorbed LAGs, we utilized the correction factor formulated by Petitet *et al.* (2012) based upon skeletochronological analysis of loggerhead turtles from the southern coast of Rio Grande do Sul and of the oceanic zone between the latitudes 29° and 38°S and longitudes 45° and 51°W. This correction factor (Equation 1) is derived from the relationship between the LAG number and its respective diameter, on the basis of turtles that show the *annulus* (Petitet *et al.* 2012). Equation 1 was applied for the turtles analyzed in the present study utilizing the diameter of the innermost LAG, to obtain an estimate of the number of resorbed LAGs. This number was added to the number of observed LAGs to estimate the age of individuals.

$$\text{Diameter of LAG} = 2.18 \star (\text{number of line})^{0.35} \quad (1)$$

Back-calculation and growth rates

Spacing between sucessive growth marks is not constant during life, but reveals the variation in growth of bone and body and is a record of individual growth curves over time (Castanet & Smirina 1990). It has already been demonstrated in loggerhead turtles, through analysis of humeri from tagged turtles that later stranded dead that the diameter of LAGs can be converted to measurements of carapace length, representing the size of the turtle in the period of the deposition of a particular LAG (Snover *et al.* 2007; Avens *et al.* 2013). This method, called back-calculation, has been utilized for loggerhead turtles in various studies (Snover *et al.* 2007; Snover *et al.* 2010; Casale *et al.* 2011; Petitet *et al.* 2012; Avens *et al.* 2013).

To enable the prediction of CCL from the diameters of LAGs, the relation between CCL and diameter of humerus section was modeled using Equation 2. This model had the best fit to the data for loggerhead turtles of the North Atlantic (Snover *et al.* 2007; Avens *et al.* 2013) and South Atlantic (Petitet *et al.* 2012).

$$\text{CCL} = \text{CCL}_\text{h} + b(D - D_\text{h})^c \quad (2)$$

where CCL = curved carapace length of individual; CCL_h = curved carapace length of hatchling; D = diameter of humerus section; D_h = diameter of humerus section of hatchling; b = angular coefficient; c = coefficient of proportionality.

Measurements of carapace length and diameter of the humerus in hatchling loggerhead turtles from Brazil are not available. Therefore, we utilized the measurements reported by Snover *et al.* (2007), where the straight carapace length (SCL) (4.6 cm) was converted to curved length by the linear equation developed by Snover *et al.* (2010) (Eqn 4) [CCL_h = 4.77 cm; D_h = 1.9 mm].

After application of Equation 2, the values of b and c were obtained, and it was possible to apply the back-calculation model in the succeeding section to determine the CCL of the individual at each LAG, that is, at each year of life. This model was developed by Francis (1990) and adapted for the utilization in sea turtles by Snover *et al.* (2007).

$$\text{CCL}_\text{est} = [\text{f}(D_\text{line})] \star [\text{CCL}_\text{final}] \star [\text{f}(D_\text{final})] \quad (3)$$

where CCL_est = estimated curved carapace length; $\text{f}(D_\text{line})$ is Equation 2 applied to the diameter of the growth line in question; CCL_final = CCL of turtle at time of death; $\text{f}(D_\text{final})$ = Equation 2 applied to the diameter of the humerus at time of death.

After obtaining estimated CCL of the individual for each LAG, it was possible to calculate the annual growth rates by calculating the difference between adjacent pairs of estimated CCLs (Avens *et al.* 2012, 2013). Individual growth rates were grouped in accordance with the estimated initial CCL into categories of 10 cm and the mean growth rates calculated for each size range. For comparison with other published studies that utilize SCL, CCL was converted to SCL using the equation 4, obtained for loggerhead turtles of the eastern coast of the USA (Snover *et al.* 2010).

$$\text{SCL} = 0.923 \star \text{CCL} + 0.189 \quad (4)$$

Each back-calculated growth rate was assigned to the mean CCL calculated for the growth interval, as well as the age estimate and calendar year assigned to the initial LAG for the LAG pair used to calculate the growth rate. To account for incorporation of multiple annual growth intervals from each turtle and potential non-linear relationships, generalized additive mixed models (GAMMs) were applied to investigate the potential influence of continuous covariates such as size (CCL), age and year on somatic growth response. The GAMM models included turtle ID as a random, individual-specific effect, incorporated an identity link and robust quasi-likelihood function and were implemented using the *mgcv* and *nlme* packages in the statistical program R version 3.1.1 (Wood 2006; R Core Team 2014). Significance of continuous covariates was determined using non-parametric F ratio tests. Because low levels of co-concurvity were found between CCL and age (0.46), CCL and year (0.07) and age and year (0.08), all covariates were initially incorporated into a single model ($\text{GAMM}_{\text{CCL} + \text{Age} + \text{Year}}$). Stepwise removal of covariates in subsequent model runs was conducted, and model fits were evaluated using AIC values.

RESULTS

Age estimation

Representative humeri of 84 specimens (mean CCL = 71.5 ± 10.13 cm, range 53.5–101 cm) were selected for skeletochronology from a larger sample of 294 loggerhead turtles found dead-stranded in the study area between 1994 and 2010 (mean CCL of 75.1 cm ± 11.85 cm, range 43–120 cm). The CCL of the 84 selected specimens did not differ statistically from the entire sample (Kolmogorov–Smirnov = 0.16324; $P = 0.0662$). Many specimens displayed signs of interaction with coastal fishing. Five humeri were discarded because of problems during processing; therefore, age was estimated in 79 specimens. The number of LAGs in the humerus ranged between 3 and 19. Estimated ages ranged between 10 and 29 years (mean = 15 ± 3.2 years; Fig. 3), corresponding to sizes between 53.5 and 101 cm CCL (mean = 71.4 ± 9.99). Most individuals (85%) ranged between 11 and 18 years (Fig. 3). CCL and age were positively related ($r^2 = 0.21$; $P = 0.0002$; Fig. 4).

Growth

CCL showed a strong correlation with humerus diameter ($r = 0.85$; $P < 0.0001$; Fig. 5). Applying Equation 2 to our data, we obtained the angular coefficient ($b = 5.578$) and the coefficient of proportionality ($c = 0.789$) and back-calculated the size of the individual for each measurable LAG. Taking the difference between successive back-calculated or measured lengths ($n = 665$) allowed estimation of annual growth rates for 75 loggerhead turtles on the basis of 588 total growth increments. Mean overall annual growth rate was 2.1 cm CCL year^{-1} (Table 1), corresponding to 1.9 cm SCL year^{-1}.

Fig. 4. Relationship between curved carapace length and estimated age of loggerhead turtles from southern Brazil ($n = 75$; $r^2 = 0.21$; and $P = 0.0002$).

Results of GAMM analysis indicated that the model that best fitted the data incorporated all available covariates (CCL, age and year) and each had a significant influence on growth response (Table 2). Individual, random effects were significant as well ($P < 0.001$; Log-likelihood ratio test). Growth response relative to CCL was variable, increasing overall through approximately 80 cm CCL and subsequently decreasing (Fig. 6(a)). In contrast, growth response decreased consistently with age (Fig. 6(b)) and increased during the entire time frame for which back-calculated growth rates were available (1985–2009; Fig. 6(c)).

DISCUSSION

Age estimation

The ranges of sizes (53.5–101 cm CCL) and ages (10–29 years) recorded on the northern coast of Rio Grande do Sul are similar to the sizes reported for the southern coast of the state (45–102 cm CCL,
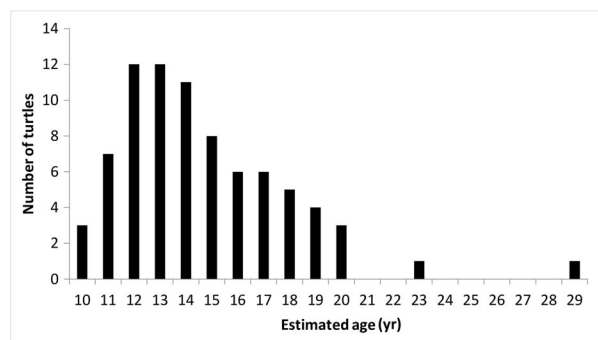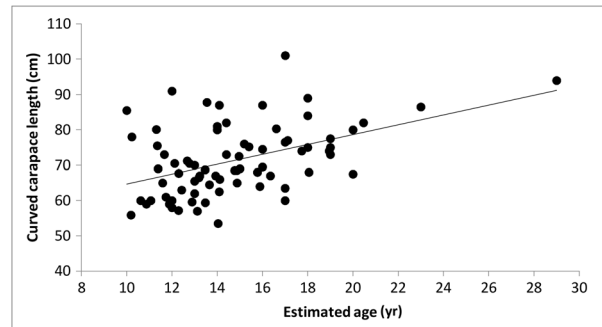

Fig. 3. Age distribution of loggerhead turtles analyzed by skeletochronology from southern Brazil ($n = 79$).
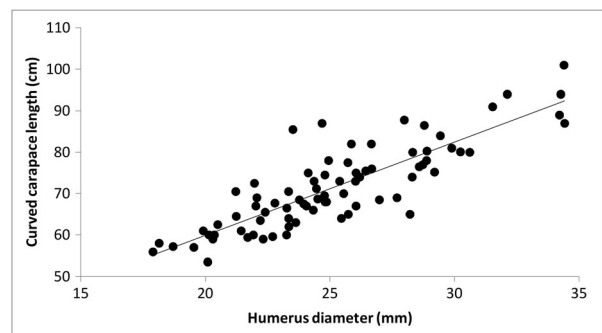

Fig. 5. Relationship between curved carapace length and humerus diameter of loggerhead turtles from southern Brazil ($n = 79$; $r = 0.85$; $P < 0.0001$). The solid line represents a linear model.

Table 1. Size class-specific CCL growth rates back-calculated from all skeletal growth increments ($n = 588$) retained in the humeri of the loggerhead turtles ($n = 75$) stranded dead in southern Brazil

Size class (cm CCL)	N	Growth rates (cm yr^{-1})	SD	Min	Max
40–49.9	41	3	1.12	0.40	6.48
50–59.9	207	1.77	1.25	0.00	5.98
60–69.9	236	1.63	1.51	0.00	10.01
70–79.9	63	2.76	1.93	0.15	10.01
80–89.9	21	2.71	1.38	0.19	5.69
90–99.9	21	0.63	0.88	0.02	3.38
100–109	1	2.44	—	—	—

CCL, curved carapace length; n, number of growth rates back-calculated from skeletal growth increments.

Table 2. Statistical output from generalized additive mixed models (GAMMs) used to investigate the potential influence of different covariates on growth response of loggerhead turtles

Model	Adjusted r^2	AIC	Variable	Edf	F	Prob(F)	Variable	Estimate	Std Error	t	Pr > |t|
GAMM$_{CCL + Age + Year}$ ($n = 583$)	0.25	1987	*CCL* (cm)	5.66	19.19	<0.001	*Intercept*	1.90	0.08	24.03	<0.001
			Age (yr)	4.06	23.41	<0.001					
			Year	1.00	27.14	<0.001					
GAMM$_{CCL + Age}$ ($n = 583$)	0.17	2008	*CCL* (cm)	5.96	16.60	<0.001	*Intercept*	1.97	0.09	21.51	<0.001
			Age (yr)	3.85	17.00	<0.001					
GAMM$_{CCL + Year}$ ($n = 583$)	0.10	2061	*CCL* (cm)	5.92	12.49	<0.001	*Intercept*	1.99	0.09	21.70	<0.001
			Year	1.00	0.09	0.76					
GAMM$_{Age + Year}$ ($n = 583$)	0.15	2069	*Age* (yr)	4.11	16.93	<0.001	*Intercept*	1.93	0.08	24.84	<0.001
			Year	3.61	6.49	<0.001					
GAMM$_{CCL}$ ($n = 583$)	0.11	2057	*CCL* (cm)	5.91	12.49	<0.001	*Intercept*	1.99	0.09	21.96	<0.001
GAMM$_{Age}$ ($n = 583$)	0.08	2080	*Age* (yr)	4.05	10.81	<0.001	*Intercept*	1.99	0.09	22.21	<0.001
GAMM$_{Year}$ ($n = 583$)	0	2111	*Year*	1.00	0.34	0.56	*Intercept*	1.99	0.09	21.14	<0.001

Edf, estimated degrees of freedom; CCL, curved carapace length.

Petitet *et al.* 2012), as well as the age range (9–24 years). The age and size ranges recorded in southern Brazil confirm that the coastal zone is utilized principally by neritic juveniles, making it an important developmental area for the species. Studies of the diet of loggerhead turtles stranded on the coast corroborate that this region is an important area for feeding and development of loggerhead turtles in the neritic phase (Bugoni *et al.* 2003; Lenz, unpublished data, 2009). We propose that the individuals typically migrate to other foraging areas to the north to attain an age and size close to that of sexual maturity.

The data presented herein demonstrate that the recruitment of juveniles from oceanic habitat to the neritic environment in southern Brazil can occur starting at 10 years of age upon reaching about 50 cm CCL. However, the majority of individuals appear to recruit at about 12 years and CCL greater than 55 cm. In southern Rio Grande do Sul, Petitet *et al.* (2012) estimated the duration of the oceanic stage as 11.5 years. Bjorndal *et al.* (2000, 2003) estimated the duration of oceanic

stage of loggerhead turtles between 7 and 11.5 years and the recruitment to neritic habitats at 46–64 cm CCL in the North Atlantic. In a recent study, Avens *et al.* (2013) estimated the mean oceanic stage duration and size of neritic recruitment for loggerhead sea turtles in the North Atlantic at 12 to 13 years and 55.3 cm SCL. Barceló *et al.* (2013) tracked 26 juvenile loggerhead turtles in the western South Atlantic with a mean length of 61 cm and on the basis of the movement patterns found, suggested that the individuals were in a transition stage. The majority of turtles tracked remained in the oceanic environment, while others moved from oceanic to neritic regions and later either remained in this habitat or returned to the oceanic environment. These data not only support our conclusion with respect to the recruitment sizes in the western South Atlantic but also indicate that this transition may not be definitive and can vary greatly among individuals, which has already been noted in the North Atlantic by McClellan and Read (2007). This individual and temporal variability for recruitment age and size demonstrates the potential plasticity of this
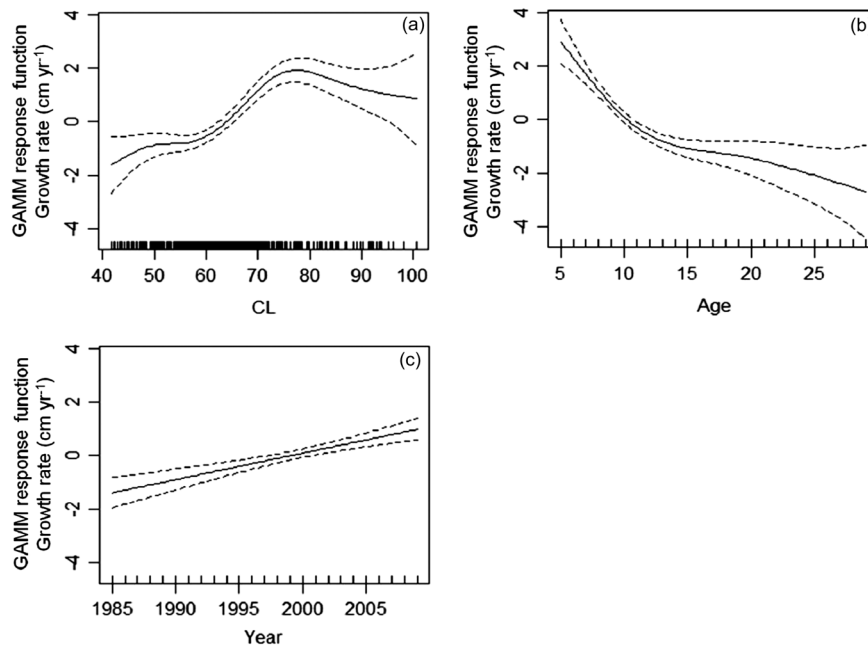
Fig. 6. Summary of generalized additive mixed model (GAMM) growth response relative to potential covariates (a) curved carapace length (CCL); (b) age; and (c) calendar year of loggerhead turtles from southern Brazil. Within each plot, the solid line represents mean growth response (on a scale centered around 0) for the covariate conditioned on other covariates included in the model and dashed lines represent the extent of the 95% Bayesian credible interval.

life history trait, which can potentially be affected by a number of biological and physical factors (Avens *et al.* 2013).

Necropsy of individuals did not include analysis of gonads to determine sexual maturity. On the basis of the minimal size of female turtles that lay their eggs on the shore of the state of Espirito Santo (83 cm CCL, Baptistotte *et al.* 2003), 86% of our sample would be classified as juveniles and 14% as possible adults. However, Bjorndal *et al.* (2001) affirm that many individuals can mature at lengths much greater than the minimal length of female turtles in nesting areas, and because of low growth rates in these subadults, mean age at sexual maturition for individuals maturing at larger sizes could be substantially greater than the mean for reaching 83 cm CCL. In addition, high variability in size at sexual maturity has been observed for captive green and Kemp's ridley sea turtles (Bjorndal *et al.* 2013a, 2014) and inferred for loggerheads (Avens *et al.* 2015). As a result, application of minimal size at maturation would likely understimate age at sexual maturation for these populations. If we considered the mean length of female turtles laying their eggs in Espirito Santo (102.7 cm CCL, Baptistotte *et al.* 2003), as suggested by Bjorndal *et al.* (2001, 2013a), none of the individuals analyzed in this study would be classified as adult.

Estimates of age at sexual maturition can vary between geographic regions and even between different studies in the same region, depending on the technique utilized.

Petitet *et al.* (2012) estimated the age at sexual maturity for loggerhead turtles of the South Atlantic to be over 32 years. In the North Atlantic, age at maturity was determined to be between 22 and 45 years (Klinger & Musick 1995; Bjorndal *et al.* 2000, 2001; Scott *et al.* 2011; Avens *et al.* 2015). On the basis of these data and considering the mean size of female turtles in egg-laying areas of Brazil (102.7 cm CCL, Baptistotte *et al.* 2003), we believe that the large majority of the individuals analyzed here still had not reached sexual maturity.

Halliday and Verrel (1988) affirm that age and body size are generally positively related in amphibians and reptiles, but this relationship is usually weak and considerable variation in body size occurs within the same age class, as demonstrated here (Fig. 4). Consequently, the use of body size is not justified as a reliable indicator of the age of an individual (Halliday & Verrel 1988). Accordingly, studies of skeletochronology are very relevant and can provide more precise estimates of age.

Growth

The strong correlation between CCL and humerus diameter yielded by the analyses presented herein was expected and indicates a positive relation between somatic and bone growth, which allows us to back-calculate earlier CCLs from the diameters of LAGs and thereby obtain measurements of annual growth of individuals. Mean annual growth rate observed (2.1 cm CCL year^{-1} or 1.9 cm

SCL year^{-1}) was similar to the rate recorded in the southern region of Rio Grande do Sul including oceanic and neritic individuals (2.45 cm CCL year^{-1}; Petitet et al. 2012). Comparing the growth rates per size class between different populations of the Atlantic (Table 3), we can see a large variation within and between the size classes. However, these growth rates are comparable overall with those reported most recently (Braun-McNeill et al. 2008; Petitet et al. 2012; Bjorndal et al. 2013b; Avens et al. 2015). Differences in environmental factors, such as temperature and food resources, may be responsible for geographic variation in the growth rates. In fact, a significant effect of latitude on the growth of juvenile loggerhead turtles (SCL between 50 and 80 cm) was observed in the western North Atlantic (Bjorndal et al. 2013b). A temporal factor also may explain part of the regional variation: the studies in Georgia (Parham & Zug 1997), Chesapeake Bay (Klinger & Musick 1995) and southern Rio Grande do Sul (Petitet et al. 2012) only considered individuals stranded during a short period of 1 or 2 years, while our sample encompasses 16 years of collection, registering growth rates attributed to the years 1985 to 2009. The GAMM model indicated that year was a factor that significantly influenced growth rates, with growth response increasing during the entire time frame for which back-calculated growth rates were available (1985–2009). Other studies have also found significant inter-annual variation in growth rates of loggerhead turtles in the North Atlantic (Bjorndal et al. 2003, 2013b; Avens et al. 2013, 2015). It is difficult to identify the causes of inter-annual variations in growth rates, but they may be associated with changes in environmental parameters and/or density-dependent effects (Bjorndal et al. 2013b;. Avens et al. 2015).

The coastal and marine region of Rio Grande do Sul is temperate-warm because of the influence of the South Atlantic Subtropical Convergence and stabilizing action of the extensive Patos–Mirim lagunar system (Seeliger & Odebrecht 1998). Growth in temperate zones can be influenced by the additional expenditure of energy associated with migratory habits and by the seasonal availability of food resources (Klinger & Musick 1995), which can be related to the large number of nearly zero growth rates recorded in this study. The first available satellite telemetry data for juvenile loggerhead turtles in the western South Atlantic indicate a strong seasonal variation in the movements in the oceanic zone, where lower-latitude (26 to 41°S) areas are utilized in the winter (Barceló et al. 2013). Our area of study (29–31°S) is within the wintering area of juvenile loggerhead turtles tagged by Barceló et al. (2013). However, this previous study involved relatively smaller specimens (mean 61.8 cm CCL) captured in the oceanic zone, while the present study is focused on dead-stranded loggerheads in neritic habitat; the two life stages may possibly exhibit different movement patterns. Neritic juveniles of some populations of sea turtles in temperate zones undergo seasonal migrations between foraging areas at high latitudes in the summer and lower latitudes in the winter (Musick & Limpus 1997). Petitet et al. (2012) suggested that the wide seasonal variation in water temperatures in southern Brazil might induce the migrations of loggerhead turtles to the north during cold months. A more pronounced seasonal migration for neritic juveniles loggerhead turtles is a plausible hypothesis, supported by our results, as

Table 3. Comparison of size class-specific growth rates (straight carapace length (SCL), cm yr^{-1}) of loggerhead turtle populations of Atlantic. Growth rates from neritic turtles. Mean growth rates or ranges presented, as available

Size class (cm SCL)	Northern RS, Brazil (present study)[†]	Southern RS, Brazil (Petitet et al. 2012)[†]	Chesapeake Bay, USA (Klinger & Musick 1995)[†]	Georgia, USA (Parham & Zug 1997)[†]	North Carolina, USA (Braun-McNeill et al. 2008)[‡]	Western North Atlantic (Avens et al. 2015)[†]	North Atlantic (Bjorndal et al. 2013b)[‡]
30–39.9	2.24	—	—	—	—	2.6	5.9
40–49.9	2.41	2.2	5.28	3.5–3.6	—	2.8	—
50–59.9	1.47	2.4	5.29	2.8–3.8	1.58	3.1	2.4
60–69.9	1.83	2.4	5.27	2.7–3.1	1.82	2.2	1.5
70–79.9	2.68	2.3	4.35	1.6–2.4	1.63	2.1	1.1
80–89.9	0.84	2.1	3.08	0.9–3.5	—	1.0	0.2
90–99.9	2.04	2	2.87	—	—	0.2	0.2

[†]Skeletochronology.
[‡]Mark-recapture.
RS, Rio Grande do Sul state; SCL, straighline carapace length.

only 8% of individuals were recorded in the colder months of the year (June to September; GEMARS, unpublished data, 2013). Telemetry studies involving neritic juveniles could elucidate these migratory patterns.

The large variation in growth rates presented herein has been reported in different populations of loggerhead turtles, among individuals as well as between successive years in the same individual (Bjorndal *et al.* 2003; Braun-McNeill *et al.* 2008; Casale *et al.* 2011; Avens *et al.* 2013, 2015). Each habitat that turtles utilize during their ontogeny has different environmental parameters, such as food availability and temperature, which can influence growth rates (Heppell *et al.* 2003). In addition, factors related to the timing of development and hatching (early or late during the reproductive season) and latitude of birth (which may reflect genetic differences) can influence the growth rates of posthatchling loggerhead turtles (Stokes *et al.* 2006). However, it is still not known if these factors can affect growth over other stages of life. The large variability in growth rates between different years in the same individual suggests that turtles may frequently move between different areas or habitats with different thermal and trophic characteristics (Casale *et al.* 2011). This opportunistic behaviour, probably driven by the search for food resources and favourable environmental conditions, is likely to be one of the factors responsible for the large variation among the growth rates observed in the present study. McClellan and Read (2007) demonstrated that the change made by juveniles between oceanic and neritic environments may not be definitive. Some young neritic individuals returned to the ocean habitat after recruitment and remained there for a variable period, as has also been observed in the western South Atlantic (Barceló *et al.* 2013). Thus, recruitment to the neritic environment in the southwest Atlantic does not appear to be a definitive change but rather a period in which individuals may shift between environments in search of more favourable conditions. This behavioural plasticity could potentially affect the growth rate of these individuals.

The GAMM model indicated that growth rate decreased consistently with age, as observed in other studies with this same model (Avens *et al.* 2013, 2015), while the growth response relative to CCL was variable, increasing overall through approximately 80 cm CCL and subsequently decreasing. Loggerhead populations in the North Atlantic showed a small increase in growth rates around 50–60 cm SCL (Avens *et al.* 2013, 2015); however, nothing is so conspicuous as reported in our study. These increases were observed in the size class just before reaching sexual maturity (83 cm of minimum CCL, Baptistotte *et al.* 2003). Loggerheads in the Mediterranean appear to increase growth rates until reaching sexual maturation (Piovano *et al.* 2011). The increased growth rates observed may be the result of the increase in size allowing turtles to exploit different, higher-nutrition prey items (e.g. having the gape and jaw strength to crush large, hard molluscan prey instead of feeding on jellies (Pajuelo *et al.* 2010).

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