The following supplements accompany the article

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

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Marine Ecology Progress Series 491: 235–251 (2013)

SUPPLEMENT 1. METHODS

Skeletochronology

Sample preparation and analysis

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 former, 2 sequential cross-sections were taken from each humerus, just distal to the delto-pectoral muscle insertion scar and perpendicular to the long axis of the bone, using a low-speed Isomet saw with a diamond-coated wafering blade (Buehler). The section processed for skeletochronological analysis was allowed to soak for 1 to 9 d (depending on humerus size) in Cal Ex II fixative/decalcifier (Fisher Scientific) and 25 µm thin sections were cut from the side of the section that had been proximal to the stable isotope section using a freezing stage microtome (Leica Microsystems). Thin sections were stained using Ehrlich’s hematoxylin and mounted in 100% glycerin on microscope slides under glass cover slips sealed with Cytoseal (Thermo-Scientific/Richard Allen Scientific).

The stained, thin sections of humeri from previous studies were histologically processed using the methods outlined in Snover & Hohn (2004) and Goshe et al. (2009). However, these thin sections had faded significantly over time and therefore needed to be rinsed sequentially in RDO (a dilute, commercially prepared decalcifying solution containing hydrochloric acid; Apex Engineering) and tap water, before being re-stained and mounted on microscope slides.

Calibrated, digital images of entire humerus sections at 4x magnification were obtained by first taking sequential, partial images using a CCD digital camera in conjunction with Microsuite image analysis software (Olympus America) and then combining those into a composite image using Adobe Photoshop (Adobe Systems). Counts of the lines of arrested growth (LAGs) that delimit the outer edge of each skeletal growth mark (Fig. 2a) within each digital image were first conducted by 3 independent readers (L. Avens, L. R. Goshe, and M. Pajuelo), followed by a joint assessment to reach consensus. LAG and humerus section diameters were measured along the axis parallel to the dorsal edge of the bone and, to ensure consistency, the diameter of the innermost measurable LAG was used as a proxy for resorption core diameter.

Assigning age

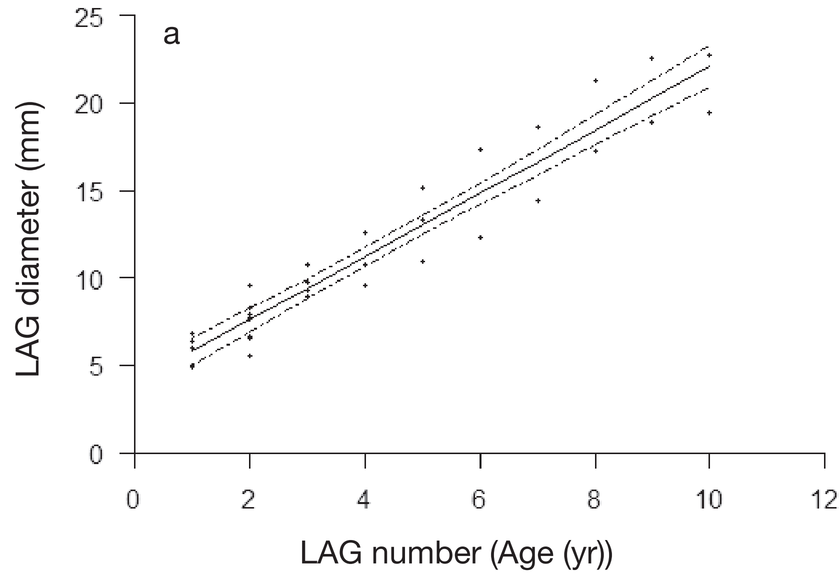
Provided that LAG deposition in loggerheads occurs in late winter/early spring, as demonstrated for Kemp's ridleys (Snover & Hohn 2004), a mean August/September hatch date in the western North Atlantic yields ages at LAG deposition of ~0.75, 1.75, 2.75 yr, and so on. The last growth increment in each humerus was examined taking into account stranding month; for spring strandings (April to June) where bone growth to the outside of the last LAG was near 0 (i.e. the LAG was marginally differentiated from the edge of the bone), the LAG was assigned to the year of stranding. In contrast, for spring strandings exhibiting incremental growth greater than 0 exterior to the last discernible LAG, this LAG was assigned the year prior to stranding, as it was assumed that the current year's LAG had not yet differentiated from the edge. Initial age calculations made using whole numbers (observed + estimated numbers of resorbed LAGs) were modified by first rounding downward to the previous $x + 0.75$ yr and then ultimately assigned an age corresponding with the nearest 0.25 yr to the date of stranding. For example, if a turtle whose humerus did not exhibit resorption and retained 8 LAGs stranded in late December, its initial age estimate based on LAG count and hatch date would be 7.75 yr, but its final age estimate would be 7.75 yr + 0.5 yr = 8.25 yr, based on its stranding date.

Stable isotope analysis

Approximately 0.6 mg of bone dust resulted from each increment and samples were immediately packed into sterilized tin capsules, then analyzed by a continuous-flow isotope-ratio mass spectrometer in the Stable Isotope Laboratory at the University of Florida, Gainesville, USA. This system consisted of a Costech ECS 4010 elemental combustion system interfaced via a ConFlo III device (Finnigan MAT, Bremen, Germany) to a Deltaplus gas isotope-ratio mass spectrometer (Finnigan MAT, Bremen, Germany). Sample stable isotope ratios relative to the isotope standard are expressed in the following conventional delta (δ) notation in parts per thousand (‰)

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

where R_{sample} and $R_{standard}$ are the corresponding ratios of heavy to light isotopes ($^{15}N/^{14}N$) in the sample and standard, respectively. $R_{standard}$ for ^{15}N was atmospheric N_2. The reference materials IAEA N1 ammonium sulfate ($(NH_4)_2SO_4$; $\delta^{15}N = +0.4‰$) and/or USGS 40-L-glutamic acid ($C_5H_9NO_4$; $\delta^{15}N = -4.52‰$) were used as calibration standards in all runs. All analytical runs included samples of standard materials inserted every 6 to 7 samples to calibrate the system and compensate for any drift over time. Replicate assays of standard materials indicated measurement errors of 0.095‰ for nitrogen and, in addition to stable isotope ratios, %N was measured for each tissue sample. Samples were combusted in pure oxygen in the elemental analyzer and resultant CO_2 and N_2 gases were passed through a series of thermal conductivity detectors and element traps to determine percent composition. Acetanilide standards (10.36% N) were used for calibration.



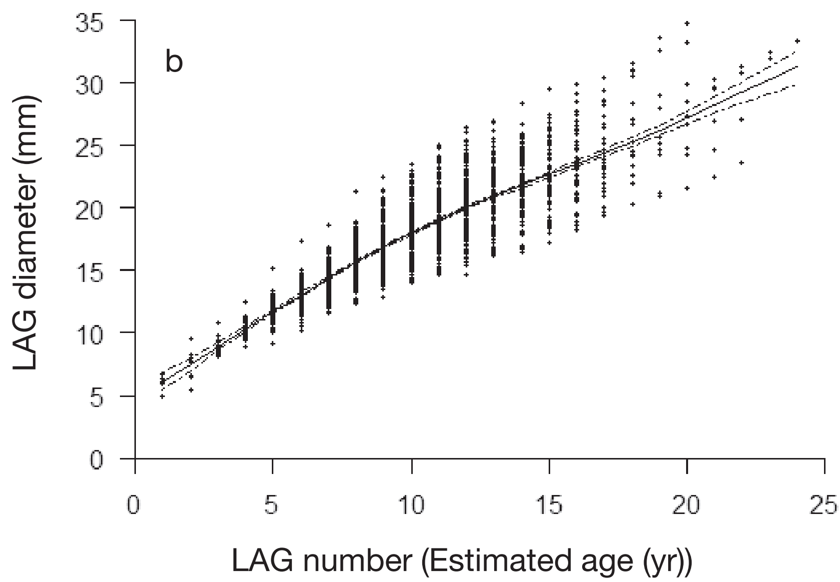


Fig. S1. *Caretta caretta*. Correction factors based on models of Line of Arrested Growth (LAG) number:LAG diameter relationships to account for any LAGs lost to resorption at the core of the humerus. (a) First order correction factor based on all humeri in which diffuse first-year LAG or 'annulus' was retained, allowing direct assignment of age based on total LAG number (*n* = 10 humeri, 34 LAGs); (b) 2nd order correction factor based on combination of (a) and additional humeri for which LAG number could be estimated using (a) (*n* = 225 humeri, 2031 LAGs) (see 'Age' in 'Results' in the main article for additional information). Dotted lines represent 95% confidence intervals

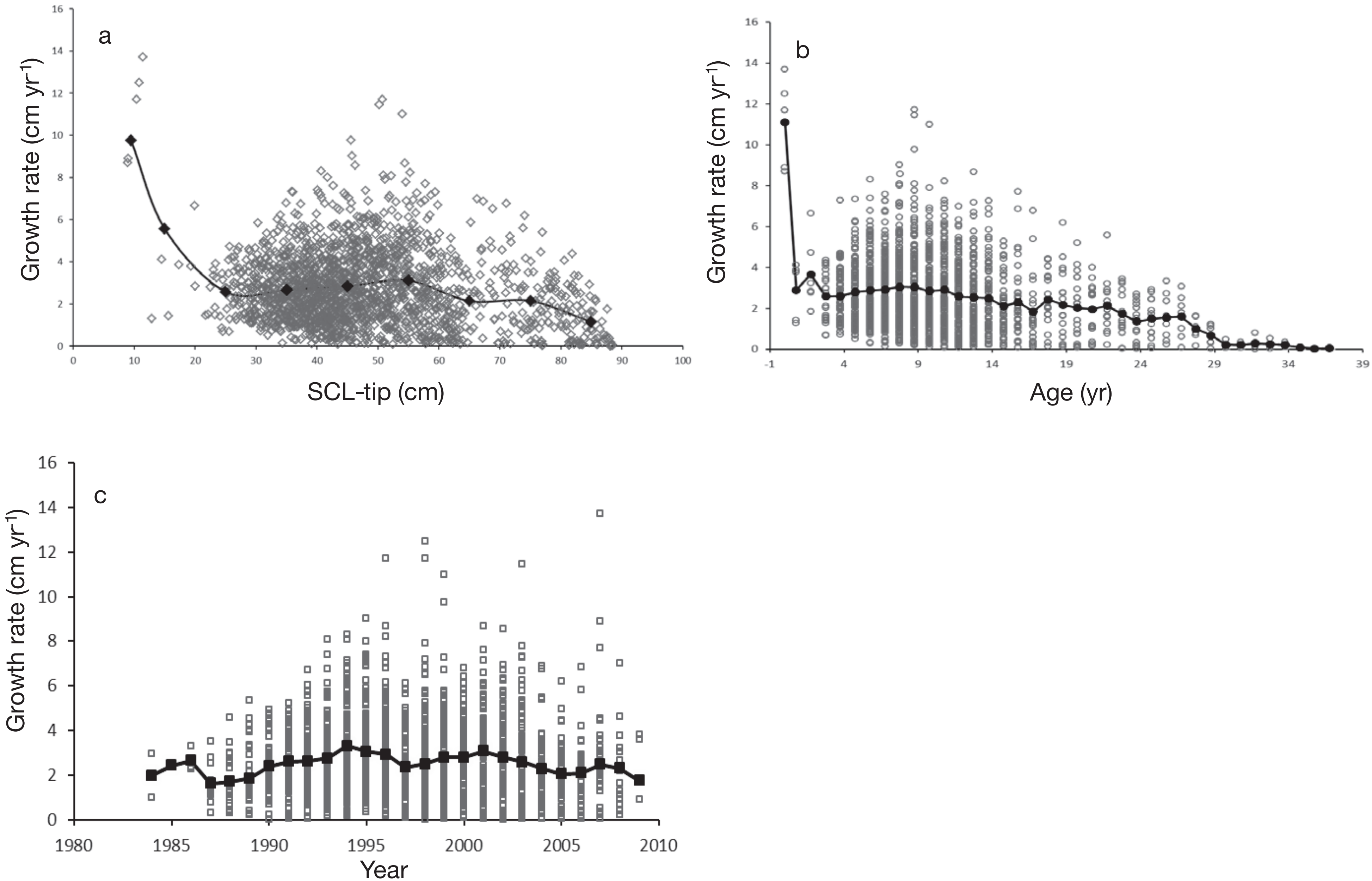


Fig. S2. *Caretta caretta*. Size, age, and calendar year-specific growth data for all back-calculated (n = 1877) growth increments. Open symbols denote individual growth rates; filled symbols connected by the continuous line signify means. Total sample size is less than that for all back-calculated length-at-age relationships (Fig. 3 in the main article) because growth intervals could not be calculated when 2 consecutive LAG measurements were not available

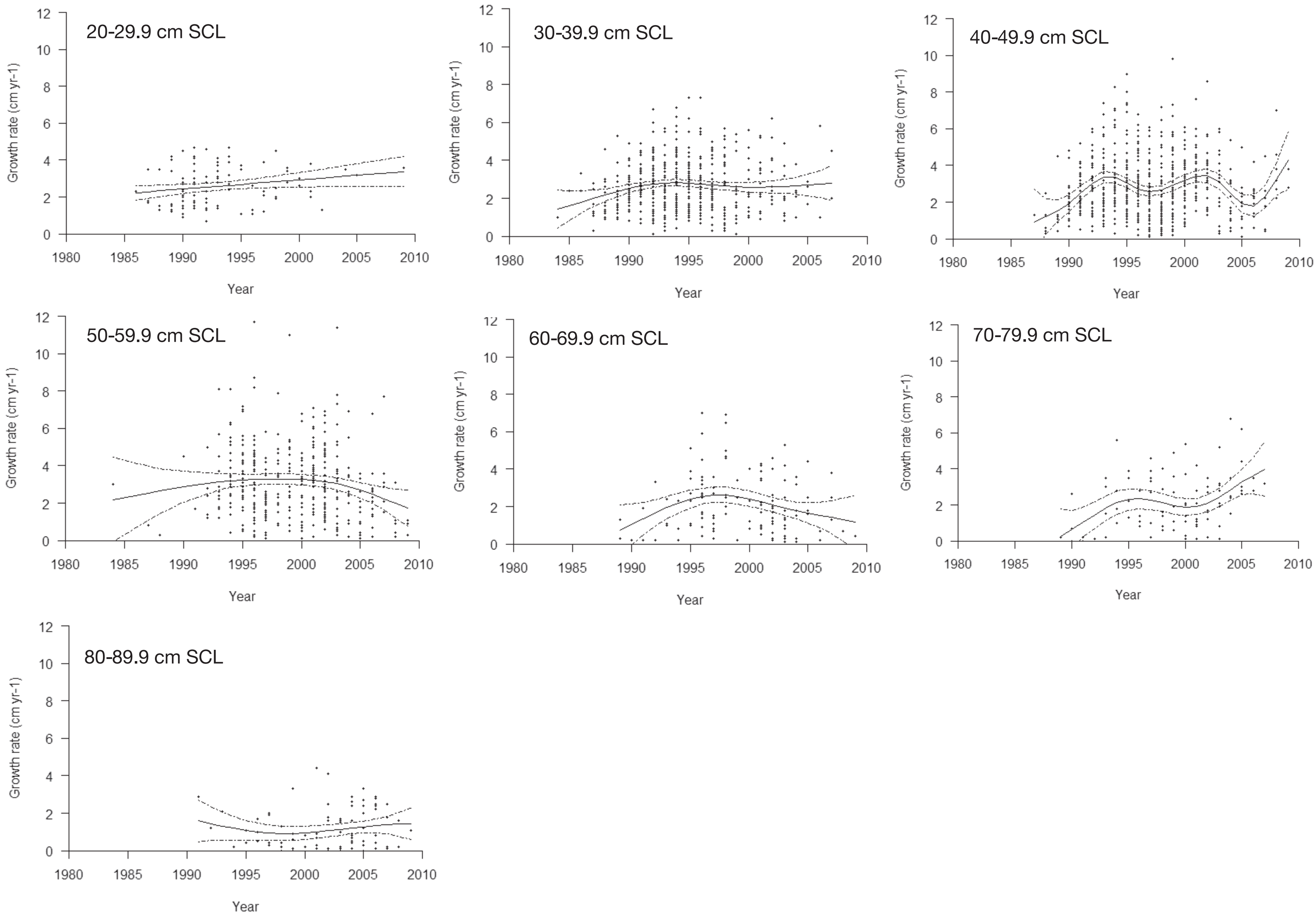


Fig. S3. *Caretta caretta*. Smoothing spline fits to back-calculated growth trajectories by calendar year for each size class represented in the sample. Dotted lines represent 95% confidence intervals. Sample sizes provided in Table S3

Table S1. *Caretta caretta*. Summary of geographic distribution, sample sizes, and straightline carapace lengths (SCL) of loggerhead sea turtles from which humeri were collected for the present study. NA: not applicable

Location	*n*	SCL (cm) range (mean ± SD)
Azores Islands	22	8.2-63.3 (23.2 ± 17.7)
Massachusetts (MA)	1	33.3 (NA)
New Jersey (NJ)	4	61.6-88.6 (73.6 ± 11.8)
Maryland (MD)	5	51.8-66.2 (62.0 ± 8.0)
Virginia (VA)	35	44.0-88.5 (61.8 ± 14.7)
North Carolina (NC)	159	43.6-88.4 (58.5 ± 8.9)
South Carolina (SC)	1	53.6 (NA)
Georgia (GA)	6	52.1-81.9 (59.9 ± 11.2)
Florida Atlantic (FL)	13	23.1-87.0 (52.8 ± 20.4)

Table S2. *Caretta caretta*. Comparison of size class-specific growth rates back-calculated using skeletochronology in the present study to those yielded by other skeletochronology, mark-recapture, and length-frequency studies in the western North Atlantic. NC = North Carolina; VA = Virginia; FL = Florida; SCL = straightline carapace length; NA = not available; - = not provided

Area	Growth Interval	Mean growth rate ± SD (range) (cm yr^{-1})																	
Size class (cm SCL):		*n*	4.6-9.9	*n*	10-19.9	*n*	20-29.9	*n*	30-39.9	*n*	40-49.9	*n*	50-59.9	*n*	60-69.9	*n*	70-79.9	*n*	80-89.9
Azores & Atlantic US (NC focus; current study)[a]	1 yr	*2*	8.8±0.1 (8.7-8.9)	*10*	6.2±4.7 (1.3-13.7)	*99*	2.6±1.1 (0.7-4.7)	*490*	2.7±1.4 (0.1-7.3)	*614*	2.8±1.7 (0.1-9.8)	*363*	3.1±2.0 (0.1-11.7)	*123*	2.1±1.6 (0.1-7.0)	*94*	2.1±1.5 (0.0-6.8)	*82*	1.1±1.1 (0.0-4.4)
Azores Islands (Bjorndal et al. 2000)[b]	4 mo-4.2 yr	*6*	12			*1*	4.2	*3*	4.7 (4.5-5.1)	*2*	4 (3.4-4.6)	*1*	6.1	*2*	2.9 (2.8-3.0)				
Azores Islands (Bjorndal et al. 2000)[c]	NA					-	5.3	-	4.6	-	3.9	-	3.1						
Core & Pamlico Sounds, NC (Braun-McNeill et al. 2008)[b]	>0.9 yr											*44*	1.6±0.4	*122*	1.8±0.2	*43*	1.6±0.4		
Chesapeake Bay, VA (Klinger & Musick 1995)[a]	1 yr									*6*	5.3±2.8	*13*	5.3±1.4	*29*	5.3±1.6	*24*	4.4±2.0	*2*	3.1±1.2
Chesapeake Bay, VA (Klinger & Musick 1995)[b]	NA											*2*	3.0±0.1	*9*	1.5±1.2	*1*	0.3	*6*	1.2±0.9
Georgia (Parham & Zug 1997) [a]	1 yr					*3*	4 (3.4-4.9)	*10*	3.4 (1.9-7.0)	*9*	3.6 (2.6-4.3)	*8*	3.3 (1.7-5.2)	*6*	2.9 (2.2-4.1)	*5*	2.1 (1.6-2.9)	*9*	2.1 (0.3-4.6)
Mosquito Lagoon, FL (Mendonça 1981)[b]	3.5-22 mo											*2*	7.4±1.4	*7*	6.0±2.3	*4*	5.0±3.5		
Hutchinson Island, FL (Herren et al. 2004)[b]	>11 mo											*12*	2.3±2.2	*42*	0.9±1.0	*4*	0.1±0.4	*3*	0.3±0.1
Florida (modified Bjorndal et al. 1983)[b]	NA																	*10*	0.5 (0-1.3)
Florida (Bjorndal et al. 2001)[c]	NA									-	3.2	-	2.8	-	2.3	-	1.9	-	1.6

[a]Skeletochronology [b]Mark-recapture [c]Length-frequency

Table S3. *Caretta caretta*. Size class (SCL, cm) and calendar year-specific growth (cm yr^{-1}) rates back-calculated from all measurable growth increme
(*n* = 1877) in humeri from juvenile loggerhead sea turtles in the western North Atlantic. (-) indicates no data are available

Growth rates (cm yr^{-1})	Size class																									
	4.6-9.9			10-19.9			20-29.9			30-39.9			40-49.9			50-59.9			60-69.9			70-79.9			80-79.9	
Year	*n*	Mean (cm/yr)	SD	*n*	Mean (cm/yr)	SD	*n*	Mean (cm/yr)	SD	*n*	Mean (cm/yr)	SD	*n*	Mean (cm/yr)	SD	*n*	Mean (cm/yr)	SD	*n*	Mean (cm/yr)	SD	*n*	Mean (cm/yr)	SD	*n*	Mean (cm/yr)
1984	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	1	3	-	-	-	-	-	-	-	-	-
1985	-	-	-	-	-	-	-	-	-	1	2.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1986	-	-	-	-	-	-	1	2.3	-	2	2.8	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1987	-	-	-	-	-	-	3	2.3	1.1	8	1.5	0.8	1	1.3	-	1	0.8	-	-	-	-	-	-	-	-	-
1988	-	-	-	-	-	-	4	1.9	1.0	13	2.1	1.1	5	0.8	1.0	1	0.3	-	-	-	-	-	-	-	-	-
1989	-	-	-	-	-	-	10	2.1	1.2	20	2.1	1.1	9	1.5	1.2	-	-	-	2	0.8	0.7	1	0.2	-	-	-
1990	-	-	-	-	-	-	16	2.3	0.9	26	2.7	1.0	17	2.2	1.1	1	4.5	-	1	0.2	-	3	1.1	1.3	-	-
1991	-	-	-	-	-	-	13	2.9	1.1	36	2.8	1.0	27	2.6	1.1	1	1.7	-	2	1.0	1.2	2	0.1	0.1	1	2.9
1992	-	-	-	-	-	-	8	2.1	1.3	45	2.7	1.5	33	2.8	1.4	5	2.6	1.5	2	1.7	2.2	2	0.1	0.0	1	1.2
1993	-	-	-	-	-	-	9	2.8	0.9	50	2.5	1.2	37	3.0	1.8	11	3.4	2.1	3	1.5	0.8	3	2.0	1.8	1	2.1
1994	-	-	-	-	-	-	8	3.7	0.9	51	3.2	1.5	40	3.6	1.9	26	3.5	1.9	3	1.1	1.1	4	2.8	2.0	1	0.2
1995	-	-	-	-	-	-	3	2.8	1.4	50	2.8	1.4	53	3.2	1.9	30	3.3	1.9	7	2.8	1.5	4	2.7	1.2	2	0.7
1996	-	-	-	-	-	-	3	1.7	0.8	43	2.7	1.4	58	2.9	1.6	39	3.5	2.3	15	2.9	1.8	4	1.5	0.9	3	1.1
1997	-	-	-	-	-	-	4	2.4	1.1	35	2.5	1.3	57	2.5	1.5	28	2.2	1.4	9	1.7	1.2	7	2.7	1.6	4	1.1
1998	1	11.7	-	1	12.5	-	4	2.7	1.2	28	2.6	1.7	52	2.1	1.8	18	3.4	1.8	9	4.0	1.9	6	1.9	1.3	4	0.5
1999	-	-	-	2	2.6	1.7	4	3.3	0.4	19	2.2	1.6	56	3.0	1.9	22	3.4	2.3	3	2.0	1.6	7	2.3	1.6	5	1.0
2000	-	-	-	-	-	-	2	2.8	0.3	16	2.5	1.4	42	3.3	1.5	27	3.0	1.8	5	1.8	1.4	10	1.9	1.6	4	0.3
2001	1	8.7	-	-	-	-	3	2.7	0.9	12	2.9	1.5	30	3.8	1.3	37	3.6	1.9	12	2.4	1.4	11	1.4	1.2	6	1.1
2002	-	-	-	1	4.1	-	1	1.3	-	11	3.2	1.5	25	3.3	1.8	39	3.2	1.7	17	2.0	1.4	8	2.0	1.1	7	1.5
2003	-	-	-	1	6.7	-	-	-	-	9	2.3	1.4	18	3.0	1.7	26	3.6	2.8	16	1.6	1.6	8	2.4	1.6	9	0.7
2004	-	-	-	-	-	-	1	3.5	-	5	2.3	1.0	15	2.1	1.4	13	2.9	1.8	6	1.8	1.4	5	3.4	2.0	10	1.3
2005	-	-	-	-	-	-	1	3.2	-	-	-	-	13	1.7	1.3	11	1.6	1.2	4	2.6	1.3	5	3.8	1.5	8	1.6
2006	-	-	-	-	-	-	-	-	-	-	-	-	12	2.1	0.9	12	2.3	1.7	2	0.4	0.4	2	3.1	0.5	9	1.6
2007	1	8.9	-	1	1.3	-	-	-	-	2	3.4	3.4	7	2.0	1.4	7	3.4	2.0	2	2.5	1.7	1	3.2	-	4	1.1
2008	-	-	-	2	2.5	1.8	-	-	-	2	3.3	1.8	6	3.9	1.7	5	1.1	1.1	1	0.7	-	-	-	-	2	0.9
2009	-	-	-	-	-	-	1	3.6	-	-	-	-	2	3.3	0.7	3	0.8	0.4	1	0.4	-	-	-	-	1	1.1

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SUPPLEMENT 2. FURTHER ACKNOWLEDGEMENTS

We are grateful for the sample collection conducted by participants in the National Sea Turtle Stranding and Salvage Network; without their assistance this work would not have been possible. Special thanks go to: Baldhead Island Conservancy, Carolina Beach Turtle Program, Caswell Beach Sea Turtle Program, Duke University, Ecological Associates Inc., Florida Fish and Wildlife Research Institute, Fort Fisher State Recreation Area, Georgia Department of Natural Resources, Karen Beasley Sea Turtle Rescue and Rehabilitation Center, Mackay Island and Pea Island National Wildlife Refuges, Marine Mammal Stranding Center, Marine Science Center, Miami-Dade Parks, Marine Corps Base Camp Lejeune, Maryland Department of Natural Resources, Ocean Isle Beach Turtle Watch, National Marine Fisheries Service Southeast Fisheries Science Center, National Park Service (Cape Hatteras, Cape Lookout, Cumberland Island), Network for Endangered Sea Turtles, North Carolina Aquariums, North Carolina Maritime Museum, North Carolina Wildlife Resources Commission, Ocracoke Wildlife Rehabilitation, Progress Energy Carolinas, REMSA Inc., Sea Turtle Preservation Society, South Carolina Department of Natural Resources, South Carolina SeaGrant, Sunset Beach Turtle Program, Virginia Aquarium Stranding Response Program, Virginia Department of Game and Inland Fisheries, and Virginia Institute of Marine Sciences.