`The following supplement accompanies the article

Reconstructing sea turtle ontogenetic habitat shifts through trace element analysis of bone tissue

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Text S1. Materials and Methods

Skeletochronology and Age Estimation

Humeri were prepared and histologically processed as described by Avens and Snover (2013). Briefly, a knife was used to extract humerus bones from flippers and remove soft tissue. Bones were then boiled to remove any remaining soft tissue and air dried. A low-speed isomet saw (Beuhler) was then used to cut two 2–3 mm thick cross-sections from each humerus bone at the site of the deltopectoral muscle insertion scar—one on each side. One section was reserved for skeletochronology and the other was reserved for complementary chemical analysis. The 'skeletochronology' section was decalcified over multiple days using Cal-Ex II fixative/decalcifier (Fisher Scientific), then thin sectioned (25 microns) on the side originally facing the 'biogeochemical' section using a freezing-stage microtome. Thin sections were stained using Ehrlich's hematoxylin, mounted onto microscope slides in 100% glycerin, and digitally imaged using a digital camera fitted to a compound microscope. Growth mark analyses were performed using Microsuite image analysis software (Olympus America) and Adobe Photoshop (Adobe Systems). Two independent readers (LA, LRG) analyzed the digital bone images to determine the number and placement of lines of arrested growth (LAGs), which delimit the outer edges of each skeletal growth mark, followed by a joint assessment to reach consensus.

Previous analyses have validated that LAGs are deposited annually in Kemp's ridley sea turtles (Snover & Hohn 2004, Avens et al. 2017) and western North Atlantic loggerhead sea turtles (Klinger & Musick 1992, Coles et al. 2001, Bjorndal et al. 2003, Snover & Hohn 2004, Avens et al. 2013, 2015). Thus, assuming annual LAG deposition, a calendar year was back-assigned to each LAG based on date of stranding. Age at stranding was also estimated for each turtle through skeletochronological age estimation. Kemp's ridley and loggerhead sea turtles deposit a unique first-year growth mark, or "annulus," that differs from subsequent marks (Snover & Hohn 2004). When the annulus was visible within a bone image, an initial age estimate was determined directly from LAG counts. For larger turtles, the annulus is often lost to resorption (Zug et al. 1986), necessitating the development of a correction factor to estimate the number LAGs lost to resorption based on the relationship between LAG numbers and diameters from known-age individuals (Parham & Zug 1997). The estimated number of resorbed LAGs was then added to the number of observed LAGs to yield an initial age estimate for larger individuals. A final age estimate at stranding was made by adjusting initial age estimates to the nearest 0.25 years based on the mean hatch date for the population and individual stranding date.

Given that LAG deposition occurs in late winter/early spring and peak hatching occurs during the summer (Snover & Hohn 2004), the first-year growth mark denotes an age of ~0.75 years, the next LAG an age of 1.75 years, and so on.

Furthermore, an allometric relationship exists between humerus section diameter (HSD) and SCL for loggerhead and Kemp's ridley sea turtles (Snover et al. 2007, Avens et al. 2017). These relationships were combined with the body proportional hypothesis (Francis 1990) to estimate SCL for every measurable LAG in each humerus section, adjusted for SCL and HSD at death. A mean SCL estimate was then generated for each pair of successive LAGs that was used in all analyses.

Stable Isotope Analysis

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

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

where X is ^{15}N and R is the ratio of heavy to light isotopes ($^{15}N/^{14}N$) in the sample and standard, respectively. $R_{standard}$ for δ15N was atmospheric N2. The internal standard IAEA-600 (Caffeine; isotopic composition of δ15N = 1.00 ‰) was calibrated at regular intervals against the international standards. Analytical precision was 0.10 ‰ for loggerhead bone samples and 0.06 ‰ for Kemp's ridley bone samples. In addition to δ15N ratios, %N and %C were calculated using mass 28 and mass 44 and %N values were used to calculate C:N ratios (%C divided by %N).

Table S1. Statistical output for generalized additive mixed models (GAMMs) used to examine the effect of body size (juvenile loggerhead, $n = 35$) or age (juvenile Kemp's ridley, $n = 44$) on bone isotopic ($\delta^{15}N$) and elemental ratios (Me:Ca). General model: gamm($\delta^{15}N$ ~ body size or age, random = ~1|Bone_ID, correlation = corAR1(form = ~1| Bone_ID)

Model	*N*	Adjusted R^2	Smooth term		
			Edf	*F*	Prob(*F*)
(a) Loggerhead sea turtles					
$\delta^{15}N$ ~ SCL	230	0.66	4.14	36.27	**<0.001**
Mg:Ca[a] ~ SCL	250	0.24	2.76	10.22[b]	**<0.001**
Mn:Ca[a] ~ SCL	250	0.05	1.67	0.55	0.404
Cu:Ca[a] ~ SCL	250	–0.05	3.42	3.61	**0.014**
Zn:Ca[a] ~ SCL	250	0.03	3.44	2.77	**0.028**
Sr:Ca ~ SCL	250	0.33	4.49	19.95[b]	**<0.001**
Ba:Ca[a] ~ SCL	250	0.27	1.00	92.15	**<0.001**
(b) Kemp's ridley turtles					
$\delta^{15}N$ ~ age	130	0.22	3.41	23.24[b]	**<0.001**
Mg:Ca[a] ~ age	101	–0.03	1.00	2.89	0.092
Mn:Ca ~ age	101	0.05	1.68	2.16	0.234
Cu:Ca[a] ~ age	101	–0.01	1.00	0.05	0.823
Zn:Ca[a] ~ age	101	0.03	1.00	3.09	0.082
Sr:Ca ~ age	101	0.12	1.92	19.65[b]	**<0.001**
Ba:Ca[a] ~ age	101	0.19	3.61	10.28	**<0.001**

[a]Data $\log_{10}$-transformed prior to analysis
SCL is back-calculated straightline carapace length
N is number of unique bone growth layers included in analysis

Table S2. Tukey's *post hoc* multiple comparison adjusted *P* values for linear mixed models used to examine the effect of back-calculated SCL size class (juvenile loggerhead, n = 35) or age class (juvenile Kemp's ridley, n = 44) on bone isotopic ($\delta^{15}N$) and elemental ratios (Me:Ca)

Size or age class comparison	$\delta^{15}N$	Mg:Ca	Sr:Ca	Ba:Ca
(a) Loggerhead sea turtles				
30 vs. 40	0.621	1.000	0.906	0.473
30 vs. 50	**<0.001**	0.090	**0.024**	**0.002**
30 vs. 60	**<0.001**	**0.011**	**<0.001**	**<0.001**
30 vs. 70	**<0.001**	**0.035**	**<0.001**	**<0.001**
30 vs. 80	**<0.001**	0.144	**0.004**	**0.013**
40 vs. 50	**<0.001**	**0.002**	**0.006**	**0.002**
40 vs. 60	**<0.001**	**<0.001**	**<0.001**	**<0.001**
40 vs. 70	**<0.001**	**0.017**	**<0.001**	**<0.001**
40 vs. 80	**<0.001**	0.121	**0.008**	**0.053**
50 vs. 60	**<0.001**	0.358	**0.033**	**0.043**
50 vs. 70	**<0.001**	0.488	**0.007**	**0.057**
50 vs. 80	**0.036**	0.675	0.114	0.465
60 vs. 70	0.232	0.963	0.238	0.711
60 vs. 80	0.791	0.960	0.613	0.967
70 vs. 80	0.999	0.998	1.000	1.000
(b) Kemp's ridley sea turtles				
0.00 vs. 0.75	0.978	0.814	0.573	**0.003**
0.00 vs. 1.75	**0.022**	0.983	**0.010**	**<0.001**
0.00 vs. 2.75	**<0.001**	0.931	**<0.001**	**<0.001**
0.00 vs. 3.75	**<0.001**	0.628	**<0.001**	**<0.001**
0.00 vs. 4.75	**<0.001**	0.712	**<0.001**	**<0.001**
0.75 vs. 1.75	**<0.001**	0.982	**0.013**	**0.003**
0.75 vs. 2.75	**<0.001**	1.000	**<0.001**	**<0.001**
0.75 vs. 3.75	**<0.001**	0.954	**<0.001**	**0.002**
0.75 vs. 4.75	**<0.001**	0.974	**<0.001**	**0.017**
1.75 vs. 2.75	**0.003**	0.984	**0.023**	0.771
1.75 vs. 3.75	**0.039**	0.575	**0.003**	0.761
1.75 vs. 4.75	**0.035**	0.805	**<0.001**	0.836
2.75 vs. 3.75	1.000	0.657	0.538	0.997
2.75 vs. 4.75	0.913	0.916	0.109	0.996
3.75 vs. 4.75	0.907	1.000	0.492	1.000

Table S3. Mean (± SD) $\delta^{15}N$ values (‰) and elemental ratios (mg g^{-1}) by (**a**) 10-cm size class for loggerhead sea turtles and (**b**) age class for Kemp's ridley sea turtles.

Size or age class	*N*	$\delta^{15}N$	*N*	Mg:Ca	Mn:Ca	Cu:Ca	Zn:Ca	Sr:Ca	Ba:Ca
(a) Loggerhead sea turtle (size class, cm SCL)									
20 (20–29.9) [a]	2	8.99 ± 0.64	2	35.41 ± 4.69	0.048 ± 0.004	0.041 ± 0.029	0.652 ± 0.207	11.42 ± 2.05	0.69 ± 0.01
30 (30–39.9)	31	9.60 ± 0.74	35	30.95 ± 4.53	0.047 ± 0.011	0.039 ± 0.042	0.913 ± 0.856	10.42 ± 1.21	0.43 ± 0.15
40 (40–49.9)	63	9.86 ± 1.03	64	32.79 ± 5.53	0.047 ± 0.016	0.038 ± 0.040	0.942 ± 0.673	10.61 ± 1.15	0.42 ± 0.19
50 (50–59.9)	72	11.50 ± 2.21	80	29.86 ± 6.53	0.050 ± 0.027	0.035 ± 0.041	0.671 ± 0.429	9.59 ± 1.42	0.29 ± 0.18
60 (60–69.9)	37	14.32 ± 1.97	43	26.94 ± 4.45	0.063 ± 0.040	0.060 ± 0.084	0.685 ± 0.471	8.61 ± 1.24	0.20 ± 0.14
70 (70–79.9)	21	16.08 ± 1.58	20	22.63 ± 3.05	0.076 ± 0.032	0.119 ± 0.096	1.067 ± 0.644	8.04 ± 1.33	0.20 ± 0.17
80 (80–89.9)	4	16.36 ± 0.54	6	23.52 ± 3.36	0.066 ± 0.024	0.090 ± 0.043	1.037 ± 0.472	7.91 ± 1.10	0.17 ± 0.04
(b) Kemp's ridley sea turtle (age class, year)									
0.00 (0.00–0.75)	3	9.22 ± 1.13	6	24.79 ± 1.20	0.073 ± 0.017	0.114 ± 0.155	0.822 ± 0.80	9.87 ± 0.45	0.12 ± 0.03
0.75 (0.75–1.75)	23	11.27 ± 2.49	15	28.06 ± 3.82	0.077 ± 0.024	0.081 ± 0.117	0.712 ± 0.443	9.23 ± 0.83	0.09 ± 0.03
1.75 (1.75–2.75)	29	13.44 ± 2.19	21	27.60 ± 3.67	0.066 ± 0.030	0.068 ± 0.069	0.663 ± 0.593	8.96 ± 0.85	0.07 ± 0.02
2.75 (2.75–3.75)	33	14.23 ± 2.52	26	27.12 ± 3.53	0.059 ± 0.027	0.072 ± 0.085	0.519 ± 0.243	8.57 ± 1.09	0.07 ± 0.03
3.75 (3.75–4.75)	25	14.04 ± 2.22	20	27.00 ± 3.65	0.054 ± 0.015	0.088 ± 0.091	0.487 ± 0.275	8.61 ± 0.91	0.07 ± 0.03
4.75 (4.75–5.75)	11	14.49 ± 2.27	9	27.81 ± 4.71	0.063 ± 0.025	0.093 ± 0.087	0.428 ± 0.082	8.12 ± 1.27	0.08 ± 0.04
5.75 (5.75–6.75) [a]	3	14.85 ± 1.37	2	29.86 ± 8.42	0.051 ± 0.000	0.027 ± 0.030	0.401 ± 0.065	8.14 ± 0.49	0.11 ± 0.06
6.75 (6.75–7.75) [a]	2	14.81 ± 2.85	1	24.45	0.053	0.005	0.351	8.72	0.05

N is number of unique bone growth layers sampled.

[a]Data excluded from statistical comparisons among size and age classes due to small sample size.

Table S4. Statistical output for repeated measure correlation analyses used to examine the relationship between bone $\delta^{15}N$ values and trace element ratios for juvenile loggerhead (n = 35) and juvenile Kemp's ridley (n = 28) sea turtles

Comparison	*N*	*df*	r_{rm}	95% CI	*P*-value
(a) Loggerhead sea turtles					
$\delta^{15}N$ – Mg:Ca[a]	235	199	–0.48	–0.58 to –0.37	**<0.001**
$\delta^{15}N$ – Mn:Ca[a]	235	199	+0.21	+0.07 to +0.34	**0.003**
$\delta^{15}N$ – Cu:Ca[a]	235	199	–0.28	–0.40 to –0.15	**<0.001**
$\delta^{15}N$ – Zn:Ca[a]	235	199	–0.30	–0.42 to –0.17	**<0.001**
$\delta^{15}N$ – Sr:Ca	235	199	–0.74	–0.80 to –0.67	**<0.001**
$\delta^{15}N$ – Ba:Ca[a]	235	199	–0.70	–0.76 to –0.62	**<0.001**
(b) Kemp's ridley turtles					
$\delta^{15}N$ – Mg:Ca[a]	79	50	–0.09	–0.36 to +0.20	0.539
$\delta^{15}N$ – Mn:Ca	79	50	–0.05	–0.32 to +0.24	0.746
$\delta^{15}N$ – Cu:Ca[a]	79	50	+0.08	–0.20 to +0.35	0.555
$\delta^{15}N$ – Zn:Ca[a]	79	50	–0.02	–0.30 to +0.26	0.890
$\delta^{15}N$ – Sr:Ca	79	50	–0.50	–0.69 to –0.26	**<0.001**
$\delta^{15}N$ – Ba:Ca[a]	79	50	–0.19	–0.44 to +0.10	0.182

[a]Data $\log_{10}$-transformed prior to analysis
N is number of unique bone growth layers included in analysis
df is degrees of freedom
r_{rm} is repeated measures correlation coefficient

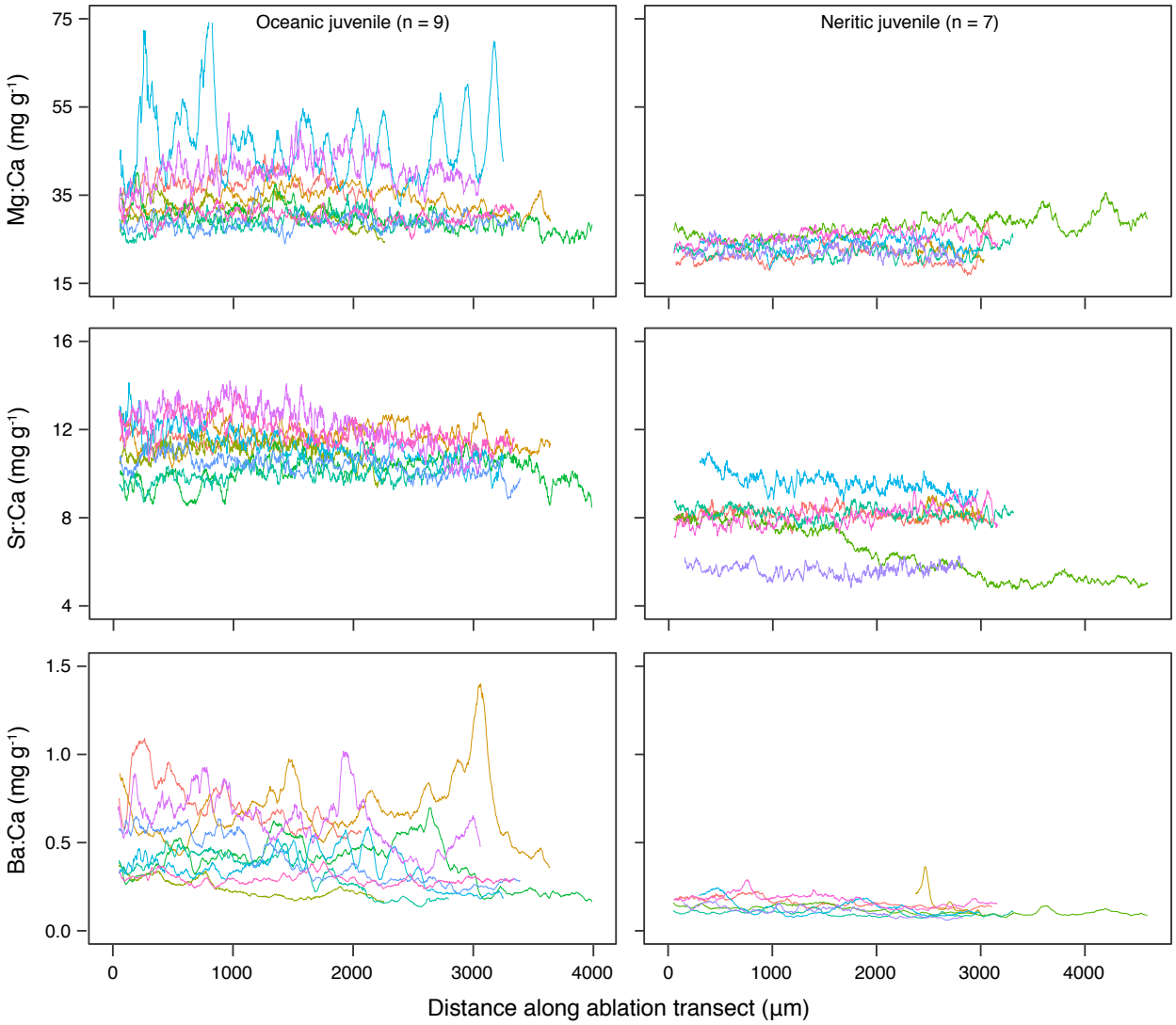

Fig. S1. Loggerhead sea turtle Mg:Ca, Sr:Ca, and Ba:Ca profiles (rows) for oceanic juveniles (left column; all bone $\delta^{15}N$ values < 11.0 ‰) and neritic juveniles (right column; all bone $\delta^{15}N$ values > 13.0 ‰). Profiles presented are rolling means (width = 10, or ~25 microns for juveniles) for individual turtles.

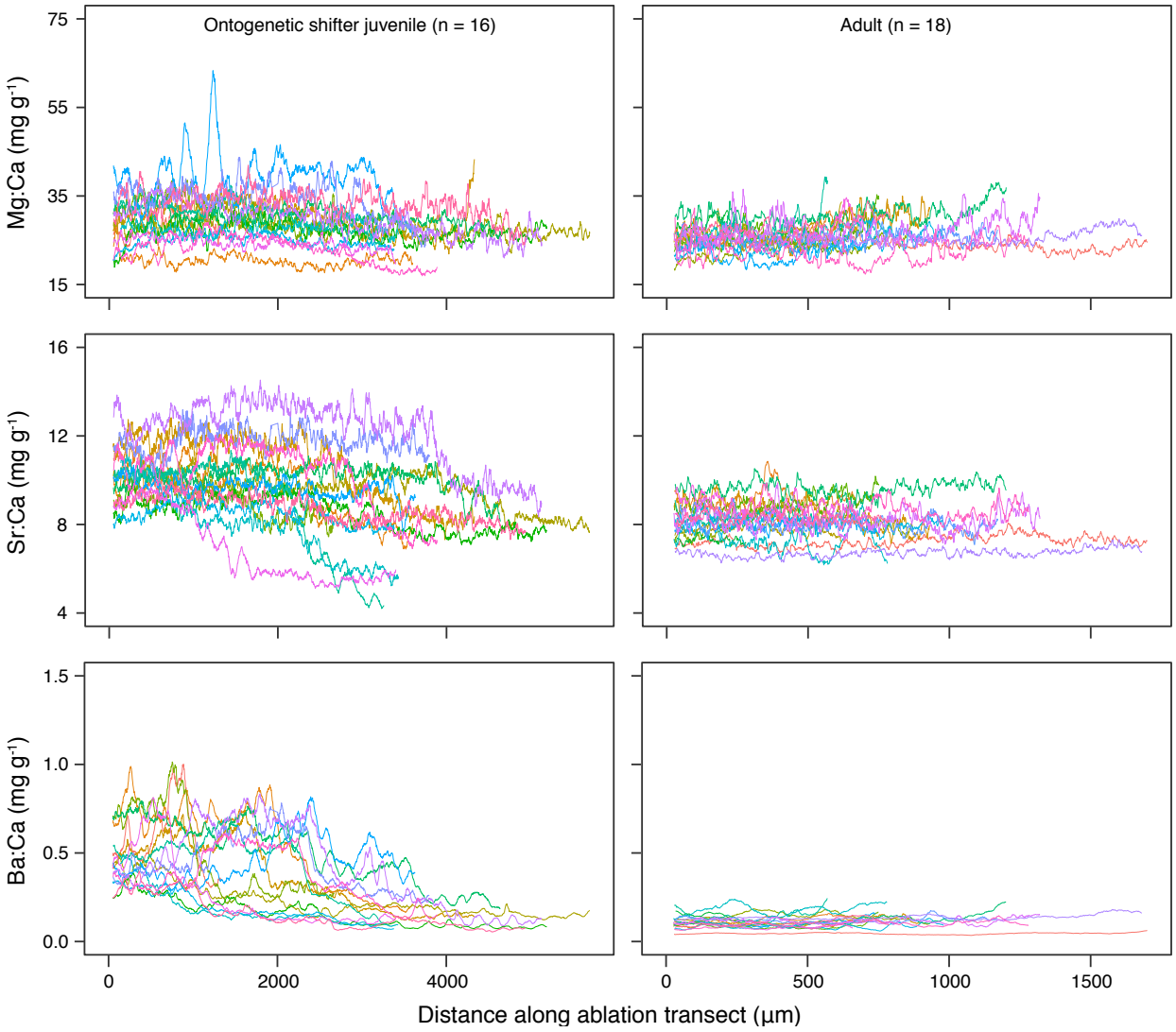

Fig. S2. Loggerhead sea turtle Mg:Ca, Sr:Ca, and Ba:Ca profiles (rows) for individual ontogenetic shifter juveniles (left column; bone $\delta^{15}N$ values increased sharply) and adults (right column). Lines are rolling means for individual turtles (width = 10, or ~25 microns for juveniles and ~15 microns for adults).

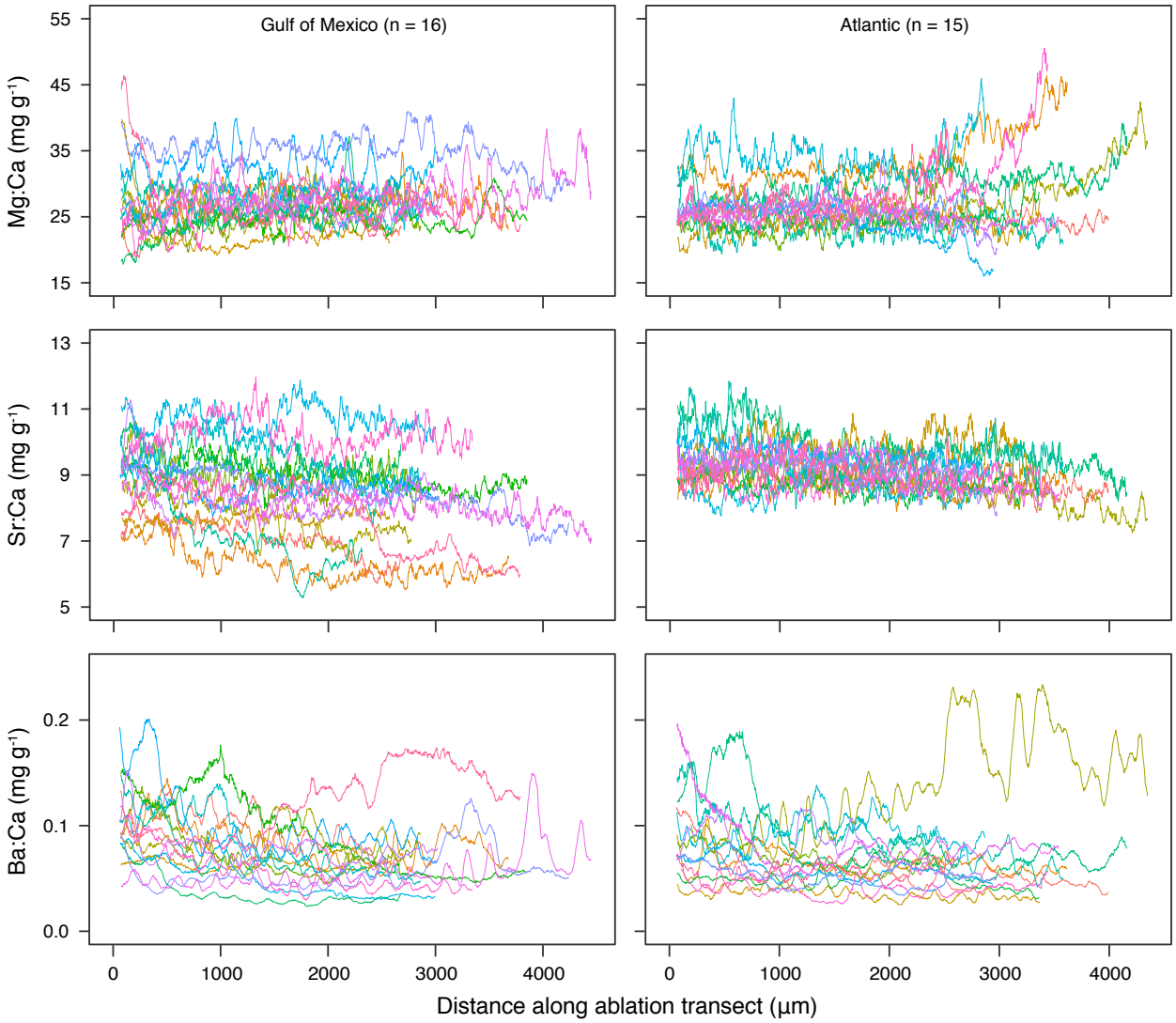

Fig. S3. Juvenile Kemp's ridley sea turtle Mg:Ca, Sr:Ca, and Ba:Ca profiles (rows) for individuals stranded in the Gulf of Mexico (left column) and U.S. Atlantic Coast (right column). Lines are rolling means for individual turtles (width = 10, or ~25 microns).

References

Avens L, Goshe LR, Coggins L, Shaver DJ, Higgins B, Landry AM, Bailey R (2017) Variability in age and size at maturation, reproductive longevity, and long-term growth dynamics for Kemp's ridley sea turtles in the Gulf of Mexico. PLOS ONE 12:e0173999

Avens L, Goshe LR, Coggins L, Snover ML, Pajuelo M, Bjorndal KA, Bolten AB (2015) Age and size at maturation- and adult-stage duration for loggerhead sea turtles in the western North Atlantic. Mar Biol 162:1749–1767

Avens L, Goshe LR, Pajuelo M, Bjorndal KA, MacDonald BD, Lemons GE, Bolten AB, Seminoff JA (2013) Complementary skeletochronology and stable isotope analyses offer new insight into juvenile loggerhead sea turtle oceanic stage duration and growth dynamics. Mar Ecol Prog Ser 491:235–251

Avens L, Snover ML (2013) Age and age estimation in sea turtles. In: Wyneken J, Lohmann KJ, Musick JA (eds) The Biology of Sea Turtles. CRC Press, Boca Raton, FL, p 97–134

Bjorndal KA, Bolten AB, Dellinger T, Delgado C, Martins HR (2003) Compensatory growth in oceanic loggerhead sea turtles: response to a stochastic environment. Ecology 84:1237–1249

Coles WC, Musick JA, Williamson LA (2001) Skeletochronology validation from an adult loggerhead (*Caretta caretta*). Copeia 2001:240–242

Francis R (1990) Back-calculation of fish length: a critical review. J Fish Biol 36:883–902

Klinger RC, Musick JA (1992) Annular growth layers in juvenile loggerhead turtles (*Caretta caretta*). Bull Mar Sci 51:224–230

Parham JF, Zug GR (1997) Age and growth of loggerhead sea turtles (*Caretta caretta*) of coastal Georgia: an assessment of skeletochronological age-estimates. Bull Mar Sci 61:287–304

Snover ML, Avens L, Hohn AA (2007) Back-calculating length from skeletal growth marks in loggerhead sea turtles *Caretta caretta*. Endanger Species Res 3:95–104

Snover ML, Hohn AA (2004) Validation and interpretation of annual skeletal marks in loggerhead (*Caretta caretta*) and Kemp's ridley (*Lepidochelys kempii*) sea turtles. Fish Bull 102:682–692

Zug GR, Wynn AH, Ruckdeschel C (1986) Age determination of loggerhead sea turtles, *Caretta caretta*, by incremental growth marks in the skeleton. Smithson Contrib Zool 427:1–44