

**Supplementary Materials for:**

**Effects of demineralization on the stable isotope analysis of bone samples**

**Calandra Turner Tomaszewicz<sup>1,2\*</sup>, Jeffrey A. Seminoff<sup>2</sup>, Matthew D. Ramirez<sup>3</sup>, Carolyn M. Kurle<sup>1</sup>**

<sup>1</sup>Division of Biological Sciences, Ecology, Behavior, and Evolution Section, University of California, San Diego, La Jolla, California 92093-0116, USA

<sup>2</sup>Southwest Fisheries Science Center, NOAA, National Marine Fisheries Service, La Jolla, California 92037, USA

<sup>3</sup>Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon 97330, USA

Running title: *Sampling bone for SIA: Effects of demineralization*

Keywords: *stable isotope analysis, collagen, acidification, protocol, marine turtles*

\*Corresponding Author: CTT, cturnert@ucsd.edu, 858-334-2842

Division of Biological Sciences; Ecology, Behavior, and Evolution Section  
University of California San Diego, 9500 Gilman Dr., #0116, La Jolla, CA 92093-0116, USA

**Supplementary Material**

**Table S1 - Micromilled powder replicates**

Results from bone powder acidification technical variation test using x3 replicates from Pacific green turtles (*Chelonia mydas*), “Cm” (n=5), Pacific loggerheads (*Caretta caretta*), “Cc” (n=5), and Atlantic loggerheads, “Cc” (n=20). a)  $\delta^{13}\text{C}$  values in permil (‰) units, b)  $\delta^{15}\text{N}$  values in permil (‰) units, c) %C, d) %N, and e) C:N.

a)		Acidified samples				
		$\delta^{13}\text{C}$ (‰)				
		Replicate 1	Replicate 2	Replicate 3	Mean	SD
Bone ID	Species					
<b>Pacific</b>						
<b>A</b>	Cm	-14.65	-14.72	-14.57	-14.64	0.07
<b>B</b>	Cm	-15.39	-15.41	-15.46	-15.42	0.03
<b>C</b>	Cm	-15.33	-15.45	-15.29	-15.36	0.09
<b>D</b>	Cm	-15.39	-15.25	-15.32	-15.32	0.07
<b>E</b>	Cm	-16.46	-16.44	-16.49	-16.46	0.02
<b>F</b>	Cc	-16.32	-16.47	-16.45	-16.41	0.08
<b>G</b>	Cc	-16.40	-16.45	-16.27	-16.38	0.09
<b>H</b>	Cc	-15.29	-15.33	-15.31	-15.31	0.02
<b>I</b>	Cc	-16.02	-15.83	-15.96	-15.93	0.10
<b>J</b>	Cc	-16.57	-16.58	-16.65	-16.60	0.04
<b>Atlantic</b>						
<b>A</b>	Cc	-14.85	-15.01	-14.81	-14.89	0.11
<b>B</b>	Cc	-14.27	-14.34	-14.26	-14.29	0.04
<b>C</b>	Cc	-15.27	-15.40	-15.33	-15.33	0.07
<b>D</b>	Cc	-15.81	-15.89	-15.83	-15.84	0.04
<b>E</b>	Cc	-12.97	-12.99	-13.02	-12.99	0.03
<b>F</b>	Cc	-15.05	-14.92	-15.02	-15.00	0.07
<b>G</b>	Cc	-15.04	-14.90	-15.07	-15.00	0.09
<b>H</b>	Cc	-16.26	-16.76	-16.58	-16.53	0.25
<b>I</b>	Cc	-15.36	-15.18	-15.06	-15.20	0.15
<b>J</b>	Cc	-16.02	-15.98	-15.84	-15.95	0.09
<b>K</b>	Cc	-14.57	-14.73	-14.64	-14.64	0.08
<b>L</b>	Cc	-15.45	-15.19	-15.37	-15.34	0.13
<b>M</b>	Cc	-15.13	-15.28	-15.20	-15.20	0.08
<b>N</b>	Cc	-15.45	-15.42	-15.41	-15.43	0.02
<b>O</b>	Cc	-15.30	-15.08	-15.34	-15.24	0.14
<b>P</b>	Cc	-15.10	-15.11	-15.07	-15.10	0.02
<b>Q</b>	Cc	-15.18	-15.28	-15.33	-15.26	0.08
<b>R</b>	Cc	-15.61	-15.56	-15.60	-15.59	0.03
<b>S</b>	Cc	-15.23	-15.15	-15.34	-15.24	0.10
<b>T</b>	Cc	-14.41	-14.36	-14.43	-14.40	0.04

b)		Acidified samples				
		$\delta^{15}\text{N}$ (‰)				
		Replicate 1	Replicate 2	Replicate 3	Mean	SD
Bone ID	Species					
<b>Pacific</b>						
A	Cm	15.95	15.82	15.95	15.91	0.07
B	Cm	18.47	18.66	18.53	18.55	0.10
C	Cm	13.58	13.75	13.79	13.70	0.11
D	Cm	15.95	16.27	16.16	16.12	0.16
E	Cm	17.56	17.67	17.71	17.64	0.08
F	Cc	16.99	16.71	16.65	16.78	0.19
G	Cc	16.24	16.35	16.43	16.34	0.10
H	Cc	18.41	18.47	18.40	18.42	0.04
I	Cc	17.13	17.17	17.08	17.13	0.05
J	Cc	14.36	14.68	14.55	14.53	0.16
<b>Atlantic</b>						
A	Cc	13.92	13.89	13.99	13.93	0.05
B	Cc	13.13	12.99	13.02	13.05	0.07
C	Cc	13.95	13.95	14.06	13.98	0.06
D	Cc	11.97	12.04	12.06	12.02	0.05
E	Cc	14.77	14.62	14.64	14.68	0.08
F	Cc	13.92	13.85	13.92	13.90	0.04
G	Cc	15.42	15.45	15.35	15.41	0.05
H	Cc	10.09	10.22	10.22	10.18	0.08
I	Cc	12.15	12.22	12.22	12.20	0.04
J	Cc	13.26	13.30	13.34	13.30	0.04
K	Cc	11.95	11.96	11.96	11.96	0.01
L	Cc	15.74	15.76	15.69	15.73	0.04
M	Cc	11.58	11.65	11.70	11.64	0.06
N	Cc	15.98	15.91	15.91	15.93	0.04
O	Cc	16.20	16.25	16.19	16.21	0.03
P	Cc	13.85	13.90	13.76	13.84	0.07
Q	Cc	12.54	12.46	12.46	12.49	0.05
R	Cc	16.64	16.66	16.76	16.69	0.06
S	Cc	10.37	10.39	10.34	10.37	0.03
T	Cc	15.71	15.76	15.82	15.76	0.06

c)		Acidified samples				
		% C				
		Replicate 1	Replicate 2	Replicate 3	Mean	SD
Bone ID	Species					
<b>Pacific</b>						
<b>A</b>	Cm	48.0	71.1	47.8	55.6	13.4
<b>B</b>	Cm	43.0	38.2	37.4	39.5	3.1
<b>C</b>	Cm	32.5	41.5	34.7	36.2	4.7
<b>D</b>	Cm	27.7	46.5	43.0	39.1	10.0
<b>E</b>	Cm	33.1	42.5	44.1	39.9	5.9
<b>F</b>	Cc	29.9	48.0	47.8	41.9	10.4
<b>G</b>	Cc	31.0	39.5	51.6	40.7	10.3
<b>H</b>	Cc	32.8	38.9	39.2	37.0	3.6
<b>I</b>	Cc	51.1	26.3	24.5	34.0	14.9
<b>J</b>	Cc	23.9	41.3	65.2	43.4	20.7
<b>Atlantic</b>						
<b>A</b>	Cc	46.5	46.3	46.2	46.3	0.2
<b>B</b>	Cc	45.1	45.4	45.6	45.4	0.3
<b>C</b>	Cc	46.1	46.1	45.7	46.0	0.2
<b>D</b>	Cc	45.9	46.1	45.5	45.9	0.3
<b>E</b>	Cc	46.0	45.5	45.8	45.8	0.3
<b>F</b>	Cc	46.0	46.3	45.9	46.1	0.2
<b>G</b>	Cc	46.2	46.6	45.5	46.1	0.6
<b>H</b>	Cc	45.8	46.8	46.2	46.3	0.5
<b>I</b>	Cc	45.4	45.6	45.5	45.5	0.1
<b>J</b>	Cc	46.9	46.9	46.6	46.8	0.2
<b>K</b>	Cc	44.9	46.5	45.9	45.8	0.8
<b>L</b>	Cc	45.7	46.1	46.1	46.0	0.2
<b>M</b>	Cc	46.6	46.7	46.1	46.5	0.3
<b>N</b>	Cc	44.3	44.8	44.9	44.7	0.3
<b>O</b>	Cc	47.5	47.0	47.1	47.2	0.3
<b>P</b>	Cc	45.9	45.5	45.3	45.6	0.3
<b>Q</b>	Cc	47.0	46.8	46.4	46.7	0.3
<b>R</b>	Cc	46.5	46.7	46.4	46.6	0.2
<b>S</b>	Cc	45.7	46.3	46.4	46.1	0.4
<b>T</b>	Cc	46.2	46.3	46.2	46.2	0.1

d)		Acidified samples				
		% N				
		Replicate 1	Replicate 2	Replicate 3	Mean	SD
Bone ID	Species					
<b>Pacific</b>						
<b>A</b>	Cm	16.0	23.9	15.8	18.6	4.6
<b>B</b>	Cm	14.6	13.1	12.5	13.4	1.1
<b>C</b>	Cm	10.9	13.5	11.5	12.0	1.4
<b>D</b>	Cm	9.3	15.9	14.6	13.3	3.5
<b>E</b>	Cm	11.2	14.2	14.5	13.3	1.8
<b>F</b>	Cc	10.2	16.2	16.1	14.1	3.4
<b>G</b>	Cc	10.2	12.9	16.9	13.3	3.4
<b>H</b>	Cc	11.0	13.1	13.0	12.4	1.2
<b>I</b>	Cc	17.1	8.9	8.2	11.4	4.9
<b>J</b>	Cc	7.9	13.7	21.6	14.4	6.8
<b>Atlantic</b>						
<b>A</b>	Cc	15.7	15.5	15.6	15.6	0.1
<b>B</b>	Cc	15.3	15.5	15.5	15.5	0.1
<b>C</b>	Cc	15.2	15.2	15.0	15.1	0.1
<b>D</b>	Cc	16.3	16.3	16.1	16.2	0.1
<b>E</b>	Cc	15.8	15.8	15.9	15.8	0.1
<b>F</b>	Cc	15.6	15.9	15.9	15.8	0.2
<b>G</b>	Cc	16.4	16.5	16.1	16.3	0.2
<b>H</b>	Cc	15.2	14.7	14.7	14.9	0.3
<b>I</b>	Cc	15.5	15.7	15.7	15.6	0.1
<b>J</b>	Cc	16.4	16.4	16.2	16.3	0.1
<b>K</b>	Cc	15.2	15.6	15.7	15.5	0.3
<b>L</b>	Cc	15.1	15.5	15.5	15.4	0.2
<b>M</b>	Cc	15.3	15.2	15.2	15.2	0.1
<b>N</b>	Cc	15.7	15.9	15.7	15.8	0.1
<b>O</b>	Cc	15.0	15.1	14.9	15.0	0.1
<b>P</b>	Cc	15.8	16.0	15.8	15.9	0.1
<b>Q</b>	Cc	16.5	16.5	16.4	16.4	0.1
<b>R</b>	Cc	15.2	15.4	15.3	15.3	0.1
<b>S</b>	Cc	16.1	16.0	16.0	16.0	0.1
<b>T</b>	Cc	15.6	15.8	15.7	15.7	0.1

e)		Acidified samples						
		C:N					Mean	SD
		Replicate 1	Replicate 2	Replicate 3				
Bone_ID	Species							
<b>Pacific</b>								
<b>A</b>	Cm	3.0	3.0	3.0	3.0	0.0		
<b>B</b>	Cm	2.9	2.9	3.0	3.0	0.0		
<b>C</b>	Cm	3.0	3.1	3.0	3.0	0.0		
<b>D</b>	Cm	3.0	2.9	2.9	2.9	0.0		
<b>E</b>	Cm	3.0	3.0	3.0	3.0	0.0		
<b>F</b>	Cc	2.9	3.0	3.0	3.0	0.0		
<b>G</b>	Cc	3.0	3.1	3.1	3.1	0.0		
<b>H</b>	Cc	3.0	3.0	3.0	3.0	0.0		
<b>I</b>	Cc	3.0	2.9	3.0	3.0	0.0		
<b>J</b>	Cc	3.0	3.0	3.0	3.0	0.0		
<b>Atlantic</b>								
<b>A</b>	Cc	3.0	3.0	3.0	3.0	0.0		
<b>B</b>	Cc	2.9	2.9	2.9	2.9	0.0		
<b>C</b>	Cc	3.0	3.0	3.0	3.0	0.0		
<b>D</b>	Cc	2.8	2.8	2.8	2.8	0.0		
<b>E</b>	Cc	2.9	2.9	2.9	2.9	0.0		
<b>F</b>	Cc	2.9	2.9	2.9	2.9	0.0		
<b>G</b>	Cc	2.8	2.8	2.8	2.8	0.0		
<b>H</b>	Cc	3.0	3.2	3.1	3.1	0.1		
<b>I</b>	Cc	2.9	2.9	2.9	2.9	0.0		
<b>J</b>	Cc	2.9	2.9	2.9	2.9	0.0		
<b>K</b>	Cc	3.0	3.0	2.9	3.0	0.0		
<b>L</b>	Cc	3.0	3.0	3.0	3.0	0.0		
<b>M</b>	Cc	3.0	3.1	3.0	3.1	0.0		
<b>N</b>	Cc	2.8	2.8	2.9	2.8	0.0		
<b>O</b>	Cc	3.2	3.1	3.2	3.1	0.0		
<b>P</b>	Cc	2.9	2.9	2.9	2.9	0.0		
<b>Q</b>	Cc	2.9	2.8	2.8	2.8	0.0		
<b>R</b>	Cc	3.1	3.0	3.0	3.0	0.0		
<b>S</b>	Cc	2.8	2.9	2.9	2.9	0.0		
<b>T</b>	Cc	3.0	2.9	2.9	2.9	0.0		

**Table S2 – Micromilled acidification experiment**

Results ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, %C and %N) from bone powder acidification experiment of a) untreated and b) acidified (treated). Stable isotope values,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , are in permil (‰) units. C:N is a ratio of %C to %N. See Table 2 for effect differences.

<b>a) Micromilled Powder: Untreated</b>						
<b>Bone ID</b>	<b>Species</b>	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	<b>%C</b>	<b>%N</b>	<b>C:N</b>
<b>Pacific</b>						
<b>F</b>	Cc	-16.16	16.22	15.03	4.78	3.1
<b>G</b>	Cc	-15.91	16.37	14.43	4.39	3.3
<b>H</b>	Cc	-14.93	17.75	13.87	4.23	3.3
<b>I</b>	Cc	-15.67	16.78	14.60	4.55	3.2
<b>J</b>	Cc	-16.23	14.30	14.46	4.47	3.2
<b>K</b>	Cc	-17.15	15.36	12.06	3.80	3.2
<b>L</b>	Cc	-17.23	15.21	14.54	4.42	3.3
<b>M</b>	Cc	-16.60	16.58	15.02	4.49	3.3
<b>N</b>	Cc	-16.40	16.52	14.23	4.34	3.3
<b>O</b>	Cc	-16.66	15.75	14.63	4.48	3.3
<b>P</b>	Cc	-16.89	16.09	13.92	4.20	3.3
<b>Q</b>	Cc	-17.10	16.24	15.43	4.36	3.5
<b>R</b>	Cc	-15.77	15.49	14.64	4.39	3.3
<b>S</b>	Cc	-16.12	15.20	13.52	4.15	3.3
<b>T</b>	Cc	-16.30	15.10	15.89	4.85	3.3
<b>A</b>	Cm	-14.56	15.73	16.76	5.32	3.2
<b>AA</b>	Cm	-16.01	17.86	14.02	4.37	3.2
<b>B</b>	Cm	-15.09	17.78	14.89	4.96	3.0
<b>BB</b>	Cm	-18.36	10.78	13.43	4.02	3.3
<b>C</b>	Cm	-15.06	13.02	12.46	3.91	3.2
<b>CC</b>	Cm	-15.65	13.52	14.87	4.58	3.2
<b>D</b>	Cm	-15.31	16.37	15.53	4.98	3.1
<b>DD</b>	Cm	-15.87	16.95	12.75	4.03	3.2
<b>E</b>	Cm	-16.42	17.24	14.28	4.44	3.2
<b>U</b>	Cm	-17.20	17.29	12.78	3.80	3.4
<b>V</b>	Cm	-15.81	17.97	13.21	4.27	3.1
<b>W</b>	Cm	-14.69	16.32	13.43	4.44	3.0
<b>X</b>	Cm	-15.19	16.50	13.33	4.25	3.1
<b>Y</b>	Cm	-14.80	15.14	14.24	4.50	3.2
<b>Z</b>	Cm	-15.53	16.41	14.03	4.47	3.1

<b>Atlantic</b>						
<b>A</b>	Cc	-14.01	13.97	16.10	4.90	3.3
<b>B</b>	Cc	-13.44	13.07	15.10	4.60	3.3
<b>C</b>	Cc	-14.10	13.88	14.60	4.80	3.1
<b>D</b>	Cc	-15.11	11.95	15.00	4.70	3.2
<b>E</b>	Cc	-12.37	14.60	14.80	5.00	3.0



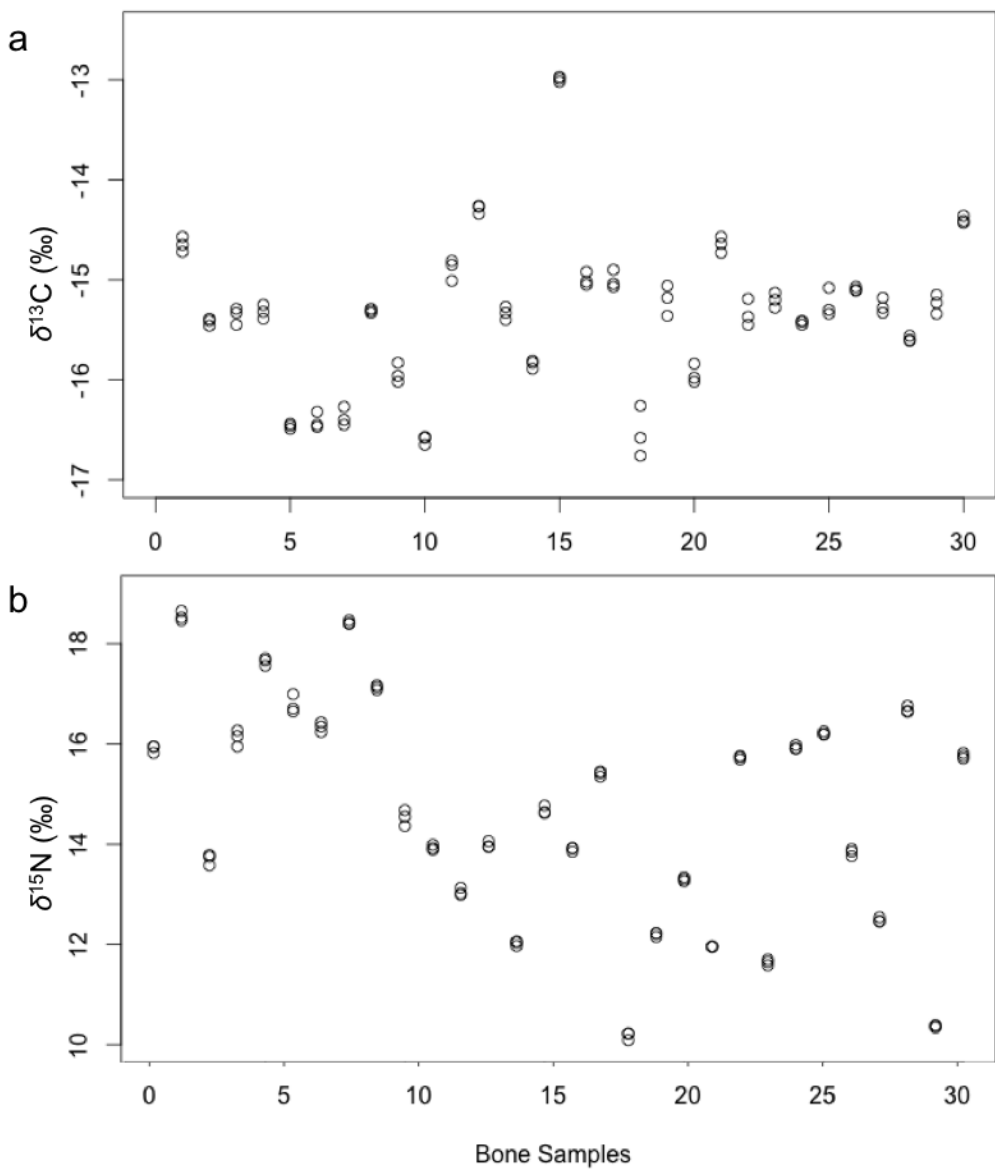
<b>F</b>	Cc	-14.18	13.81	14.20	4.30	3.3
<b>G</b>	Cc	-14.38	15.70	15.60	4.80	3.3
<b>H</b>	Cc	-15.08	10.10	13.40	4.60	2.9
<b>I</b>	Cc	-14.52	12.20	13.90	4.60	3.0
<b>J</b>	Cc	-15.15	13.13	15.00	4.50	3.3
<b>K</b>	Cc	-13.85	11.85	13.40	4.50	3.0
<b>L</b>	Cc	-14.70	15.63	14.70	5.00	3.0
<b>M</b>	Cc	-14.06	11.69	14.70	4.90	3.0
<b>N</b>	Cc	-14.34	15.89	13.70	4.70	3.0
<b>O</b>	Cc	-14.23	16.49	15.40	5.00	3.1
<b>P</b>	Cc	-14.49	13.75	14.30	4.70	3.0
<b>Q</b>	Cc	-14.48	12.69	15.40	4.60	3.4
<b>R</b>	Cc	-14.71	16.85	14.60	4.80	3.0
<b>S</b>	Cc	-14.59	10.14	13.60	4.70	2.9
<b>T</b>	Cc	-13.82	15.55	14.00	4.70	3.0
<b>All</b>	AVG:	-15.33	15.02			
	SD:	1.17	2.05			

<b>b) Micromilled Powder: Acidified</b>						
<b>Bone ID</b>	<b>Species</b>	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	<b>%C</b>	<b>%N</b>	<b>C:N</b>
<b>Pacific</b>						
<b>F</b>	Cc	-16.32	16.99	29.86	10.17	2.9
<b>G</b>	Cc	-16.40	16.24	31.00	10.23	3.0
<b>H</b>	Cc	-15.29	18.41	32.83	11.00	3.0
<b>I</b>	Cc	-16.02	17.13	51.11	17.07	3.0
<b>J</b>	Cc	-16.57	14.36	23.86	7.94	3.0
<b>K</b>	Cc	-18.52	15.18	47.86	13.85	3.5
<b>L</b>	Cc	-17.79	14.83	43.18	13.37	3.2
<b>M</b>	Cc	-16.73	16.45	44.02	14.57	3.0
<b>N</b>	Cc	-17.82	16.04	46.80	13.79	3.4
<b>O</b>	Cc	-18.18	16.03	46.20	13.64	3.4
<b>P</b>	Cc	-17.51	15.89	44.07	14.00	3.1
<b>Q</b>	Cc	-17.73	16.41	44.49	12.88	3.5
<b>R</b>	Cc	-16.12	15.62	46.64	15.34	3.0
<b>S</b>	Cc	-16.86	14.99	31.17	9.86	3.2
<b>T</b>	Cc	-16.83	14.79	43.78	14.53	3.0
<b>A</b>	Cm	-14.65	15.95	47.97	16.02	3.0
<b>AA</b>	Cm	-16.48	17.76	41.79	13.90	3.0
<b>B</b>	Cm	-15.39	18.47	43.02	14.63	2.9

<b>BB</b>	Cm	-18.86	11.05	44.25	14.23	3.1
<b>C</b>	Cm	-15.33	13.58	32.54	10.90	3.0
<b>CC</b>	Cm	-16.02	13.58	44.70	14.44	3.1
<b>D</b>	Cm	-15.39	15.95	27.74	9.30	3.0
<b>DD</b>	Cm	-16.70	17.06	44.11	14.57	3.0
<b>E</b>	Cm	-16.46	17.56	33.08	11.19	3.0
<b>U</b>	Cm	-18.42	16.73	45.06	13.97	3.2
<b>V</b>	Cm	-16.73	17.54	44.58	14.85	3.0
<b>W</b>	Cm	-15.19	15.76	44.88	15.20	3.0
<b>X</b>	Cm	-15.86	16.08	45.42	15.16	3.0
<b>Y</b>	Cm	-15.42	15.53	43.47	14.52	3.0
<b>Z</b>	Cm	-16.02	16.25	44.59	14.82	3.0

<b>Atlantic</b>						
<b>A</b>	Cc	-14.85	13.92	46.50	15.70	3.0
<b>B</b>	Cc	-14.27	13.13	45.10	15.30	2.9
<b>C</b>	Cc	-15.27	13.95	46.10	15.20	3.0
<b>D</b>	Cc	-15.81	11.97	45.90	16.30	2.8
<b>E</b>	Cc	-12.97	14.77	46.00	15.80	2.9
<b>F</b>	Cc	-15.05	13.92	46.00	15.60	2.9
<b>G</b>	Cc	-15.04	15.42	46.20	16.40	2.8
<b>H</b>	Cc	-16.26	10.09	45.80	15.20	3.0
<b>I</b>	Cc	-15.36	12.15	45.40	15.50	2.9
<b>J</b>	Cc	-16.02	13.26	46.90	16.40	2.9
<b>K</b>	Cc	-14.57	11.95	44.90	15.20	3.0
<b>L</b>	Cc	-15.45	15.74	45.70	15.10	3.0
<b>M</b>	Cc	-15.13	11.58	46.60	15.30	3.0
<b>N</b>	Cc	-15.45	15.98	44.30	15.70	2.8
<b>O</b>	Cc	-15.30	16.20	47.50	15.00	3.2
<b>P</b>	Cc	-15.10	13.85	45.90	15.80	2.9
<b>Q</b>	Cc	-15.18	12.54	47.00	16.50	2.9
<b>R</b>	Cc	-15.61	16.64	46.50	15.20	3.1
<b>S</b>	Cc	-15.23	10.37	45.70	16.10	2.8
<b>T</b>	Cc	-14.41	15.71	46.20	15.60	3.0
<b>All</b>		<b>AVG:</b>	-16.00	15.03		
		<b>SD:</b>	1.20	2.03		

**Figure S1** – Sample variation observed in stable isotope values from x3 replicates of acidified micromilled bone powder samples (n = 30) a)  $\delta^{13}\text{C}$  values, b)  $\delta^{15}\text{N}$  values.



**Figure S2** – C:N ratios, calculated by %C divided by %N, of micromilled bone powder samples, black open circles = untreated bone powder, grey filled circles = acid treated bone powder. All samples restricted to C:N values below 3.5, due to low lipid content.<sup>[55]</sup> Threshold of C:N = 3.5 shown as dashed diagonal line.

