Chemistry of bone remodelling preserved in extant and fossil Sirenia

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1. Specimen Information

Extant material is represented by an isolated partial rib from an adult *Trichechus manatus* (Figure S1). The specimen is housed at the National Museum of Scotland.

Figure S1: Lateral and transverse views of a partial rib from an adult (?) *T. manatus* (NMS.Z.2015.9). Scale bar is 1 cm.

Fossil material is represented by an isolated rib fragment from the extinct dugong, *Metataxytherium* (PAS11-04; Late Miocene, USA), which is on loan from the Delaware Valley Paleontological Society (DVPS) and housed at the Department of Earth and Environmental Science at Temple University (PA, USA).

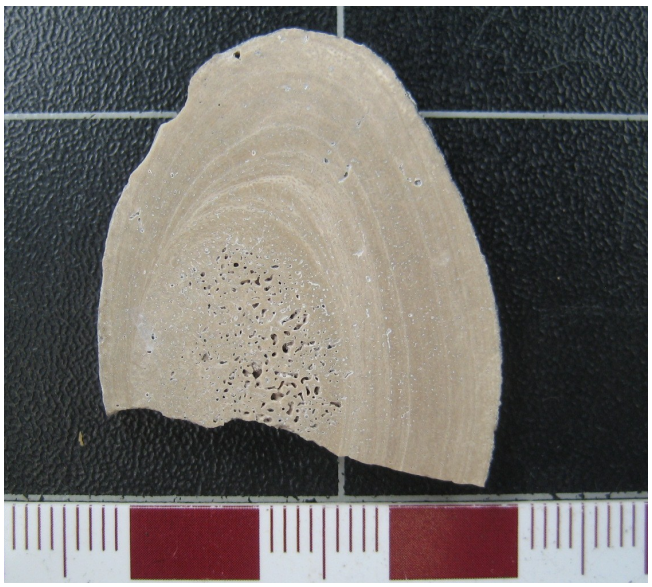

Figure S2: Transverse view of the *Metaxytherium sp*. rib section (PAS11-04).

2. Particle-Induced X-ray Emission (PIXE)/Rutherford Backscatter Spectroscopy (RBS), Surrey Ion Beam Centre Experimental Setup

PIXE/RBS analysis was used to verify synchrotron concentrations determined using PyMCA (especially Zn) and to determine the organic/apatite stoichiometry as marine mammal bones are known to maintain high levels of organics for extended periods of time. Analysis was performed at the Surrey Ion Beam Centre (University of Surrey, Guildford, UK) using a dual detector setup that allows for both PIXE and RBS to be taken simultaneously, yielding simultaneous elemental and 3D depth profiling information from a sample. Specimens were mapped using a 2.5 MeV proton beam (2MV Tandetron accelerator), with the beam focused to a 20 μm area. Maps of 2 x2 mm areas of interest were run for 2-8 hours in order to obtain adequate statistics for the low-concentration elements. Concentrations were calculated using the raw X-ray and RBS data using a combination of the OMDAQ2007 and GUPIXWIN programs[1-2] using a Pb glass and Durango apatite standard for

calibration of beam parameters. The statistical and fitting error for elemental concentrations was calculated by GUPIXWIN using the peak and background counts for each element.

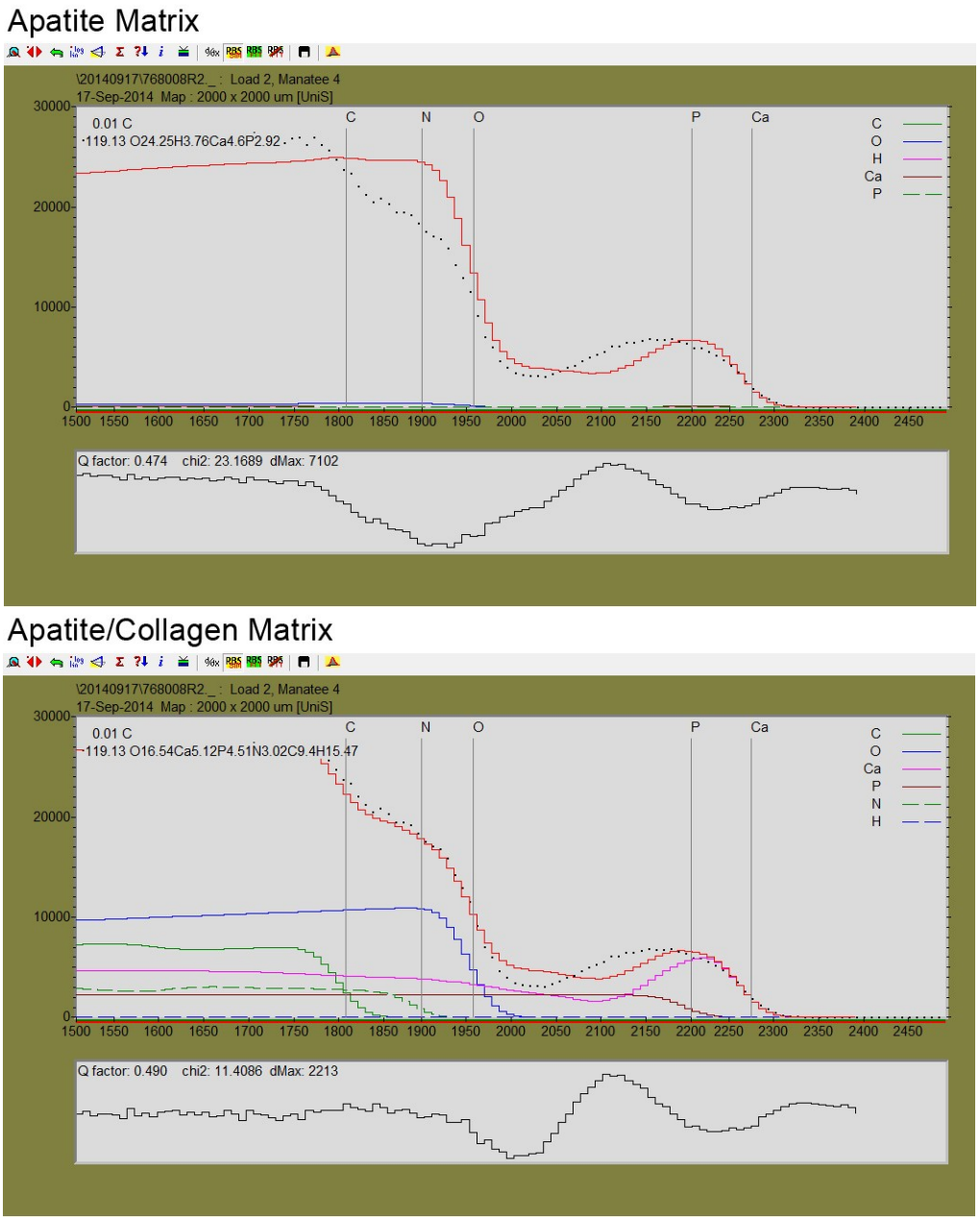

Figure S3: RBS profiles showing fits and chi^2 for pure apatite (top) as compared to a mixture of collagen (~33%) and apatite (~66%; bottom) of *T. manatus*. However, the fit between 2000 and 1700 is greatly improved with the incorporation of collagen into the stoichiometry, which a change in chi^2 from 23.17 (apatite) to 11.41 (apatite and collagen). The poor fit in both spectra between 2200 and 2000 is caused by non-Rutherford scatter from Ca that has not been corrected for properly in the software.[2]

3. Diamond Light Source (DLS) Experimental Setup

The microfocus beam line I18 at DLS allows for small-scale elemental mapping (millimetre^2) at micron resolution. The combination of Kirkpatrick-Baez mirrors, a double crystal Si(111) monochromator, and a 4-element Vortex silicon drift detector allow for full EDS spectrum to be recorded for each pixel of the elemental map and for the collection of full EXAFS. Flux was estimated to range between 10^{11} and 10^{12} photons s^{-1}. Maps were processed using the ROI imaging tool in PyMCA freeware[3] by defining the X-ray emission energy of an element in the recorded EDS spectra.

4. Quantification from EDS Spectra

Quantification using the synchrotron data was accomplished using point analyses, which were selected by identifying an area of interest within the scan, driving to those coordinates, and collecting for 30 seconds. EDS spectra were fitted with PyMCA from fundamental parameters of the experiment using a Durango apatite mineral standard with known elemental concentrations for calibration (Figure S4).

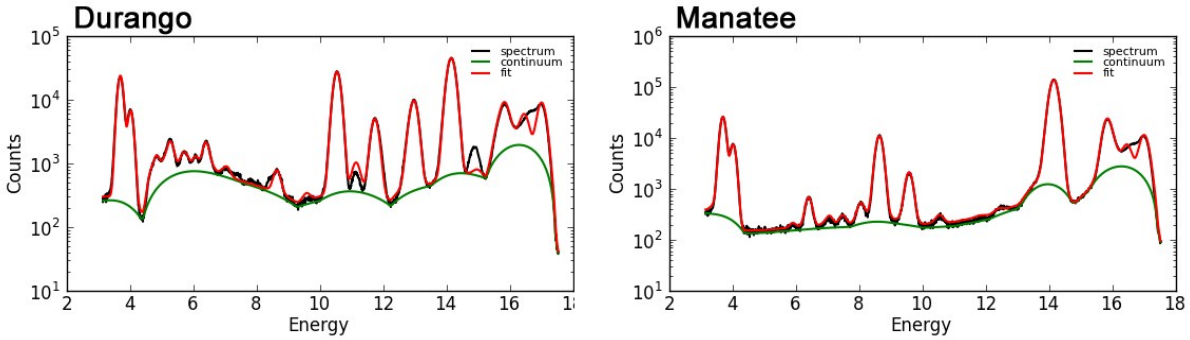

Figure S4: Example of an EDS spectrum fitted using PyMCA from the Durango standard (left) and bone sample (right).

The addition of collagen to the sample stoichiometry matrix affected the calculated quantification of trace elements, with roughly a 30% decrease in Zn and 60% decrease in Sr (Table S1). The collagen

matrix quantifications are within the range seen in other marine vertebrates, while the apatite matrix concentrations are elevated compared to marine vertebrates.[4-5] Combined with the results seen in the RBS fit, this suggests that it is important to include both organic and inorganic constituents when quantifying trace elements in fresh bone or bone that has retained much of its organics.

Element	*T. manatus* osteon high Zn Apatite Matrix	*T. manatus* osteon high Zn Apatite/Collagen Matrix
Ca	43.27%	39.86%
Mn	19	18
Fe	168	117
Cu	25	18
Zn	699	477
Sr	2130	1444

Table S1: Comparison between using a pure apatite matrix versus an apatite/collagen matrix when quantifying trace elements using synchrotron analysis.

Element	Bone	Osteon
V	240	322
Cr	279	255
La	36	132
Ce	85	97
Nd	45	70
Pb	37	37

Table S2: Additional elemental concentrations from *Metaxytherium* not detected within extant material (*T. manatus*). Concentrations are given in ppm.

References:

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