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Stable isotopes in barnacles as a tool to understand green sea turtle (*Chelonia mydas*) regional movement patterns

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Detailed methods used in the preparation and analysis of sea turtle barnacle calcite.

1. Barnacle Collection, Dissection and Milling

Platylepas spp. barnacles were collected from adult green sea turtles caught around PANWR between July and August 2011. All barnacles were found embedded in the turtles' soft tissue (Gómez et al., 2011). Barnacles were removed from the turtle's skin and stored in vials with 90% ethanol until analysis.

The barnacles were identified as belonging to the genus *Platylepas* . Barnacles deemed an appropriate size for the milling runs were selected (≥2mm height). We picked three specimens per turtle in order to assess the consistency of recorded isotope ratios of different barnacles on a given turtle. A total of 12 barnacles were selected from 4 different turtles (3 barnacles each). The barnacles were then dissected under a microscope removing any soft tissue and other contaminants, so that only the calcite endoskeleton of the barnacle was left. The dissections were performed in 90% ethanol using two fine-point tweezers, after which the clean barnacle endoskeletons were stored in marked vials in 90% ethanol.

The calcite endoskeletons were broken in half creating two half circles, so that half of each barnacle could be glued onto a glass slide and the mill could pass along the outer surface of the endoskeleton. 5-minute epoxy was used to attach the barnacles to the slide with the epoxy being applied on the glass and the broken ends of the endoskeleton, so that the halved endoskeleton created an arch with the outside surface facing upward. Glue was also applied to the hollow space below the arch in order to give support during milling.

A Merchantek MicroMill (Electro Scientific Industries, Inc., Portland, United States) was used to mill the calcite samples along the growth trajectory of the barnacles. The glass slides

holding the barnacle endoskeletons were fixed to the computer-operated mill. The mill was programed to take passes perpendicular to the growth trajectory of the endoskeleton that were 0.3-0.4 mm apart. The milling was performed using a ball end mill with a diameter of 0.7 mm. The samples were taken from the outermost part of the endoskeleton to exclude any calcite deposits that might have been the result of ageing and thickening of the individual plates.

The milled material was then collected after each pass of the mill using the static of a scalpel blade and transferred into glass vials. These were marked with the barnacle number and layer identifier. The slide with the barnacle was cleared of any residue millings by blowing over it with a can of compressed air, so that the subsequent layer sample would not be contaminated.

The calcite samples were sent to the Keck Paleoenvironmental & Environmental Stable Isotope Laboratory at the University of Kansas, where they were isotopically analyzed for oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) stable isotope ratios. A Kiel Carbonate Device III and a Finnigan MAT253 isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) were used to perform the lab analysis. To prepare each analyzed sample the lab weighed out 20 to 80 microgram of pure carbonate in stainless steel boats. The calcite samples in the boats were roasted under vacuum for one hour at 200°C to release any volatile compounds. In order to measure the samples' oxygen and carbon isotope ratios, the calcite samples and corresponding standards were moved individually into glass vials and reacted with three drops of 100% prepared phosphoric acid (ρ=1.8860 g/cm3) under vacuum for 4 minutes at 75°C. CO2 was released and trapped cryogenically, after which it was transferred online to an IRMS instrument where it was measured 8 fold against a calibrated CO^2 benchmark tank for δ

ratio analysis and reported versus the VPDB (Vienna Pee Dee Belemnite) scale. Sample data were reported as being more accurate than 0.03‰ for $\delta^{13}C$ and 0.06‰ for $\delta^{18}O$.

2. Mapping of Calcite Oxygen Isotope Ratios

ArcGIS (Version 10) was used to create a map showing the calcite oxygen isotope ratios one would expect to see on a large scale throughout the Pacific Ocean to put the oxygen isotope results into geographic context. The layer of predicted oxygen isotope signatures for the Pacific Ocean was created by rearranging and applying the isotopic calcite conversion formula (Killingley and Lutcavage, 1983). The formula has three variables - sea surface temperature (T), oxygen stable isotope levels in the water (W), and the stable isotope levels in the calcite (C). The formula is used to convert the water and calcite stable isotope values into expected sea surface temperature. However, if sea surface temperature is known, the formula can be rearranged and solve for predicted calcite values using the quadratic equation.

Original Formula:

$$T = 16.5 - 4.3C + 4.3\,W + 0.14\,C^2 - 0.28\,WC + 0.14\,W^2$$

Rearranged Formula:

$$0 = [0.14]C^2 + [-0.28W - 4.3]C + [0.14W^2 + 4.3W - T + 16.5]$$

Quadratic Equation:

$$\frac{-[-0.28W - 4.3] - \sqrt{[-0.28W - 4.3]^2 - 4\times[0.14]\times[0.14W^2 + 4.3W - T + 16.5]}}{2\times[0.14]}$$

Annual average sea surface temperature data from NOAA's World Ocean Database were used for the temperature variable (NOAA, 2005). These figures most closely matched the time frame of the data on water oxygen isotopes. Published water oxygen isotope figures from 2006 were used to create the oxygen isotope layer (LeGrande and Schmidt, 2006). These layers were imported into ArcGIS and converted to raster. The above quadratic equation was entered into ArcGIS's Raster Calculator tool to create the calcite oxygen isotope layer of the values one would expect to find throughout the Pacific Ocean. This calculation included the necessary +1.3 correction in the oxygen isotope ratio compared to the original formula above, due to the difference in the fractioning in barnacles (Killingley and Lutcavage, 1983).

References

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