



Stable hydrogen and oxygen isotope ratios in water beetle chitin

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Beetles are highly sensitive to climate change and are commonly used to estimate paleotemperatures using the Mutual Climatic Range (MCR) method. Beetles also capture a stable isotope signature when the chitin of their exoskeleton is formed. Water beetles are thought to be in isotopic equilibrium with the water in which they live. In this study we have used water beetles of the genera *Helophorus* (scavenger) and *Hydroporus* (predator). The specimens come from museum collections and represent 50 locations on a latitudinal transect ranging from 20-80°N in North America. In addition, beetle specimens and water samples were collected from Lake Ontario to correct for exchangeable hydrogen and fractionation effects. To monitor exchangeable hydrogen an internal standard of homogenized crab chitin was used. A 1M NaOH treatment was used to remove all proteinaceous material and a chloroform/ethanol mixture was used to remove waxes: this produced a crude chitin. This is different to the nitration method, which is much more destructive and inhibits the processing of small samples. Analyses were performed using a TCEA connected to a ThermoFinnigan Delta Plus XP. Isotopic standards were monitored continuously throughout the analytical time using international and internal standards. This study shows a relationship between isotopic ratios in the chitin of water beetles with latitude and MAT (from nearby weather stations). The isotope values in both beetle genera show a trend from high to low latitudes in δD from -160 to +60 per mil and in $\delta^{18}O$ from 8 to 27 per mil. Binning the isotopic data in 5° latitude averages, a very strong correlation between latitude and isotope ratios is observed ($r^2 = 0.9$ and 1.0 for δD and $\delta^{18}O$, respectively). These averages remove scatter that is potentially caused by environmental variables (e.g. lake

hydrology, trophic state, emergence time and year). A strong correlation was found between isotope ratios in *Hydroporus* and MAT ($r^2 = 0.9$ for both δD and $\delta^{18}\text{O}$), but was weaker for *Helophorus* ($r^2 = 0.4$ and 0.8). Poor correlations were observed between isotope ratios and MAP ($r^2 \leq 0.4$). This updated dataset increases our ability to isotopically analyze fossil beetles from North America to reconstruct paleotemperatures, which can then be directly compared to MCR estimates.