Evaluating Which Times of Day Mercenaria mercenaria Grows Using Oxygen Isotope Analysis

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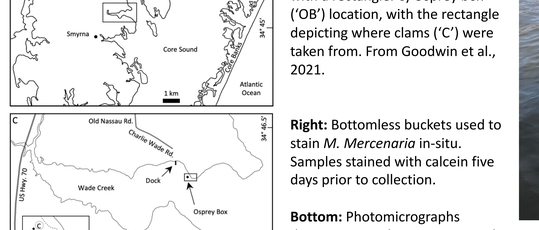
Abstract

Biogeochemical archives from bivalve mollusk shells provide important records of past environmental conditions. Stable oxygen isotopes in bivalve mollusk shell carbonate ($\delta^{18}O_{carb}$) are precipitated in equilibrium with water, and therefore reflect a combination of temperature and the oxygen isotope composition of the water ($\delta^{18}O_{water}$) in which they grew. Interpreting $\delta^{18}O_{carb}$ values, however, may be complicated by species-specific growth rate variations that can bias isotope values towards optimal growth conditions. Previous work suggests the hard clam, *Mercenaria mercenaria*, living in Jarrett Bay, North Carolina, grow preferentially during the warm hours of the day. To further investigate the influence of optimal growth temperatures paleoenvironmental reconstructions we calculated predicted $\delta^{18}O_{carb}$ values using temperatures and $\delta^{18}O$ w records collected every two hours over five days from the Jarrett Bay study site. Water samples were collected using an ISCO programmable autosampler. For two of the five days we collected paired water samples, one stored open to the atmosphere and the other stored under mineral oil to prevent evaporation. $\delta^{18}O_{water}$ values from paired samples were statistically indistinguishable, suggesting little to no evaporative enrichment took place in the autosampler. We also calculated an oxygen isotope envelope for 2021 using daily maximum and minimum water temperatures and weekly $\delta^{18}O_{water}$ values. Predicted $\delta^{18}O_{carb}$ values were then compared with observed $\delta^{18}O_{carb}$ values micromilled from three *M. mercenaria* specimens collected from the study site. To facilitate this comparison clams were stained with calcein five days prior to their collection. Calibration of the predicted and observed $\delta^{18}O_{carb}$ values is consistent with previous findings that *M. mercenaria* grows preferentially during the warmest hours of the day. At this point it is not possible to determine if shell deposition halts or simply slows during cooler temperatures. Future high-resolution sampling of shell carbonate is needed to address this question. Our findings suggest that significant portions of each day are not represented, and these biogeochemical archives should be interpreted accordingly.

Background



Previous work by Goodwin et al. (2021) revealed how the biology of *M. mercenaria* leads to preferential growth during the warmer hours of the day, and thus a biased recording of temperature.

Samples from five different *M. mercenaria* specimens are plotted along with the oxygen isotope envelope, which shows the range of potential $\delta^{18}O_c$ values each day.

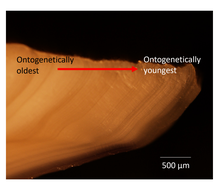
Locality & Calcein Staining

Three different *M. mercenaria* specimens which were suitably young were collected from the study site.

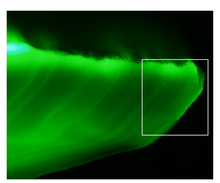
Left: Locality map. A) Map depicting the location of Carteret County in North Carolina. B) Core Sound & Jarett Bay, with the location of Wade Creek marked with a rectangle. C) Osprey box ('OB') location, with the rectangle depicting where clams ('C') were taken from. From Goodwin et al., 2021.

Right: Bottomless buckets used to stain *M. Mercenaria* in-situ. Samples stained with calcein five days prior to collection.

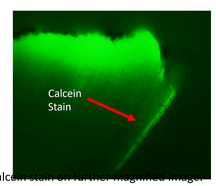
Bottom: Photomicrographs depicting growth increments and calcein stains



Visible growth increments in a magnified cross-section of a shell under visible light.



Same shell as to the left, under UV light. Calcein stain line visible to the right. White box contains portion of image which is further magnified below.

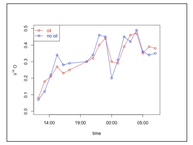


Calcein stain on further magnified image

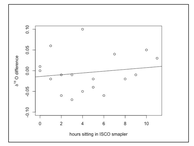
Methods

Water Sampling

Hourly water samples collected with the ISCO 3700 Portable Sampler

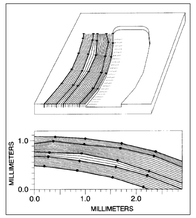


Paired hourly water samples collected. One set of samples contained mineral oil, to control evaporation, and one set with water samples exposed to the open air within the ISCO sampler. No significant difference found between samples with mineral oil, and samples without.

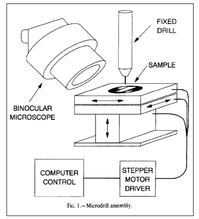


Differences in samples with mineral oil vs. samples without mineral oil, plotted according to the number of hours they sat in the ISCO Sampler before being manually collected and capped. Sample-t test showed no significant difference between the groups.

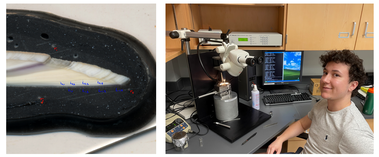
Sampling Shells For Isotopic Analysis



Virtual lines created, and coordinates recorded, to align the shell's growth intervals with the micromill's drilling pattern.



Generalized diagram of a computer controlled micromill (Dettman et al., 1995).



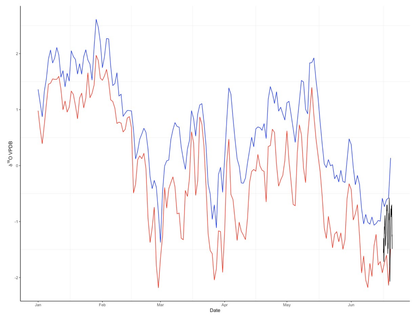
Samples drilled using the X-Y-Z computer controlled micromill procedure outlined in Dettman et al., 1995 (figures 12 and 13). Target mass of ~ 30 µg for all samples.

Samples run on Union College's Stable Isotope Laboratory using a Thermo Delta Advantage isotope ratio mass spectrometer coupled to a GasBench II.

Results

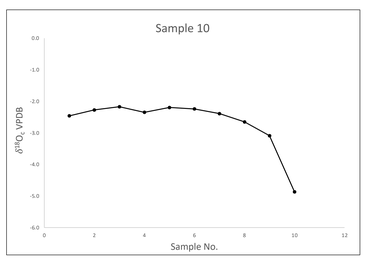
Right: Oxygen isotope envelope created for January 1st 2021, through June 21st, 2021 using daily minimum (blue line) and maximum (red line) temperatures.

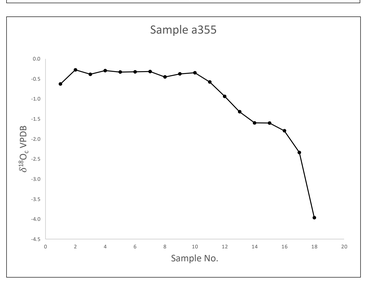
Predicted $\delta^{18}O_c$ curve calculated for the five-day study period (black line)

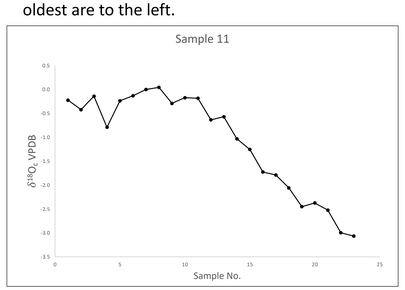


Below & to the left: Plots of each $\delta^{18}O_c$ isotope sample from all three sampled *M. mercenaria*.

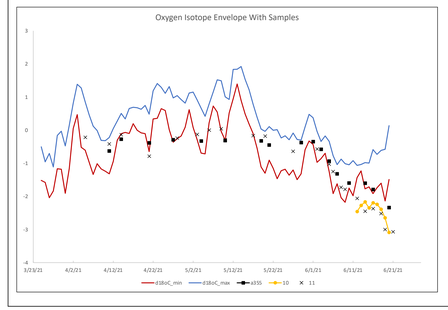
Samples which are ontogenetically youngest are to the right, and those which are ontogenetically the oldest are to the left.







Fitting Samples to The Oxygen Isotope Envelope

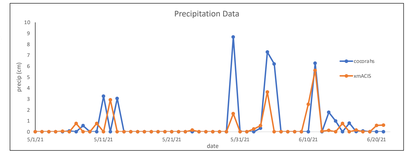


Procedure:

- Ontogenetically oldest sample assigned date of clam's collection
- Undated samples assigned dates using linear interpolation assuming a constant growth rate
- Samples falling outside of the envelope were adjusted so that they fell within the oxygen isotope envelope
- Samples were fit to only a portion of the envelope shown in the results section
- Procedure completed according to Goodwin et al., 2021

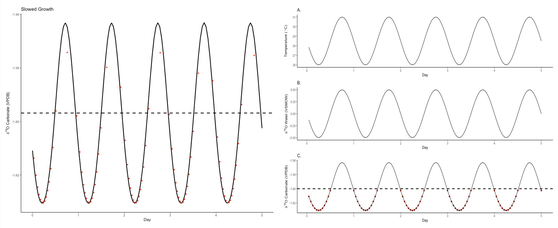
Discussion

- Samples generally follow the warmer temperatures indicated by the oxygen isotope values in the oxygen isotope envelope shown above (figure 18). This aligns with previous findings from studies of *M. mercenaria*
- Samples do not exceed a $\delta^{18}O_c$ value of approximately 0 ‰
  - Therefore, the temperature recorded by the specimens during the sampling period ranged between ~20 °C and ~38 °C
- Assuming continuous growth, sample sizes of 30-50 µg likely represent an average of around seven days.
- Samples falling outside of the oxygen isotope envelope during mid-June may reflect increased precipitation



Precipitation data from:
- xmacis.rcc-acis.org
- cocorahs.org

Is growth slowed or halted?



Left: Slowed growth model, where the clams grow more slowly when temperatures are outside of the ideal range. Recorded temperatures (red dots) compared to the complete temperature record (black line).

Right: Halted growth model, where growth ceases above a certain temperature. C) Recorded temperatures (red dots) compared to all potential isotopic values, given continuous growth (black line).

Future Directions

- To further constrain the timing of samples additional, high-resolution samples are needed to further constrain the timing of shell growth
- Future analysis using SIMS (secondary ion mass spectrometer) can be used to measure $\delta^{18}O_c$ values for much smaller samples, allowing for samples to represent smaller periods of time

Acknowledgements

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Citations:

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- Dettman, D.L., A.K. Reische, and K.C Lohmann (1999), Controls on the stable isotope composition of seasonal growth bands in aragonitic fresh-water bivalves (*Unionidae*), *Geochimica et Cosmochimica Acta*, v. 63, pp. 1049-1057.
- Goodwin, D.H., Flessa, K.W., Schone, B.R., & Dettman, D.L. (2001). Cross-Calibration of Daily Growth Increments, Stable Isotope Variation, and Temperature in the Gulf of California Bivalve Mollusk *Chione cortezi*: Implications for Paleoenvironmental Analysis. *Palaios, 16*: 387-398.
- Goodwin, David H., et al. "Comparing contemporary biogeochemical archives from *Mercenaria mercenaria* and *Crassostrea virginica*: Insights on paleoenvironmental reconstructions." *Palaeogeography, Palaeoclimatology, Palaeoecology* 562 (2021): 110110.