

PROCEEDINGS OF THE TWENTY-SIXTH ANNUAL KECK RESEARCH SYMPOSIUM IN GEOLOGY

April 2013
Pomona College, Claremont, CA

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**KECK GEOLOGY CONSORTIUM
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2012-2013 PROJECTS

**TECTONIC EVOLUTION OF THE CHUGACH-PRINCE WILLIAM TERRANE: SHUMAGIN ISLANDS
AND KENAI PENINSULA, ALASKA**

Faculty: *JOHN GARVER*, Union College, *CAMERON DAVIDSON*, Carleton College

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Washington & Lee University, *ALEXANDER SHORT*, University of Minnesota-Morris, *CARLY ROE*, Lawrence
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LAVAS AND INTERBEDS OF THE POWDER RIVER VOLCANIC FIELD, NORTHEASTERN OREGON

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Pomona College, *MICHAEL BAEZ*, California State University-Fullerton.

**BIOGEOCHEMICAL CARBON CYCLING IN FLUVIAL SYSTEMS FROM BIVALVE SHELL
GEOCHEMISTRY - USING THE MODERN TO UNDERSTAND THE PAST**

Faculty: *DAVID GILLIKIN*, Union College, *DAVID GOODWIN*, Denison University.

Students: *ROXANNE BANKER*, Denison University, *MAX DAVIDSON*, Union College, *GARY LINKEVICH*, Vassar
College, *HANNAH SMITH*, Rensselaer Polytechnic Institute, *NICOLLETTE BUCKLE*, Oberlin College, *SCOTT
EVANS*, State University of New York-Geneseo.

**METASOMATISM AND THE TECTONICS OF SANTA CATALINA ISLAND: TESTING NEW AND
OLD MODELS**

Faculty: *ZEB PAGE*, Oberlin College, *EMILY WALSH*, Cornell College.

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Colorado College, *MITCHELL AWALT*, Macalester College, *FREDY, AGUIRRE*, Franklin & Marshall College,
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**GEOLOGY, PALEOECOLOGY AND PALEOCLIMATE OF THE PALEOGENE CHICKALOON
FORMATION, MATANUSKA VALLEY, ALASKA**

Faculty: *CHRIS WILLIAMS*, Franklin & Marshall College, *DAVID SUNDERLIN*, Lafayette College.

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CURTIN*, Pomona College, *TYLER SCHUETZ*, Carleton College, *BRENNAN O'CONNELL*, Colorado College,
SHAWN MOORE, Smith College.

CRETACEOUS TO MIOCENE EVOLUTION OF THE NORTHERN SNAKE RANGE METAMORPHIC CORE COMPLEX: ASSESSING THE SLIP HISTORY OF THE SNAKE RANGE DECOLLEMENT AND SPATIAL VARIATIONS IN THE TIMING OF FOOTWALL DEFORMATION, METAMORPHISM, AND EXHUMATION

Faculty: *MARTIN WONG*, Colgate University, *PHIL GANS*, University of California-Santa Barbara.

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THE ROLE OF GROUNDWATER IN THE FLOODING HISTORY OF CLEAR LAKE, WISCONSIN

Faculty: *SUSAN SWANSON*, Beloit College, *JUSTIN DODD*, Northern Illinois University.

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PALEOENVIRONMENTAL RECORDS AND EARLY DIAGENESIS OF MARL LAKE SEDIMENTS: A CASE STUDY FROM LOUGH CARRA, WESTERN IRELAND

Faculty: *ANNA MARTINI*, Amherst College, *TIM KU*, Wesleyan University.

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INTERDISCIPLINARY STUDIES IN THE CRITICAL ZONE, BOULDER CREEK CATCHMENT, FRONT RANGE, COLORADO

Faculty: David Dethier, Williams College, Will Ouimet, U. Connecticut.

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Keck Geology Consortium: Projects 2012-2013
Short Contributions— Bivalve Geochemistry Project

**BIOGEOCHEMICAL CARBON CYCLING IN FLUVIAL SYSTEMS FROM BIVALVE SHELL
GEOCHEMISTRY - USING THE MODERN TO UNDERSTAND THE PAST**

Faculty: DAVID GILLIKIN, Union College, DAVID GOODWIN, Denison University.

**RECONSTRUCTING INTRA-ANNUAL GROWTH PATTERNS OF LAMPSILIS CARDIUM USING
STABLE ISOTOPE GEOCHEMISTRY AND ENVIRONMENTAL PARAMETERS**

ROXANNE BANKER, Denison University
Research Advisor: David Goodwin

VITAL EFFECTS ON STABLE CARBON ISOTOPES IN FRESHWATER BIVALVES

MAX I. DAVIDSON, Union College
Research Advisor: David P Gillikin

**LINEAR AND LANDMARK-BASED MORPHOMETRIC COMPARISON OF TWO POPULATIONS OF
CAMPELOMA, SP. ACROSS THE K-PG BOUNDARY**

GARY LINKEVICH, Vassar College
Research Advisor: Stephanie Peek

**CARBON ISOTOPE CYCLING: A COMPARISON BETWEEN FOSSIL SHELLS ACROSS THE
CRETACEOUS-PALEOGENE BOUNDARY AND TODAY**

HANNAH SMITH, Rensselaer Polytechnic Institute
Research Advisor: Miriam Katz

THE LIFE AND AFTERLIFE OF HELL CREEK UNIONIDS

NICOLLETTE BUCKLE, Oberlin College
Research Advisor: Karla Parsons-Hubbard

**TRACE ELEMENTS WITHIN THE FRESHWATER BIVALVE LAMPSILIS CARDIUM FROM THE
O'SHAUGHNESSY RESERVOIR, OHIO**

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VITAL EFFECTS ON STABLE CARBON ISOTOPES IN FRESHWATER BIVALVES

MAX I. DAVIDSON, Union College
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INTRODUCTION

Analysis of bivalve shells can be used to better understand the environmental conditions at the time of aragonite precipitation via analysis and interpretation of $\delta^{13}\text{C}_{\text{shell}}$ values. Shell $\delta^{13}\text{C}$ values can be used to understand a plethora of environmental parameters including: pH, stream respiration, primary production, land use patterns (Mook 2000), salinity (Gillikin et al. 2006), and upwelling (Killingley & Berger 1979; Sadler et al. 2012). The carbon in bivalve shells is thought to originate primarily from dissolved inorganic carbon (DIC) in the surrounding water; however, some of the carbon originates from the mollusks own respired or metabolic carbon (C_M) (McConnaughey & Gillikin, 2008; Gillikin et al., 2009). Bivalves precipitate aragonite via an active process involving pumping H_2O , Ca^{2+} , and CO_3 into the extra-pallial fluid (EPF) from the ambient water and pumping out H^+ ions to increase pH (Fig.1). Furthermore, a portion of the respired CO_2 enters the EPF; therefore bivalves will assimilate some of their own respired or metabolic carbon into their shell (McConnaughey and Gillikin 2008). The respired carbon can alter the $\delta^{13}\text{C}$ value of the shell, thus obscuring environmental interpretations.

Precipitation of aragonite does not occur at a constant rate over time. Unionid bivalves, for example, will only precipitate aragonite during warm portions of the year when the water is over 12°C (Dettman et al. 1999). Environmental changes such as a large discharge event can also cause bivalves to shut down and stop precipitating aragonite (Dettman et al. 2004). Therefore, seasonal variation in temperature and changes in the environment can cause gaps in the biogeochemical record preserved in bivalve shells.

The percent of metabolic carbon incorporated into bivalve shells has been debated to be somewhere between 5-35% (Gillikin 2005). C_M incorporation tends to increase through ontogeny as demand for environmental carbon decreases due to decreased shell growth rates while metabolic carbon increases as animal tissue mass increases (Lorrain et al., 2004). A simple mixing model can be used to calculate percent C_M incorporation using the $\delta^{13}\text{C}$ value of tissue as the respired carbon endmember and $\delta^{13}\text{C}_{\text{DIC}}$ as the other. It was suggested by McConnaughey et al. (1997) that environmental CO_2/O_2 ratios determine C_M incorporation in bivalve shells. As the CO_2/O_2 ratio increases C_M incorporation decreases as the bivalves are forced to filter more water to meet their oxygen

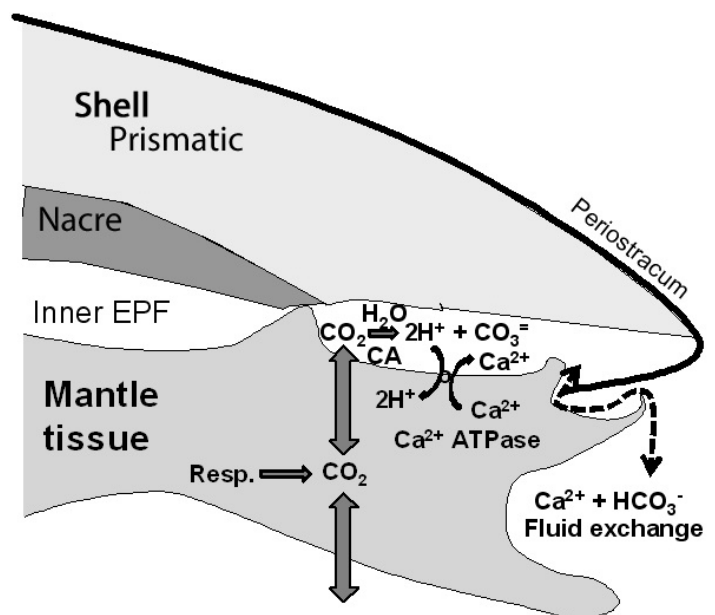


Figure 1. The active process involved in the assimilation of carbon into a bivalve shell (figure modified from McConnaughey and Gillikin 2008).

demand flushing the animal and EPF with CO₂ rich ambient water. Others believe that the disequilibrium between $\delta^{13}\text{C}_{\text{shell}}$ values and $\delta^{13}\text{C}_{\text{DIC}}$ values is attributed to a temperature dependent fractionation as proposed by Grossman and Ku (1986) and used by Sadler et al. (2012).

In order to uncover how C_M incorporation changes over time in *Lampsilis cardium* (Unionidae) bivalves were raised for one full year (2010) and a respiration experiment was conducted in July 2012. The mussels were raised adjacent to the Columbus Zoo and Aquarium Freshwater Mussel Conservation and Research Center in the O'Shaughnessy Reservoir (central Ohio) where a plethora of environmental parameters were measured on a weekly basis by researchers at the facility. We tracked how percent C_M incorporation into *L. cardium* shells changed over one year of growth. We also measured $\delta^{13}\text{C}_{\text{Respiration}}$ values, by conducting a respiration experiment in order to better understand the respiration end-member of the C_M /DIC mixing model.

METHODS

Lampsilis cardium were raised in sediment laden cages suspended from a dock in the O'Shaughnessy Reservoir in Delaware County, Ohio adjacent to the Columbus Zoo & Aquarium Freshwater Mussel Conservation & Research Center, for one full year, 2010. Onset data loggers were deployed to monitor water temperatures on an hourly basis throughout 2010. We sampled the water weekly throughout the entire year and analyzed the samples for $\delta^{18}\text{O}_{\text{water}}$ values (Picarro CRDS), [DIC] (using Pierott et al. (2006), discharge (USGS), total suspended solids, $\delta^{13}\text{C}_{\text{DIC}}$ values (Thermo Finnigan DeltaPlus XL isotope ratio mass spectrometer with a GasBench II) pH_{NBS} (MetrOhm 826, see Frankignoulle and Borges, 2001), chlorophyll a, total alkalinity (MetrOhm 888 Titrand auto-titrator), dissolved oxygen (YSI ProPlus), and $\delta^{13}\text{C}_{\text{POC}}$ values (see Lorrain et al. (2003) analyzed on an elemental analyzer – IRMS (EA-IRMS; Thermo Flash HT and DeltaV Advantage).

Bivalves, OR4 and OR6 were collected in September and December 2010, respectively. Tissues including, adductor, digestive gland, mantle, and gills were

analyzed for $\delta^{13}\text{C}$ values using the previously mentioned EA-IRMS. The shells were serial sampled every ~200 μm and samples were converted from a distance along shell axis to a time axis by Banker (this project) using $\delta^{18}\text{O}$ shell values. $\delta^{13}\text{C}_{\text{POC}}$ and $\delta^{13}\text{C}_{\text{DIC}}$ values were fit to shell dates using linear interpolation. Shells were analyzed using a Thermo MAT 252 and Kiel III automated carbonate sampling device.

To conduct the respiration experiment (Fig. 2), six open chambers were placed in the raceways at the husbandry facility. *Lampsilis cardium* were placed in four of the six chambers and allowed to acclimate for approximately one hour, while two of the chambers



Figure 2 Mussels acclimating in respiration chambers at the Columbus Zoo & Aquarium Freshwater Mussel Conservation and Research Center.

were left blank to determine background conditions. After the acclimation period the bivalves appeared to be filtering normally. Prior to the incubation, water $\delta^{13}\text{C}_{\text{POC}}$, $\delta^{13}\text{C}_{\text{DIC}}$, pH_{NBS} , DO, and total alkalinity samples were taken. Chambers were then covered and the incubation period began. One of the chambers contained an optical dissolved oxygen probe that recorded DO values based on light and did not consume O_2 . When dissolved oxygen decreased from ~ 80 to $\sim 50\%$ in the monitored chamber the incubation period ended and water samples were again taken. The respiration experiment was repeated four times. We used the following mixing model to determine $\delta^{13}\text{C}_{\text{Respiration}}$ values of *L. cardium*.

$$\delta^{13}\text{C}_{\text{DIC Final}} = \delta^{13}\text{C}_{\text{respired}} * ([\text{DIC}]_{\text{final}} - [\text{DIC}]_{\text{initial}}) + \delta^{13}\text{C}_{\text{DIC initial}} * (1 - ([\text{DIC}]_{\text{final}} - [\text{DIC}]_{\text{initial}}))$$

With this information we were able to use the following mixing model to estimate how C_M incorporation into the shells varied throughout the year (aragonite fractionation from Romanek et al., 1992).

Percent C_M Equation:

$$\% \text{C}_M = [(\delta^{13}\text{C}_{\text{shell}} - 2.7 - \delta^{13}\text{C}_{\text{DIC}}) / (\delta^{13}\text{C}_{\text{respired}} - \delta^{13}\text{C}_{\text{DIC}})] * 100$$

RESULTS/DISCUSSION

Only three animal and four trials leveraged DIC a significant amount (5%) during the respiration experiment (Table 1). These results provide limited data on the $\delta^{13}\text{C}_{\text{Respiration}}$ value of *L. cardium*. The $\delta^{13}\text{C}_{\text{Respiration}}$ values resembled both $\delta^{13}\text{C}_{\text{POC}}$ values and $\delta^{13}\text{C}_{\text{Tissue}}$ values equally with mean $\Delta_{\text{Respiration-POC}}$ and $\Delta_{\text{Respiration-Tissue}}$ values of -2.1% . The $\delta^{13}\text{C}_{\text{POC}}$ values had a median $\Delta_{\text{Respiration-POC}}$ value of -1.6% , which was closer than the median $\Delta_{\text{Respiration-Tissue}}$ value of -2.3% , thus we determined $\delta^{13}\text{C}_{\text{POC}}$ values resemble $\delta^{13}\text{C}_{\text{Respiration}}$ values best. We therefore used $\delta^{13}\text{C}_{\text{POC}}$ values as our respiration end member when calculating C_M incorporation into shells OR4 and OR6. Percent C_M incorporation ranged from $\sim 12\%$ to $\sim 43\%$ throughout the growing season (Fig. 3). Such a

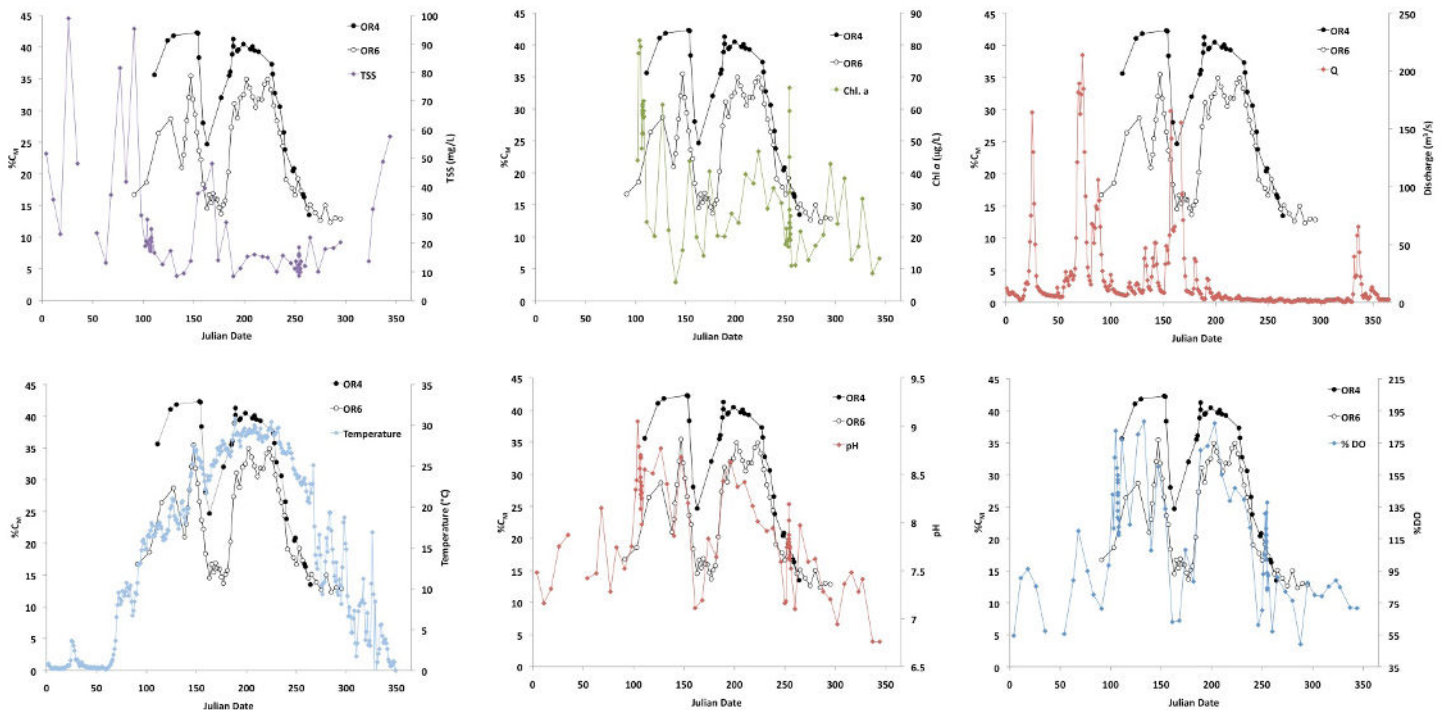


Figure 3. C_M contribution in two shells, OR4 and OR6, varies over time compared to several environmental parameters. DO and pH exhibit the best correlation with percent C_M contribution over time of all measured parameters. However, percent C_M contribution generally follows temperature except for a peak discharge period.

large variation may effect the validity and accuracy of paleoenvironmental interpretations based on stable carbon isotopes in bivalve shells that assume percent C_M is constant or a negligible factor in regards to the source of carbon in shells.

Table 1. Results from respiration experiment. Only 3 animals in 4 trials respired enough to leverage DIC. One is clearly an outlier (in grey) and is not included in the average. (Data in ‰ VPDB)

| Run | Animal | $\delta^{13}C_{Resp}$ | $\delta^{13}C_{POC}$ | $\delta^{13}C_{Tissue}$ | $\Delta_{Resp-POC}$ | $\Delta_{Resp-Tissue}$ |
|---------|--------|-----------------------|----------------------|-------------------------|---------------------|------------------------|
| 1 | IV | -35.1 | -34.1 | -32.6 | -1.0 | -2.5 |
| 3 | IV | -34 | -30.4 | -32.6 | -3.6 | -1.4 |
| 4 | IX | -35.1 | -33.4 | -32.8 | -1.6 | -2.3 |
| 4 | VII | -44 | -33.4 | -32.7 | -10.6 | -11.3 |
| Average | | -34.7 | -32.6 | | -2.1 | -2.1 |

* $\delta^{13}C_{Resp}$ is the $\delta^{13}C$ value of respired carbon, calculated using the mixing model described above. $\delta^{13}C_{POC}$ is the $\delta^{13}C$ value of the particulate organic matter of the water in the respiration chamber. $\delta^{13}C_{Tissue}$ is the $\delta^{13}C$ value of the adductor muscle in the bivalves used in the respiration experiment. $\Delta_{Resp-POC}$ is the difference in ‰ of the $\delta^{13}C$ value of respired carbon and the $\delta^{13}C$ value of the particulate organic matter of the water in the respiration chamber. $\Delta_{Resp-Tissue}$ is the difference in ‰ of the $\delta^{13}C$ value of respired carbon and the $\delta^{13}C$ value of the adductor muscle in the bivalves used in the respiration experiment.

Percent C_M incorporation correlated strongly with DO and pH (Fig. 3). It must be noted, however, that pH is most likely a function of dissolved CO_2 , which correlates with DO due to respiration and photosynthesis, therefore, pH may not be directly affecting percent C_M incorporation. Percent C_M also followed a similar trend as temperature, however, a decrease in percent C_M around Julian Day 160, the time of an observed discharge event, breaks the correlation with temperature. During the discharge event total suspended solids increased, perhaps contributing to the decrease in percent C_M contribution. As DO decreases, bivalves are forced to filter more water over their gills to meet their oxygen demand, in turn diluting their own respired carbon pool with ambient water (*cf.* McConnaughey et al. 1997). It can be inferred from our data that any environmental alteration that affects DO can also affect C_M contribution in bivalve shells. Chlorophyll *a*, did not exhibit a strong correlation with percent C_M contribution throughout the water monitoring period.

Examples of such environmental alteration include a discharge event, as we observed, algal blooms and subsequent aerobic decomposition (respiration), warming or cooling events, and topographical alterations that change water turbulence. Increasing pCO_2 levels and rising temperatures, due to anthropogenic CO_2 emissions may decrease DO levels

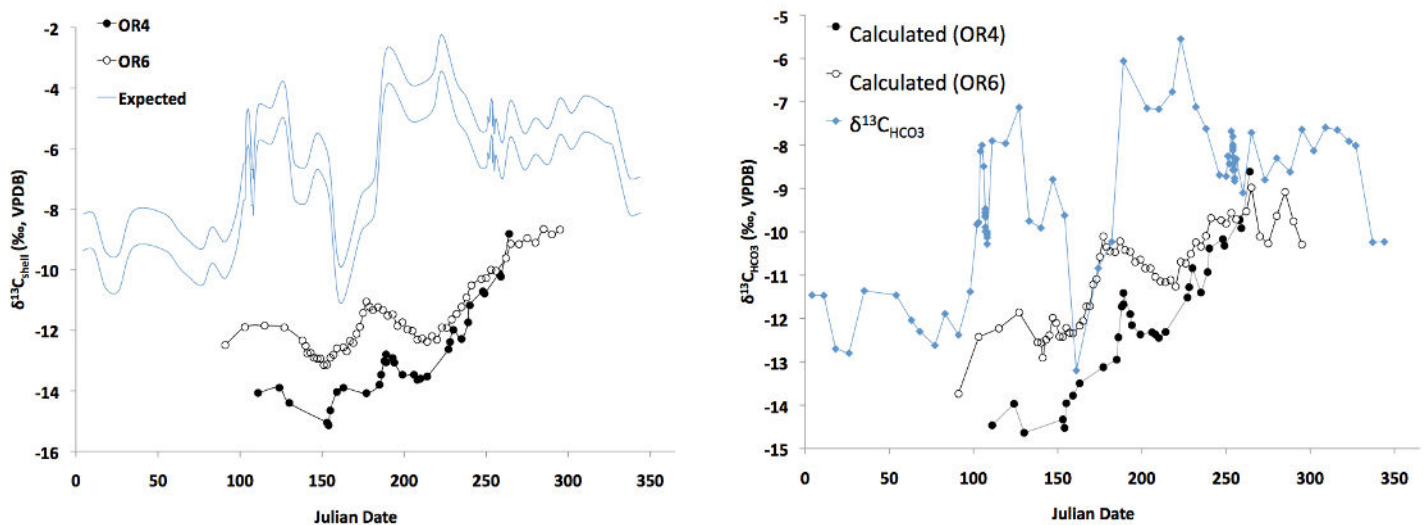


Figure 4. (Left) Expected $\delta^{13}C_{shell}$ values based on $\epsilon_{aragonite-HCO_3}$ of 2.7 ± 0.6 ‰ from Romanek et al. (1992) and $\delta^{13}C_{shell}$ values. (Right) measured and calculated $\delta^{13}C_{HCO_3}$ values. Calculated based on the temperature dependent ϵ from Grossman and Ku (1986): $\epsilon_{mollusk-DIC}$ (‰) = $2.66 - 0.131 * T(^{\circ}C)$.

in waterways therefore reducing the amount of C_M incorporated into the bivalve shells.

Our data suggests that shells OR4 and OR6, precipitated aragonite out of carbon isotopic equilibrium with the surrounding water (i.e. DIC), when comparing our data to expected $\delta^{13}C_{shell}$ values using the $\epsilon_{aragonite-HCO_3}$ of $+2.7 \pm 0.6$ ‰ from Romanek et al. (1992) (Fig. 4). The most parsimonious explanation is that C_M was incorporated into the shells, as the carbon in the shells is not solely derived from DIC. Our data also suggests that the temperature dependent ϵ from Grossman and Ku (1986), used by Sadler et al. (2012), is inaccurate (Fig. 4). The observations made by Grossman and Ku (1986) appeared to be temperature dependent, while metabolic rates, not temperature were most likely affecting the $\delta^{13}C_{shell}$ values of the shells sampled in their paper. Grossman and Ku (1986) sampled marine bivalves at varying depths, at greater depths temperature decreased and $\delta^{13}C_{shell}$ values increased suggesting a temperature effect, however, metabolic rates also decrease with depth, as endotherms require less food at colder temperatures and there tends to be less food in deeper marine waters.

The large and variable metabolic C_M contribution indicates that the stable carbon isotope archive in freshwater bivalves (Unionidae) has the potential for significant distortion. Geochemical interpretations based on stable carbon isotopes in bivalve shells should therefore assume a wide range of C_M contribution in the shells, ideally from 0 to ~40%. It is important to note that C_M contribution in bivalve shells can vary amongst species, individual animals, and through ontogeny. An understanding of bivalve biology and ecology, water chemistry, and seasonal variability in the settings where shells are collected may reduce the amount of uncertainty when assuming C_M contribution and therefore yield more accurate environmental interpretations.

CONCLUSIONS

C_M incorporation correlates strongly with DO and pH of the ambient water. Factors such as discharge, that impacts DO, most likely have an indirect impact on C_M incorporation. C_M incorporation is most likely

highly variable, throughout a year, in eutrophic settings where DO fluctuates greatly. It is unlikely to observe as much inter-annual variation in C_M incorporation in oligotrophic settings.

Lampsilis cardium precipitate aragonite out of carbon isotopic equilibrium of the ambient DIC as expected $\delta^{13}C_{shell}$ values based on ϵ from Romanek et al. (1992) do not exhibit similar trends or match measured $\delta^{13}C_{shell}$ values. Measured $\delta^{13}C_{DIC}$ values do not match calculated $\delta^{13}C_{DIC}$ values based on ϵ from Grossman and Ku (1986). Therefore, temperature based observations made by Grossman and Ku (1986) were most likely inaccurate.

$\delta^{13}C_{respiration}$ values, calculated during our respiration experiment, closely match $\delta^{13}C_{POC}$ values, therefore $\delta^{13}C_{POC}$ values can be used as the respiration endmember in percent C_M incorporation equations for *L. cardium*.

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