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

April 2013
Pomona College, Claremont, CA

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ISSN# 1528-7491

The Consortium Colleges The National Science Foundation ExxonMobil Corporation

KECK GEOLOGY CONSORTIUM
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Funding Provided by:
Keck Geology Consortium Member Institutions
The National Science Foundation Grant NSF-REU 1062720
ExxonMobil Corporation

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SCOTT EVANS, SUNY Geneseo Geology Department, 1 College Circle, Geneseo, NY 14454
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Learning Science Through Research
Published by Keck Geology Consortium
Short Contriubtions
26th Annual Keck Symposium Volume
6 April, 2013
ISBN: 1528-7491

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1. ABSTRACT

Molluscan bivalve shells record the elemental concentrations of the water in which they grow. Much is still unknown about trace element variations within freshwater bivalve shells. To better understand the relationship between shell and water concentrations, trace element water samples were collected weekly from the Columbus Zoo and Aquarium Freshwater Mussel Conservation and Research Center and the adjacent O'Shaughnessy Reservoir in 2010. Several specimens of the freshwater bivalve *Lampsilis cardium* were collected in April, September, and December 2010.

Trace elements over entire ontogenetic cycles, $\delta^{18}O$ shell values, water trace element values, and several environmental factors show evidence for possible controls on shell chemistry. It seems likely that complicated processes not indicated in these environmental data sets, and probably unrelated to environmental conditions, control Mg/Ca shell incorporation. Similarly, Mn/Ca concentrations do not vary directly with any of the parameters tested, however, ontogeny does seem to affect this element. Sr/Ca ratios within these shells exhibit a positive correlation with Sr/Ca ratios of the water in which they lived, and a negative correlation with turbidity. Values for Ba/Ca suggest a low background with peak interruptions, possibly due to variations in growth rate, as indicated by $\delta^{18}O$ comparisons, and/or chlorophyll-a concentrations.

2. INTRODUCTION

There is a wealth of information that can be gained by studying accretionary calcite and aragonite of marine and freshwater organisms. Foraminifera, corals, and gastropods have all shown useful results as geochemical indicators of environmental conditions (Lea and Boyle 1989, McCulloch et al., 2003, Sosdian et al., 2012). Studies have also focused on the value of molluscan bivalves as proxies for environmental conditions.

Trace elemental concentrations from biogenically precipitated calcite and aragonite have recently gained interest as possible proxies for paleoenvironmental reconstructions. For example Ba/Ca and Sr/Ca concentrations in calcitic foraminifera and aragonitic corals and gastropods have been shown to represent living conditions (Lea and Boyle, 1989, McCulloch et al., 2003, Sosdian et al., 2012). Trace element signatures of marine bivalves have also been studied; Sr/Ca ratios are thought to reflect growth rates, barium content in bivalve shells is thought to reflect seawater Ba/Ca concentrations, while boron and uranium inclusion seem to represent strong biological controls (Swan, 1956, Gillikin et al., 2005, McCoy et al., 2011, Gillikin and Dehairs 2013).

Few studies have examined trace elemental concentrations in freshwater bivalves. Bailey and Lear (2006) examined Sr/Ca concentrations in freshwater bivalves and were only able to conclude that the Sr/Ca levels in the shells did not change with Sr/Ca levels of water, possibly indicating strong biological controls. Similarly, Carroll and Romanek (2008) examined trace elements in the inner and outer nacreous layers of freshwater bivalves, and while they did find a significant difference between the layers, they did not identify any controlling factors for the compositions

they found. Izumida et al. (2011) examined the freshwater bivalve *Hyriopis* sp., they found that Sr/Ca concentrations decrease with age and are elevated during warm periods, Ba/Ca precipitation is controlled by growth rates, and that Mg/Ca incorporation is the result of complicated processes that are still poorly understood.

This research examines the variations in trace elemental concentrations of several specimens of the freshwater aragonitic bivalve *Lampsilis cardium* from the Columbus Zoo and Aquarium Freshwater Mussel Conservation and Research Center on the O'Shaughnessy Reservoir, Delaware county, Ohio. Several shells were collected and analyzed for concentrations of the trace elements magnesium, manganese, strontium, barium, and $\delta^{18}O$. Environmental conditions for the O'Shaughnessy Reservoir were carefully monitored during the year 2010.

3. METHODS

3-1. Study Period

Eighteen specimens of *Lampsilis cardium* from the collection at the Columbus Zoo facility were selected in 2010. Twelve of these specimens lived in contained cages directly in the O'Shaughnessy Reservoir; herein referred to as OR2-A(1-3), OR4-A(1-3), OR6-A(1-3) and OR7-A(1-3). The remaining six specimens were kept indoors in the facility, grown in water pumped directly from the reservoir; these are OR1-A(1-3), OR3-A(1-3). These shells were collected and all tissues were removed at different points throughout the year; OR1 and OR2 were collected on April 22, 2010, OR3 and OR4 were collected on September 22, 2010, OR6 was collected on January 7, 2011, and OR7 was collected on December 10, 2010. Water samples were collected weekly from December 10, 2009 until December 10, 2010. Environmental data for the reservoir, such as turbidity and temperature were monitored using Yellow Springs Instrument (YSI) company probes. Chlorophyll-a content was determined using a standard photospectrometric technique.

3-2. Sample preparation and analysis

Water samples for elemental determination were filtered using a 0.2 μm syringe filter (Sartorius Minisart 16532-Q) and were acidified to pH 3 using ultrapure HCl and stored until analysis. Samples were analyzed on a Perkin Elmer Elan 6100 (DRC) Inductively Coupled Mass Spectrometer (ICP-MS) using a multielement standard matched in concentration. Both SLRS-5 and NIST 1640 standards were analyzed and indicate good accuracy and precision (for standards as percent recommended values: Mg was 98.1%, Mn was 106.4%, Sr was 103.7%, and Ba was 108.0%).

For isotopic and trace elemental analysis specimens of *Lampsilis cardium* were cross-sectioned along the axis of maximum growth. When numerous cross sections were needed from one shell, deviation from maximum growth axis was less than 1 cm. Carbonate powder was micromilled from the cross sections using a custom built mill at Denison University. All carbonate isotopic analyses were performed on a Finnigan MAT 252 Isotope Ratio Mass Spectrometer (IRMS) equipped with a Kiel III automated sampling device. Samples were reacted with >100% orthophosphoric acid at 70°C. Results are reported relative to Vienna Pee-Dee Belemnite (VPDB) by calibration to the NBS-19 reference standard ($\delta^{18}O$ = +2.20% VPDB). Dates were assigned by matching $\delta^{18}O$ shell values to expected values calculated using $\delta^{18}O$ water values and temperature data (see Banker, this study, for details).

Shell cross section samples were analyzed for trace elements using a CETAC LSX-213 frequency quintupled neodymium-doped yttrium aluminum garnet laser (LA; λ = 213 nm) coupled to the aforementioned ICP-MS. LA-ICP-MS results were calibrated using the NIST 612 glass standard (values from Pearce et al., 1997). Laser samples (50 μm spots) started at the tip of the commissure and proceeded at 200 μm intervals along the outer prismatic layer (150 μm from the outermost shell edge). Approximately 100 samples were collected from most shells. To assess possible ontogenetic trends, approximately 300 samples were taken from shells OR7-A1L and OR6-A2R, sampling at 400 μm intervals in the later half of shell sections analyzed. Trace elemental sampling was also done inside micromilled troughs (used to

analyze $\delta^{18}O$ ratios) in shells OR4-A1L and OR6-A1L. Calibration (gas blank subtraction, ^{43}Ca normalization, and drift correction) was performed offline, post-collection, using GEOPRO software (CETAC). Results from calibration are initially reported in ppm, and then normalized to millimoles per mole of Ca. Accuracy was determined using micro-analytical carbonate standards (MACS) 1 and 3 (values from S. Wilson, USGS, unpublished data, 2004) standards run before and after each day of shell sampling; the sampling was determined to be accurate (Table 1). Systems operated under conditions as outlined in Gillikin and Dehairs (2013).

4. RESULTS

4-1. Consistency

To establish the reproducibility of these results comparison was made between analysis of OR4-A1L and OR6-A1L in August of 2012, and then again in October of the same year. These procedures show the same results in the same shell when run a second time. Some offset is expected because different parts of the shell with different profiles based on distance from umbo were used. Similarly, a comparison of trace elemental data taken from shells OR2-A2R, OR4-A1L, and OR6-A1L for Mn/Ca, Sr/Ca and Ba/Ca shows that similar values and patterns were preserved in these shells, offset based on date of collection.

4-2. Ontogenetic effects

Shells OR6-A2R and OR7-A1L were sampled from commissure to umbo in order to determine the effects of ontogeny on trace elemental concentrations. Mn/Ca concentrations show increased variability in amplitudes of cycles observed (Figure 1a). Ba/Ca concentrations show relatively low (approximately 0.2 mmol/mol) background concentrations with important 'peak' deviations (Figure 1b).

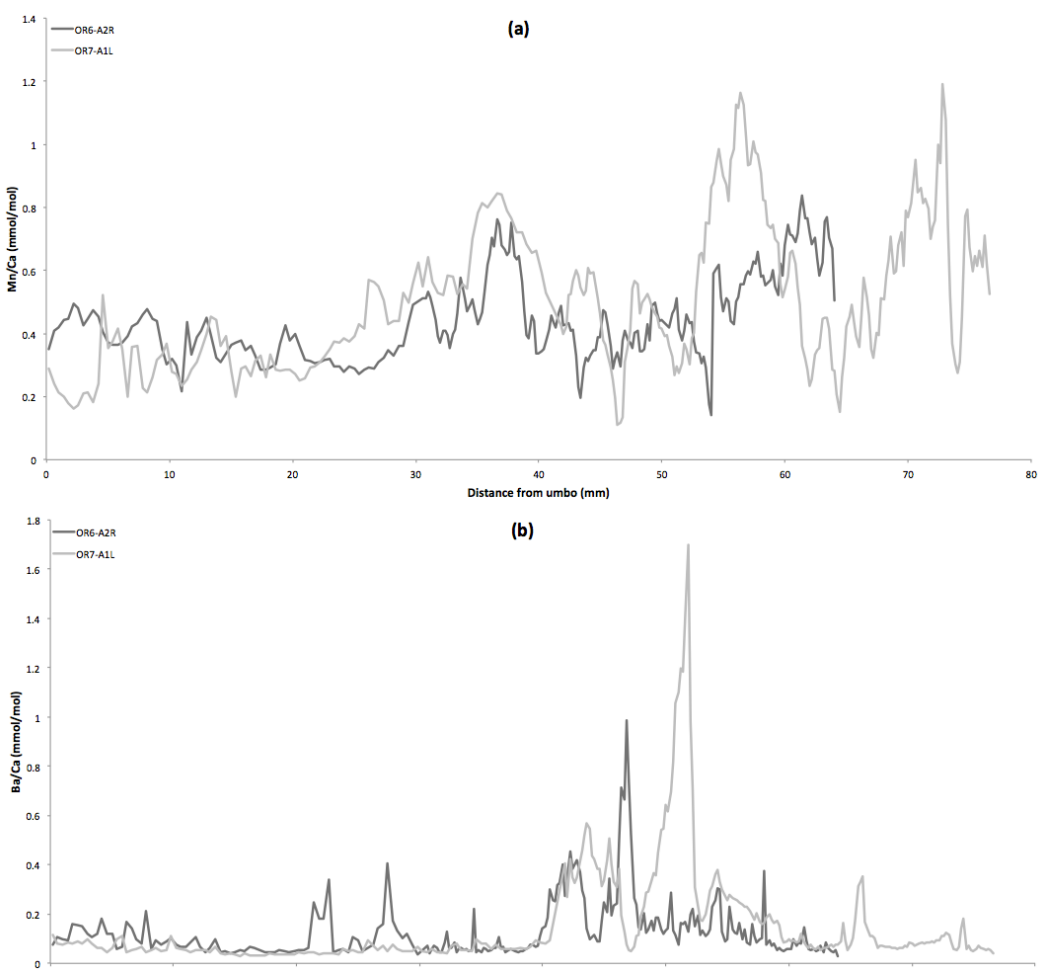

Figure 1. (a) Mg/Ca and (b) Ba/Ca ratios showing ontogenetic trends for OR6-A2R (245 data points) and OR7-A1L (278 data points)

4-3. Shell trace elements and $\delta^{18}O$

Comparisons of $\delta^{18}O$ concentrations from shells OR4-A1L and OR6-A1L show that these shells consistently recorded $\delta^{18}O$. Comparison of shell trace element concentrations with these values shows no real trend in the cases of Mg/Ca, Mn/Ca, and Sr/Ca. Ba/Ca values, however, show an inverse relationship with shell $\delta^{18}O$ values (Figure 2).

4-4. Shell and water trace elemental comparisons

Using dates determined by oxygen isotope analysis and interpolated based on oxygen isotope and trace element sample distances, the dates for trace elemental samples were calculated. A pairing of weekly water samples with shell sample trace elements can be determined using these dates. These data show no real correlation between shell and water Mg/Ca, Mn/Ca and Ba/Ca concentrations. Sr/Ca patterns in the shell, while lower, do exhibit the same trends as those patterns determined for the water samples (Figure 3).

Figure 2

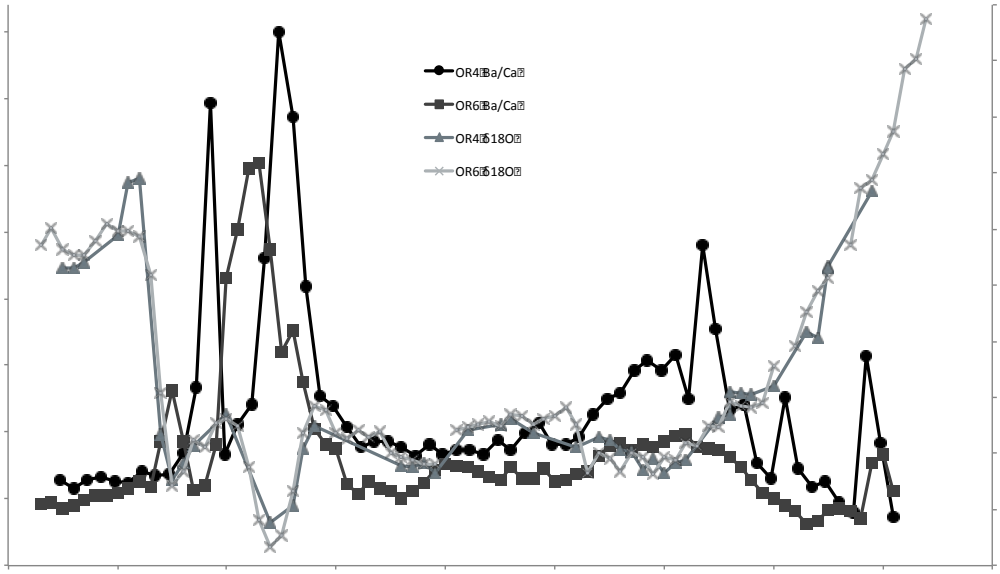

Figure 2. *Ba/Ca shell and Ba/Ca water values plotted against dates determined using $\delta^{18}O$ data.*

Figure 3

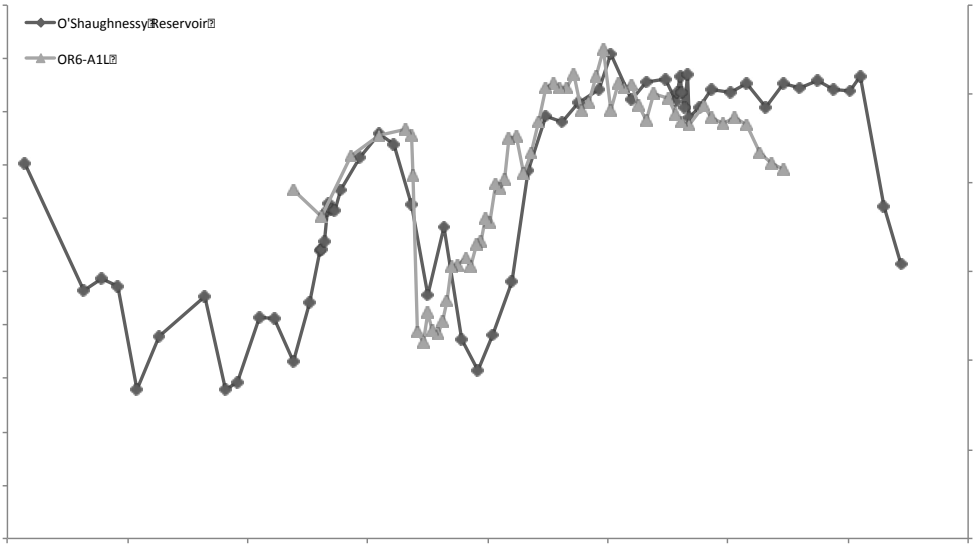

Figure 3. *Mn/Ca concentrations showing (a) ontogenetic trends for OR6-A2R (245 data points) and OR7-A1L (278 data points), (b) Mn/Ca and $\delta^{18}O$ shell values plotted against distance from umbo, and (c) Mn/Ca shell and Mn/Ca water values plotted against dates determined using $\delta^{18}O$ data.*

4-5. Trace elements and the environment

Using dates established using oxygen isotope analyses and distance relationships in the shell the weekly environmental data and shell trace element data can be aligned. Comparison of many of these factors yielded two important correlations. An examination of shell Sr/Ca with turbidity measurements shows an inverse trend (Figure 4). Similarly, shell Ba/Ca concentrations vary directly with chlorophyll-a data from the reservoir (Figure 5).

5. DISCUSSION

5-1. Reproducibility and validity

The varying trace elemental concentrations seen in these specimens recur in multiple shells (Figure 1a-d). With the exception of Mg/Ca (Figure 3a), trace elements in these shells record the same patterns amongst shells grown in the O'Shaughnessy Reservoir. The offset expected based on the dates of collection for OR2, OR4 and OR6 is evident. If trace elements were singularly controlled by biology (vital effects)

Figure 4

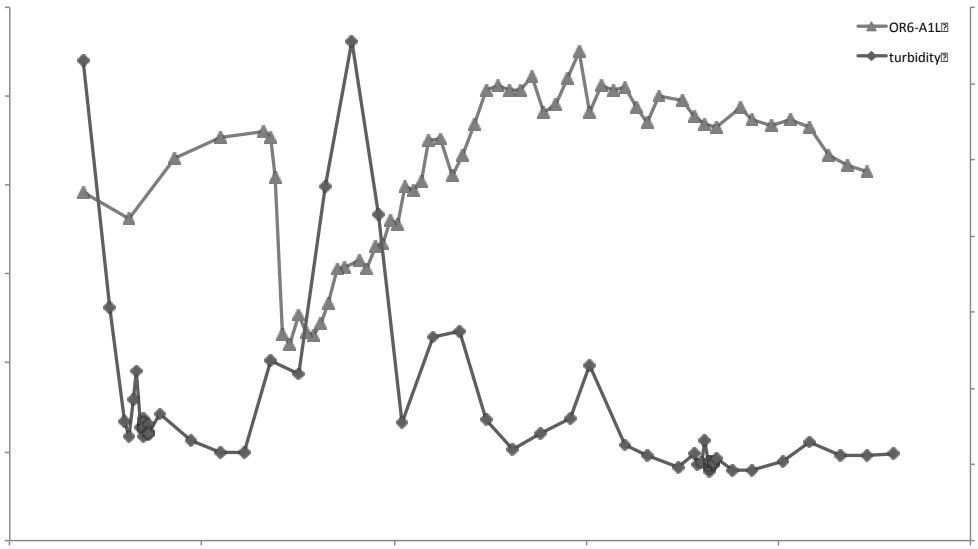

Figure 4. *Sr/Ca concentrations showing (a) ontogenetic trends for OR6-A2R (245 data points) and OR7-A1L (278 data points), (b) Sr/Ca and $\delta^{18}O$ shell values plotted against distance from umbo, (c) Sr/Ca shell and Sr/Ca water values plotted against dates determined using $\delta^{18}O$ data, and (d) Sr/Ca shell data and turbidity values plotted against dates.*

Figure 5

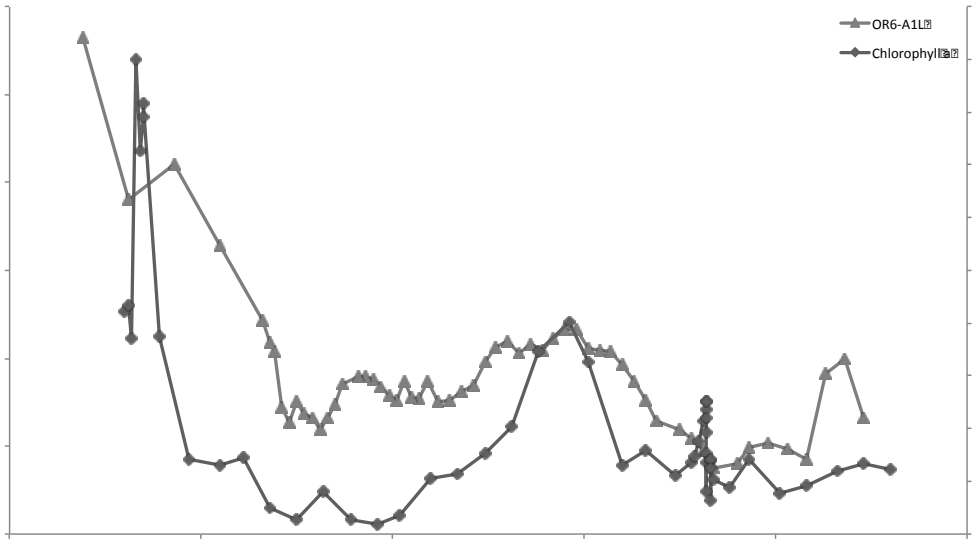

Figure 5. *Ba/Ca concentrations showing (a) ontogenetic trends for OR6-A2R (245 data points) and OR7-A1L (278 data points), (b) Ba/Ca and $\delta^{18}O$ shell values plotted against distance from umbo, (c) Ba/Ca shell and Ba/Ca water values plotted against dates determined using $\delta^{18}O$ data, and (d) Ba/Ca shell data and chlorophyll-a values plotted against dates.*

we would expect to see different concentrations and patterns in different organisms operating independently of their environment. However, because these patterns are evident in several different shells and are offset based on time of collection, this demonstrates that trace elemental concentrations of *Lampsilis cardium* are at least in part controlled by environmental factors.

5-2. Magnesium

Concentrations of Mg/Ca ratios taken from umbo to commissure show no decipherable ontogenetic effects (Figure 2a). Similarly, plots of shell Mg/Ca ratios versus $\delta^{18}O$ and water Mg/Ca ratios do not show any definitive correlations (Figure 2b, c) suggesting that magnesium incorporation in these shells represents complicated processes not strongly related, or entirely unrelated, to environmental conditions. This conclusion is similar to that proposed by Izumida et al. (2011), who suggested that Mg/Ca ratios in *Hyriopis* sp. were related to vital effects such as organic substance contamination or adsorption during crystal precipitation.

5-3. Manganese

Unlike magnesium, Mn/Ca ratios in the studied bivalves do seem to represent some form of environmental control (Figure 1b). With ontogeny, Mn/Ca concentrations become increasingly varied (Figure 3a). This is possibly a result of the decreased shell precipitation exhibited as these bivalves proceed further into ontogenetic development. Although we interpret Mn/Ca to be environmentally controlled, the environmental factors studied here could not explain the variations in Mn/Ca values within *Lampsilis cardium*. Comparison of shell Mn/Ca ratios with $\delta^{18}O$ shell values and Mn/Ca values of the O'Shaughnessy Reservoir do not show any significant trends (Figure 3b, c). Also, comparisons of environmental factors such as dissolved oxygen, dissolved inorganic carbon, pH, chlorophyll-a content, turbidity and discharge with Mn/Ca did not show any pertinent relationships.

5-4. Strontium

Sr/Ca ratios in *Lampsilis cardium* can be linked to several environmental factors. There was no observable trend exhibited on shell Sr/Ca ratios with ontogeny, and with changing shell $\delta^{18}O$ values (Figure 4a, b). Shell Sr/Ca ratios do show a direct correlation with Sr/Ca ratios recorded for the O'Shaughnessy Reservoir (Figure 4c). While the values, in mmol/mol, of the shell and water do not match, the overall trends observed in shell OR6-A1L and water Sr/Ca ratios are in good agreement. This suggests that water chemistry, at least in regards to Sr/Ca, is recorded in the shell Sr/Ca values. There is an inverse relationship between shell Sr/Ca ratios and turbidity of the O'Shaughnessy Reservoir (Figure 4d). The data presented suggest that while the water is at its most turbid Sr/Ca incorporation is significantly lower than at most other times when Sr/Ca and turbidity seem to be in 'normal' conditions. Thus Sr/Ca ratios in freshwater bivalves could provide a useful tool for paleoreconstructions in determining water Sr/Ca and levels of turbidity.

5-5. Barium

Shell Ba/Ca ratios show a steady background with peak deviations at certain points throughout ontogeny (Figure 5a). This is similar to the background concentrations reported by Gillikin et al. (2006) of 0.10 ± 0.02 mmol/mol for the estuarine bivalve *Mytilus edulis*. While a comparison of water Ba/Ca concentration with shell Ba/Ca concentration does not show direct correlations (Figure 5c), $\delta^{18}O$ shell values do vary inversely with Ba/Ca concentrations among the shells studied (Figure 5b). This correlation suggests that peak Ba/Ca concentrations correspond to higher temperatures. This is in good agreement with the results of Izumida et al. (2011) who suggested that Ba/Ca varied with temperature, and suggested that Ba/Ca incorporation was directly related to fluctuating growth rates in *Hyriopis* sp.

Several comparisons of other environmental factors with Ba/Ca concentrations show that Ba/Ca varies directly with chlorophyll-a content (Figure 5d). These data indicate that peak values of Ba/Ca incorporation in these bivalves are directly related to the photosynthetic activities of microscopic organisms. Several studies have suggested that barium uptake is related to phytoplankton productivity, but these claims have not been substantiated (Gillikin et al., 2006, Gillikin et al., 2008). This hypothesis may in fact be true for *Lampsilis cardium*.

6. CONCLUSIONS

There is some level of environmental control exhibited on manganese, strontium, and barium trace elemental concentrations found in the modern freshwater bivalve, *Lampsilis cardium*. Mg/Ca values are the result of complicated processes not easily correlated to the environment; Mn/Ca amplitudes become increasingly varied with ontogeny; Sr/Ca values are directly related to water Sr/Ca values; and Sr/Ca concentrations also decrease during times of heightened turbidity. Ba/Ca data show low background values with distinct periods of marked deviation. These periods are possibly related to temperature fluctuations and growth rates, represented by shell $\delta^{18}O$ values, or times of increased photosynthetic production, represented by chlorophyll-a content. If more data can be gathered on other freshwater bivalves, freshwater bivalves could prove an extremely important tool in recreating paleoenvironmental conditions.

ACKNOWLEDGEMENTS

To Dr. David P. Gillikin and Dr. David H. Goodwin I thank them for their introduction to subject matter, advisement, and instruction throughout this project as for research contributions (water trace element and shell $\delta^{18}O$ data). To Dr. Jeffery Over I thank for continued advisement. For personal contributions I thank the staff of the Columbus Zoo and Aquarium Freshwater mussel facility, Dr. Joseph H. Hartman, Roxanne Banker, Dane O'Niel, and Max Davidson.

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