

Oxygen and carbon isotope ratios of *Lampsilis cardium* (Unionidae) from two streams in agricultural watersheds of Iowa, USA

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Abstract

Climate archives preserved as seasonal variation in oxygen and carbon isotope ratios ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) in unionid shells potentially provide records of pre-instrumental environmental conditions in mid- to high-latitude continental regions. This study measured $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of biogenic carbonate and $\delta^{18}\text{O}_{\text{WATER}}$ to investigate the timing of growth line formation and to examine the utility of *Lampsilis cardium* as an environmental archive. We tested the hypotheses that: (1) *L. cardium* precipitates its shell in isotopic equilibrium with the environment during biomineralization; and (2) growth lines form seasonally during winter months. These questions were addressed by monitoring water chemistry, by tagging *L. cardium* to record growth, and by combining sclerochronology and stable isotope geochemistry. Temperature and $\delta^{18}\text{O}_{\text{WATER}}$ were measured fortnightly from September 2002 to July 2004 with a break in sampling from December 2003 to February 2004 in two Iowa rivers, Boone River and Buffalo Creek. These data were used to calculate expected $\delta^{18}\text{O}$ values to compare to measured shell values. One hundred fifty-four individuals were marked at the postero-dorsal margin in September 2002 and June 2003 to monitor growth. Seven recaptured shells collected in July 2004 were analyzed for shell $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ across three growth lines (including growth lines prior to the initial marking period) from the growing edge toward the umbo. Comparison of shell $\delta^{18}\text{O}$ with expected values revealed precipitation of $\delta^{18}\text{O}$ in equilibrium with the ambient environment from early spring to early fall. Winter values were not recorded due to winter growth cessation. Change in temperature or reaching a threshold temperature appeared to control the onset and cessation of growth. Moreover, the locations of seasonal growth lines corresponded to winter growth cessation allowing the assignment of calendar years to growth lines. Seasonal growth lines were distinguished from non-seasonal disturbance lines by their seasonal location inferred from the $\delta^{18}\text{O}$ time series recorded in shell growth. Thus, we can faithfully reconstruct ontogeny through time and environmental and climate conditions from mid to high latitudes using *L. cardium*. Variation in $\delta^{13}\text{C}_{\text{SHELL}}$ followed a more or less sinusoidal trend similar to the seasonal profiles of $\delta^{18}\text{O}_{\text{SHELL}}$, suggesting that seasonal processes influenced the variation in carbon isotopes. Reduced amplitudes and more negative $\delta^{13}\text{C}_{\text{SHELL}}$ values with increasing age suggest that kinetic and/or metabolic isotope effects become important through ontogeny as observed in other bivalves. The profiles of $\delta^{13}\text{C}_{\text{SHELL}}$ potentially record local differences in landscape vegetation. However, specific factors influencing $\delta^{13}\text{C}_{\text{SHELL}}$ are unknown, and further study of water quality are required to fully assess the variation of $\delta^{13}\text{C}_{\text{SHELL}}$ as an environmental proxy.

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1. Introduction

Freshwater ecosystems are sensitive to climate change (e.g., temperature and precipitation patterns) and environmental conditions (e.g., impoundment, land-use, and hydrology). Many climate studies focus on marine sediment cores to evaluate variation in changing climate (Hays et al., 1976; Dansgaard et al., 1984; Oeschger et al., 1984; Pekar and Miller, 1996; Bond et al., 2001; Zachos et al., 2001). Few studies employ a geochemical approach to address the impact of climate change and anthropogenic alteration of rivers (Ingram et al., 1996a,b; Dettman et al., 1999; Ingram and Weber, 1999; Dettman et al., 2004). Yet, freshwater is a valuable commodity; thus, obtaining seasonal-scale proxies of climate change from mid to high latitudes are essential to reconstruct and monitor the response of freshwater habitats.

Unionids (freshwater mussels) serve as ideal candidates to decipher seasonal and inter-annual scale environmental and climate conditions from mid to high latitudes because of their accretionary mode of growth, wide geographic distribution, and sensitivity to environmental conditions and perturbations. Sclerochronology is the study of incremental growth features of shells. Growth features preserve a chronologic record of growth rates, growth patterns, age, temperature, salinity, and productivity as seasonal variations in oxygen ($\delta^{18}\text{O}$) and stable carbon ($\delta^{13}\text{C}$) isotope ratios (Dettman et al., 1999; Surge et al., 2001; Wurster and Patterson, 2001; Schöne et al., 2002; Schöne et al., 2003; Elliot et al., 2003; Dettman et al., 2004; Goodwin et al., 2004; Lorrain et al., 2004; Chauvaud et al., 2005; Gillikin et al., 2005; Surge and Walker, 2005; and others). Assuming equilibrium conditions, variations in $\delta^{18}\text{O}$ of bivalve shells reflect oxygen isotope ratios of ambient water and temperature during biomineralization (McCrea, 1950; Epstein et al., 1953; Dettman et al., 1999). Although many species of marine mollusks and unionids precipitate their shells in oxygen isotope equilibrium with the ambient environment (Epstein et al., 1953; Dettman et al., 1999; Surge et al., 2001; Elliot et al., 2003; Chauvaud et al., 2005), some species display an offset attributed to metabolic processes (e.g., vital effects) (Wefer and Berger, 1991; Fenger et al., 2007). Therefore, we must examine living species to verify isotopic equilibrium before interpreting past climate and environmental conditions preserved in their fossil shells.

This study aimed to: (1) assess whether shells of the unionid, *Lampsilis cardium*, preserve seasonal environmental records as variation in $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$; and (2) investigate the timing of growth line formation.

L. cardium was chosen because of its large, thick shell, its easily identifiable growth lines, and its abundance in Iowa rivers and streams. Here, we compared measured $\delta^{18}\text{O}_{\text{SHELL}}$ with calculated values (derived from measured $\delta^{18}\text{O}_{\text{WATER}}$, temperature, and the published equilibrium fractionation equation for aragonite and water (Grossman and Ku, 1986; Dettman et al., 1999)) to test the hypothesis that *L. cardium* precipitates its shell in oxygen isotope equilibrium with the ambient water. Previous studies show that unionids slow their growth during winter months (Howard, 1921; Chamberlain, 1931; Negus, 1966; Dettman et al., 1999). To evaluate growth history, we compared the timing of growth line formation with season inferred from the isotopic time series. We hypothesized that growth line formation occurred during winter months when temperature fell below optimal growth conditions and growth line formation coincided with peaks (i.e., highest values) in the $\delta^{18}\text{O}_{\text{SHELL}}$ record. Our findings contribute to the relatively few oxygen and carbon isotope studies on freshwater unionids and to our basic understanding of unionid ecology (Fastovsky et al., 1993; Veinott and Cornett, 1996; Tevesz et al., 1996; Tevesz et al., 1997; Tevesz et al., 1998; Dettman et al., 1999; Kaandorp et al., 2003). Moreover, our results will provide the necessary seasonal-scale proxies which can be used to further our understanding of historical climate and anthropogenic alteration of freshwater ecosystems.

2. Methods

2.1. Site description

Iowa is located in the temperate, midwestern United States and its landscape has been formed by Pleistocene glaciation. Farmers in Iowa practice intensive agriculture of row crops (corn and soybean). A common agricultural method in Iowa is to drain fields artificially by installing underground tiles to divert excess water into rivers and streams. Tile drainage creates flash flood conditions during seasonal rain and storm events. Thus, freshwater ecosystems that are drained artificially are highly impacted by rainfall, groundwater, storms, and irrigation.

This study focuses on two rivers with abundant and diverse molluscan fauna in artificially drained watersheds: Boone River (BR) in Hamilton County (north-central Iowa) and Buffalo Creek (BC) in Linn County (eastern Iowa) (Arbuckle and Downing, 2002) (Fig. 1). The headwaters of BR begin in Hancock County and empty into the Des Moines River south of Webster City. Its main channel length is 166 km and characterized by a

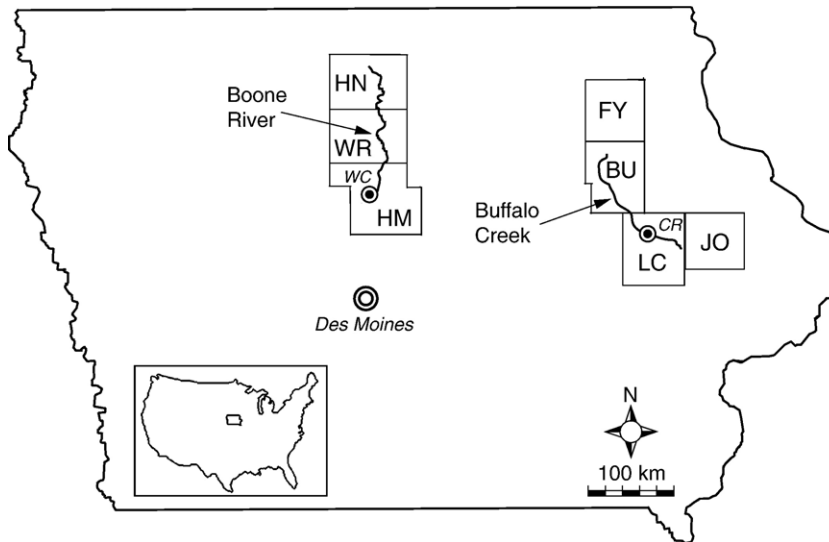


Fig. 1. Map of study area (Boone River and Buffalo Creek) located in Iowa, USA. The counties are indicated by abbreviations: BU=Buchanan County; CR=Cedar Rapids; FY=Fayette County; HM=Hamilton County; HN=Hancock County; JO=Jones County; LC=Linn County; WC=Webster City; and WR=Wright County. Filled circles identify sampling localities and the larger open bullet indicates the location of Des Moines.

riffle-pool structure with a semi-stable gravel and sand substrate. At the study locality, channel depth varied seasonally, with an average winter depth ranging from 0.5 to 1.0 m and the spring depth reaching approximately 2.5 m. Tile drains were observed at the study site. The underground drainage system exacerbated flooding conditions during spring flood. The primary row crop farmed up-river and near BR was corn (a C-4 plant) (<http://waterdata.usgs.gov/ia/nwis/rt>). Although crop rotation is a common agricultural practice, no crop rotation was observed near the study locality during the monitoring period. However, it is highly likely that crop rotation occurred within the watershed. Deciduous trees buffered the river from the corn fields.

BC is a tributary of the Wapsipinicon River starting in Jones County and ending in Buchanan County. It has a riffle-pool structure with a stable gravel and sand substrate providing ideal molluscan habitat. Channel depth at the study locality was less variable than BR, with winter depths approximately 1.0 m and summer depth approximately 1.5 m. Soybean (a C-3 plant) was the primary crop farmed near the study locality. Crop rotation likely occurred within the watershed.

2.2. Specimen marking and recapture

Mark and recapture were employed to document growth during the study. We collected and marked 92 individuals from the BR on September 1, 2002. From BC, we collected and marked 62 individuals on June 23,

2003. The left valve of each animal was cleaned of organic matter and marked with pointed plastic labels cemented with Zap-it Accelerator and Base[®], a type of dental cement (Dental Ventures of America, www.dentalventures.com), to the postero-dorsal margin of individual shells (Fig. 2). Each label identified the collection locality and the specimen number. The pointed edge of the plastic label was affixed at the



Fig. 2. Photograph of a marked *L. cardium* (specimen BR-1) in Boone River, Iowa. The label located at the postero-dorsal margin identifies the collection locality and specimen number. Growth lines are visible on the exterior of the shell. Samples of biogenic carbonate were microsampled parallel to direction of growth for isotopic analysis. The pits on the right side of the shell between the scale (in cm) and the label are the locations where the shell was microsampled.

postero-dorsal margin of the shell to indicate the beginning of the study (i.e., time zero). At the time of marking, all individuals were measured with vernier calipers (precision of 0.1 mm) for length (perpendicular to the hinge at the umbo to the margin), height (along the maximum distance of the postero-anterior axis), and thickness (parallel to the two dorsal shell sides), and then replaced into their original locations. Only a small fraction of the marked individuals were recaptured. Ten of the 91 marked unionids from BR were recaptured on July 7, 2004, after a period of 22 months. Three of the 62 marked individuals from BC were recaptured on July 15, 2004, after a growth period of 12 months. Three unmarked *L. cardium* were also collected in BC due to the low recapture percentage. Each harvested bivalve was measured for length, height, and thickness. The visceral mass was removed on-site.

2.3. Local environmental conditions

To characterize local environmental conditions, we sampled water fortnightly at 2.0 m below the water surface from September 2002 to July 2004 in BR and from June 2003 to July 2004 in BC. During winter months, we collected water samples up to 2.0 m below the water surface. However, water samples could not be consistently collected from December 2003 to February 2004 in BR and from December 2003 to January 2004 in BC because the ice froze to the river bottom (Supplementary Table 1). Water temperature was measured using a hand-held YSI multi-parameter probe (Supplementary Table 1). Fifteen milliliters of water collected for oxygen isotope analysis was stored in Nalgene polypropylene bottles.

$\delta^{18}\text{O}_{\text{WATER}}$ values were analyzed using a GasBench II auto-preparation system coupled to a Finnigan Delta Plus/XL isotope ratio mass spectrometer (IRMS) at Iowa State University. For water $\delta^{18}\text{O}$ analyses, 5 ml of water was equilibrated with CO_2 (g) at 25 °C. The standard deviation of repeated measurements of an internal water standard was better than 0.1‰. Absolute values were normalized to the international standards VSMOW and VSLAP. $\delta^{18}\text{O}_{\text{WATER}}$ values are reported in per mil (‰) relative to VSMOW standard.

2.4. Shell samples

To control for varying growth rates associated with size variability, we selected a subset of the recaptured shells from the same size class for isotopic analysis. Three individuals (BR-1, BR-2, and BR-3) from BR and

four (BC-1, BC-2, BC-3, and BC-4) from BC (one marked and three unmarked) were selected for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ analyses. The three unmarked shells were chosen preferentially over the marked individuals because of their thickness and identifiable growth lines. We removed surface organic matter from the exterior of shells with dilute bleach.

Shell microsampling and analyses were conducted at the University of Iowa's Paul H. Nelson Stable Isotope Laboratory. Microsamples were obtained using a Brasser handpiece fitted with a 0.3 mm burr mounted beneath a Nikon SMZU microscope. Transects were microdrilled perpendicular to visible growth lines (along the primary growth axis of the shell) from the outer prismatic layer. Where possible, periostracum was removed from each microsample and discarded. Approximately 20 μg of powdered aragonite was collected from each microsample pit. Individual carbonate analyses were conducted using a Kiel III automated carbonate reaction system coupled to a Finnigan-MAT 252 IRMS. Analytical precision was better than 0.1‰. $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values are reported in ‰ relative to VPDB.

2.5. Calculated $\delta^{18}\text{O}_{\text{SHELL}}$ values

To evaluate equilibrium precipitation, expected $\delta^{18}\text{O}_{\text{SHELL}}$ values were calculated for BR and BC. We calculated expected $\delta^{18}\text{O}_{\text{SHELL}}$ using water temperature, $\delta^{18}\text{O}_{\text{WATER}}$, and the following equilibrium fractionation equation (Grossman and Ku, 1986; Dettman et al., 1999):

$$1000 \ln(\alpha) = 2.559 (10^6 T^{-2}) + 0.715 \quad (1)$$

where T is temperature in Kelvin and α is the fractionation factor between water and aragonite. Calculated $\delta^{18}\text{O}_{\text{SHELL}}$ values were obtained using the following relationship:

$$\alpha_{(\text{ARAGONITE-WATER})} = \frac{(1000 + \delta^{18}\text{O}_{\text{ARAGONITE (VSMOW)})}}{(1000 + \delta^{18}\text{O}_{\text{WATER (VSMOW)})}} \quad (2)$$

The calculated $\delta^{18}\text{O}$ values were converted from VSMOW to VPDB using the equation reported by Gonfiantini et al. (1995):

$$\delta^{18}\text{O}_{\text{SHELL (VPDB)}} = (\delta^{18}\text{O}_{\text{SHELL (VSMOW)}} - 30.91)/1.03091 \quad (3)$$

To align $\delta^{18}\text{O}_{\text{SHELL}}$ with the expected $\delta^{18}\text{O}_{\text{SHELL}}$ values, we assigned dates to individual data points by anchoring the increments of growth at the time of

marking and harvest. The growth increments should record the ambient environmental conditions at or near the time of mark and harvest, respectively. We guided our assignments of dates between the known location of mark and harvest dates on the $\delta^{18}\text{O}_{\text{SHELL}}$ time series using sclerochronology (the location of seasonal growth lines). This approach allowed us to constrain the measured shell values to the predicted time series. We identified two growth lines corresponding to winter months in BR shells and one growth line in BC shells (discussed in Section 4.2).

2.6. Statistical analysis

We used a mixed model ANOVA to test whether the variance of oxygen and carbon isotope ratios of shell carbonate is different between study localities. Mixed models were employed to examine independent variables (i.e., $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) whose levels are fixed (i.e., river) by the research design and whose levels of effect are random (i.e., $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$). Our study generated two linear mixed models:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

where Y_{ij} was the dependent (response) variable, μ was the parametric mean, α_i was the fixed independent variable river, β_j was the random independent variable ($\delta^{18}\text{O}$ or $\delta^{13}\text{C}$), and ε_{ij} was the error associated with the variables. Model 1 was fit only with the isotopic composition (i.e., variance within a river), whereas model 2 was fit with isotopic composition and river (i.e., variance between rivers).

3. Results

3.1. Water temperature and chemistry

Water temperature at BR ranged from 0 to 27.7 °C, with an average summer (June to August) temperature of 20.56 ± 6.17 °C ($n=10$) and an average winter temperature (December to February) of 0.08 ± 0.07 °C ($n=6$). $\delta^{18}\text{O}_{\text{WATER}}$ varied from -15.78 to -5.27 ‰ with a mean value of -7.33 ± 1.80 ‰ ($n=35$) (Fig. 3A). The most negative $\delta^{18}\text{O}_{\text{WATER}}$ value is associated with an excursion that occurred between late February and early March, 2004.

BC water temperature ranged from 0 to 24.60 °C (Fig. 3B) with a mean summer temperature of 21.32 ± 3.30 °C ($n=9$) and mean winter temperature of 0.12 ± 0.04 °C ($n=3$). $\delta^{18}\text{O}_{\text{WATER}}$ values fluctuated seasonally from -13.66 to -6.72 ‰ with a mean value of -7.74 ± 1.57 ‰ ($n=18$) (Fig. 3). As in BR, the most negative

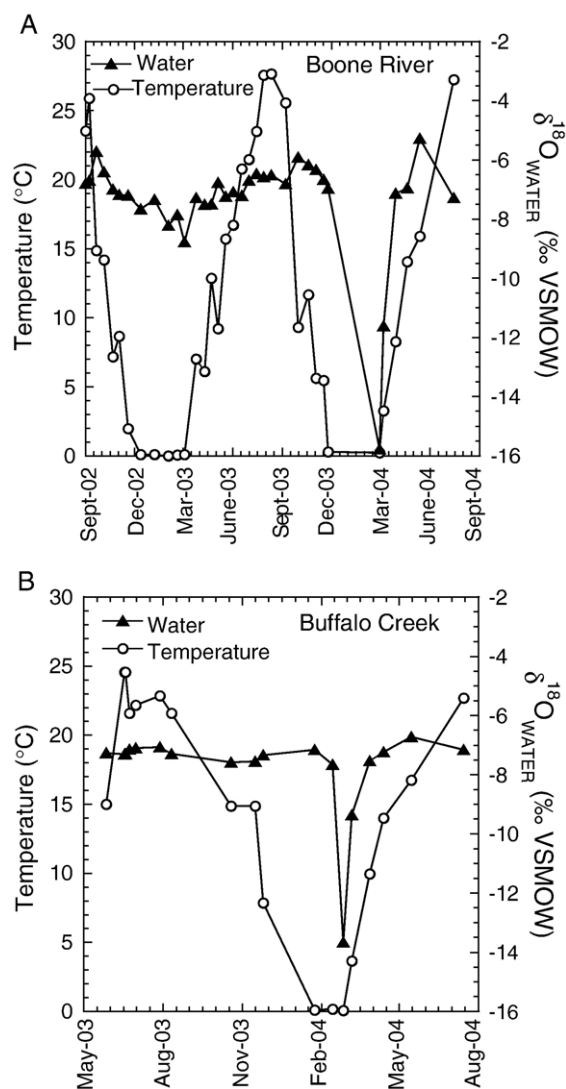


Fig. 3. Time series of temperature (°C) and $\delta^{18}\text{O}_{\text{WATER}}$ (‰) measured fortnightly at (A) Boone River from September 2002 to July 2004; and (B) Buffalo Creek from June 2003 to July 2004. Closed triangles represent $\delta^{18}\text{O}_{\text{WATER}}$ data. Open circles correspond to water temperature.

value is associated with an excursion that occurred between late February and early March, 2004.

3.2. Measured $\delta^{18}\text{O}_{\text{SHELL}}$ and $\delta^{13}\text{C}_{\text{SHELL}}$

Temporal variation in $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ within a shell follows a sinusoidal pattern with truncated peaks (Figs. 4 and 5). As shells mature, the amplitude of shell values decrease, and shell values show less seasonal variation. A significant correlation was observed between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ of four of the seven individual shells (BR-2, BR-3, BC-2, and BC-4) with r^2 values ranging from 0.31 to 0.42 (p values ranged from 0.05 to 0.001) (Table 1).

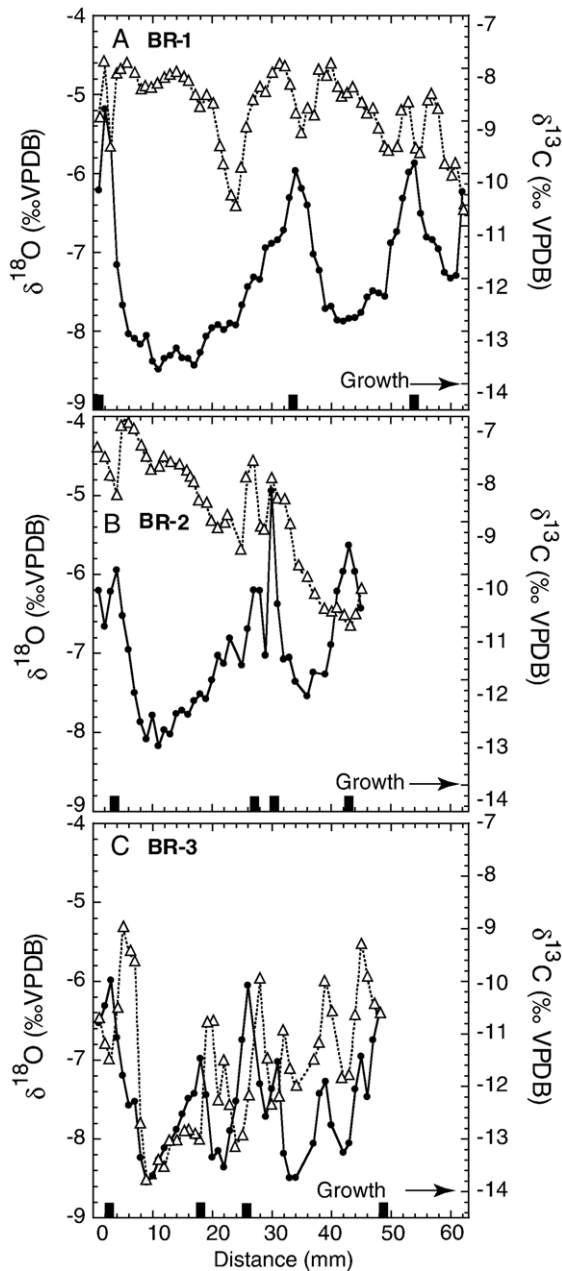


Fig. 4. Variation of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ versus distance of Boone River shells. (A) Shell BR-1; (B) Shell BR-2; and (C) Shell BR-3 were microsampled toward the growth margin (i.e., growth direction is from left to right). Filled circles represent $\delta^{18}\text{O}$ and open triangles are $\delta^{13}\text{C}$. Shaded bars at the bottom of each plot indicate the location of growth lines.

Measured $\delta^{18}\text{O}_{\text{SHELL}}$ in shells from BR (specimens BR-1, BR-2, and BR-3) ranged from -8.55 to -4.95‰ with a mean value of $-6.87 \pm 0.74\text{‰}$ ($n=39$), and those from BC (BC-1, BC-2, BC-3, and BC-4) ranged from -8.56 to -5.18‰ with an average value of $-7.29 \pm 0.44\text{‰}$ ($n=47$). Measured $\delta^{13}\text{C}_{\text{SHELL}}$ ranged from -13.75 to

-7.01‰ with an average value of $-9.84 \pm 1.15\text{‰}$ ($n=39$) in BR and from -14.41 to -9.24‰ in BC with a mean value of $-11.93 \pm 1.28\text{‰}$ ($n=47$). The result of the mixed model showed that the river effect was significant for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ ($p=0.001$ and $p=0.04$, respectively). Moreover, specimens BR-1 and BR-2 drive the difference of $\delta^{13}\text{C}$ values between the rivers.

3.3. Calculated isotopic composition

Calculated $\delta^{18}\text{O}$ values followed a seasonal trend similar to a sinusoidal curve (with the exception of the negative excursion in Spring 2004), representing the full range of possible values given the local environmental conditions throughout the water monitoring period (Figs. 6 and 7). Lower values correspond to warmer months and higher values occur in colder months. The negative excursion in February and March, 2004 corresponds to the negative excursion in measured $\delta^{18}\text{O}_{\text{WATER}}$ values when water temperature remained at 0 °C for several preceding months and a subsequent rise in temperature immediately thereafter (Fig. 3). These data suggest that the negative excursion in both rivers likely coincided with a snowmelt pulse in February or March, 2004. Calculated $\delta^{18}\text{O}$ values from BR ranged from -11.30 to -2.49‰ (average summer and winter values are $-6.85 \pm 1.37\text{‰}$ ($n=8$) and $-4.61 \pm 2.73\text{‰}$ ($n=11$), respectively), while the shell values from BC ranged from -9.16 to -3.14‰ (average summer and winter values are $-7.64 \pm 0.21\text{‰}$ ($n=8$) and $-4.96 \pm 2.36\text{‰}$ ($n=6$), respectively).

4. Discussion

4.1. Comparison of measured and expected $\delta^{18}\text{O}_{\text{SHELL}}$

The development of environmental proxy data provides a means to reconstruct paleoclimate and paleoenvironmental conditions. We examined whether *L. cardium* precipitated its shell in isotopic equilibrium with the ambient environment by comparing measured $\delta^{18}\text{O}_{\text{SHELL}}$ to calculated values (Figs. 6 and 7). Comparison of measured and expected shell values revealed that the most positive shell values are underrepresented. This observation suggests that *L. cardium* halted their growth during winter months, agreeing with published seasonal growth studies on freshwater bivalves (Chamberlain, 1931; Negus, 1966; Anthony et al., 2001). The negative excursion in early Spring 2004 was not captured in the measured shells perhaps because it reflected a brief amount of time or growth was still halted. We conclude that *L. cardium* precipitates its shell

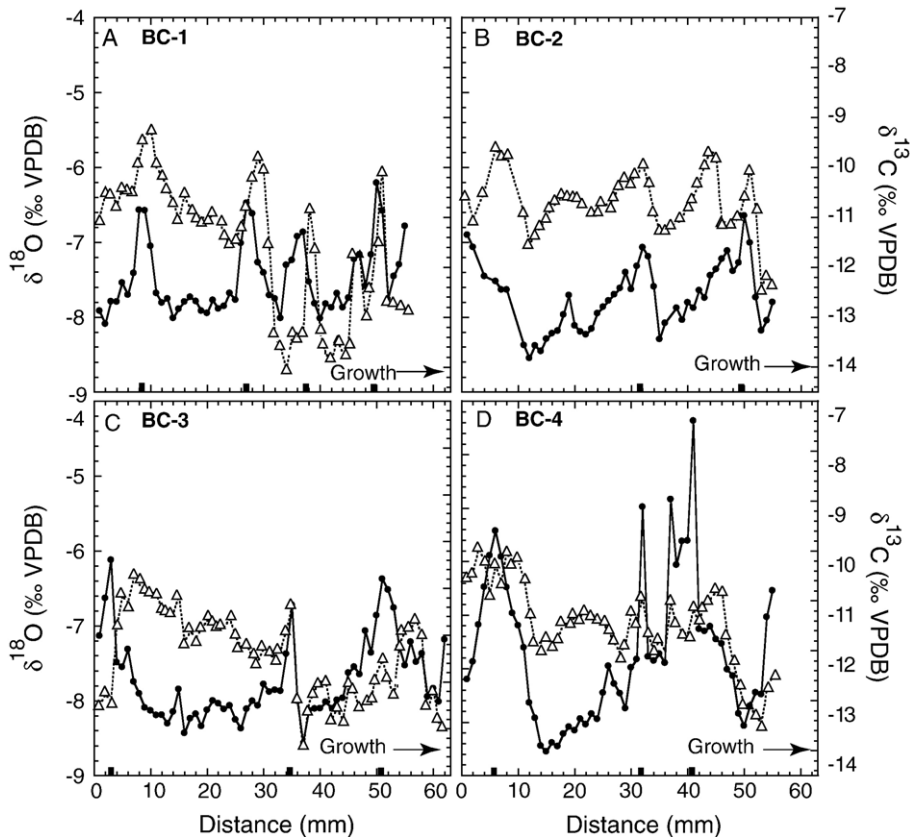


Fig. 5. Variation of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ versus distance of Buffalo Creek shells. (A) Shell BC-1; (B) Shell BC-2; (C) Shell BC-3; and (D) Shell BC-4 were microsampled toward the growth margin (i.e., growth direction is from left to right). Filled circles represent $\delta^{18}\text{O}$ and open triangles are $\delta^{13}\text{C}$. Shaded bars at the bottom of each plot indicate the location of growth lines.

in oxygen isotope equilibrium with the ambient water and that winter values are not represented due to growth cessation. Therefore, shells of *L. cardium* provide reliable archives of pre-instrumental climatic and environmental conditions.

4.2. Timing of growth line formation

Triggers responsible for the formation of annual growth lines in mollusks include extremes in temperature, food availability, and reproduction (Howard, 1921; Chamberlain, 1931; Negus, 1966; Pannella and MacClintock, 1968; Lutz and Rhoads, 1977; Jones, 1983; Goodwin et al., 2001; Surge et al., 2001; Keller et al., 2002; Elliot et al., 2003; Schöne et al., 2005). Dettman and Lohmann (1993) proposed a method to illustrate schematically patterns of growth by examining variation in oxygen isotope ratios. They identified two growth patterns: (1) continuous; and (2) seasonal. Continuous growth throughout the year was exemplified by a sinusoidal curve, while, seasonal growth (assuming

temperatures $\geq 12\text{ }^{\circ}\text{C}$) corresponded to a sinusoidal curve with truncated peaks, representing winter months or growth cessation. Their model provided a template of annual growth mediated by temperature; therefore, deviations from the model may be attributed to food supply or reproduction.

In this study, we employed the proposed modeled growth patterns of Dettman and Lohmann (1993) to address changes in growth rates (e.g., year round or seasonal growth). Variation of the measured $\delta^{18}\text{O}$ values

Table 1
Correlation by simple linear regression between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in each shell

Shells	r^2	p value
BR-1	0.19	0.15
BR-2	0.31	0.05
BR-3	0.34	0.02
BC-1	0.15	0.26
BC-2	0.43	0.002
BC-3	0.18	0.17
BC-4	0.39	0.003

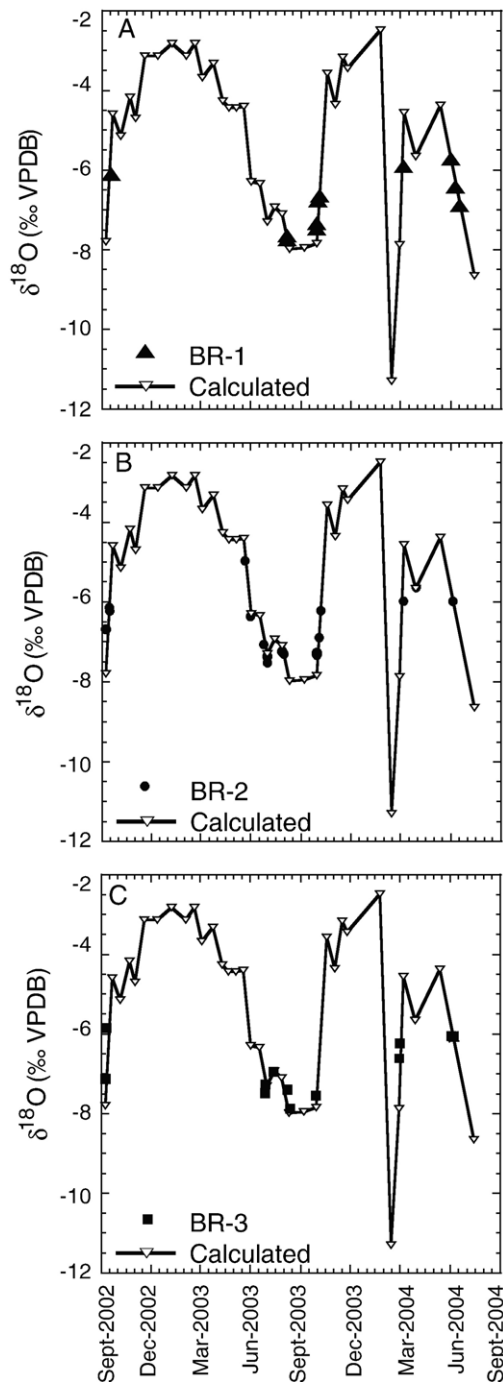


Fig. 6. Comparison of calculated to measured $\delta^{18}\text{O}_{\text{SHELL}}$ of *L. cardium* from Boone River from September 2002 to July 2004. Black line represents the calculated $\delta^{18}\text{O}$. (A) BR-1: closed triangles; (B) BR-2: closed circles; and (C) BR-3: closed squares.

followed a truncated sinusoidal pattern suggesting seasonal growth (Figs. 4 and 5). As previously discussed, we attributed the gaps in the isotope record to winter growth cessation, further supporting the obser-

vation of seasonal growth. Moreover, comparison of the measured $\delta^{18}\text{O}_{\text{SHELL}}$ against the expected values indicates that shell growth occurred from early spring to late autumn. To evaluate potential triggers, we compared the timing of winter growth cessation and onset of spring growth to the temperature records (Figs. 3, 6 and 7). Temperatures in BR from September 21, 2002 to October 5, 2002 decreased rapidly from 14.20 to 7.16 °C, which included the published cessation temperature threshold of 12 °C (Chamberlain, 1931). We observed a similar pattern in BR for the first measurable growth in Spring 2003 changing rapidly from 9.25 to 16.05 °C. Therefore, growth cessations and onset consistently correspond to rapid change in temperature reaching a temperature threshold.

To test the hypothesis that the formation of growth lines in individual shells occurred during winter months, we predicted that cessation lines would occur at the highest $\delta^{18}\text{O}$ values recorded in the shell (i.e., when temperature drops below optimal growth conditions). Periods of winter growth cessation identified from the isotope record consistently correlate to locations of prominent and complete growth lines demonstrating their seasonal formation (Figs. 4 and 5). This suggests that we can determine the age of individual shells by using the isotope record and growth lines.

Less prominent and incomplete growth lines were also present. We interpret these features as disturbance lines. Negus (1966) identified disturbance lines as incomplete and thin; whereas seasonal growth lines are complete and thick. Of the individuals sampled in our study, multiple shells (BR-2, BR-3, and BC-1) revealed incompletely formed disturbance lines (Figs. 4 and 5). These non-seasonal lines have been documented by Downing et al. (1992). Factors attributed to the formation of disturbance lines are non-seasonal temperature change (such as periodic release of reservoir water), predation, and reproduction (Krantz et al., 1987). Understanding growth patterns and distinguishing seasonal growth lines from disturbance lines is valuable when interpreting the impact of historical changes (e.g., construction of dams) on freshwater ecosystems and molluscan fauna. Furthermore, identification of seasonal growth lines aids in reconstructing variation of environmental and climate conditions.

We discriminated disturbance lines from seasonal growth lines by comparing the amplitude of $\delta^{18}\text{O}_{\text{SHELL}}$ values and the completeness of the growth line in our study to those reported by Dettman et al. (1999). They reported variations in seasonal amplitude (one peak to one valley) of $\sim 2.5\%$ in temperate regions. We observed two variations: (1) a shift in amplitude of

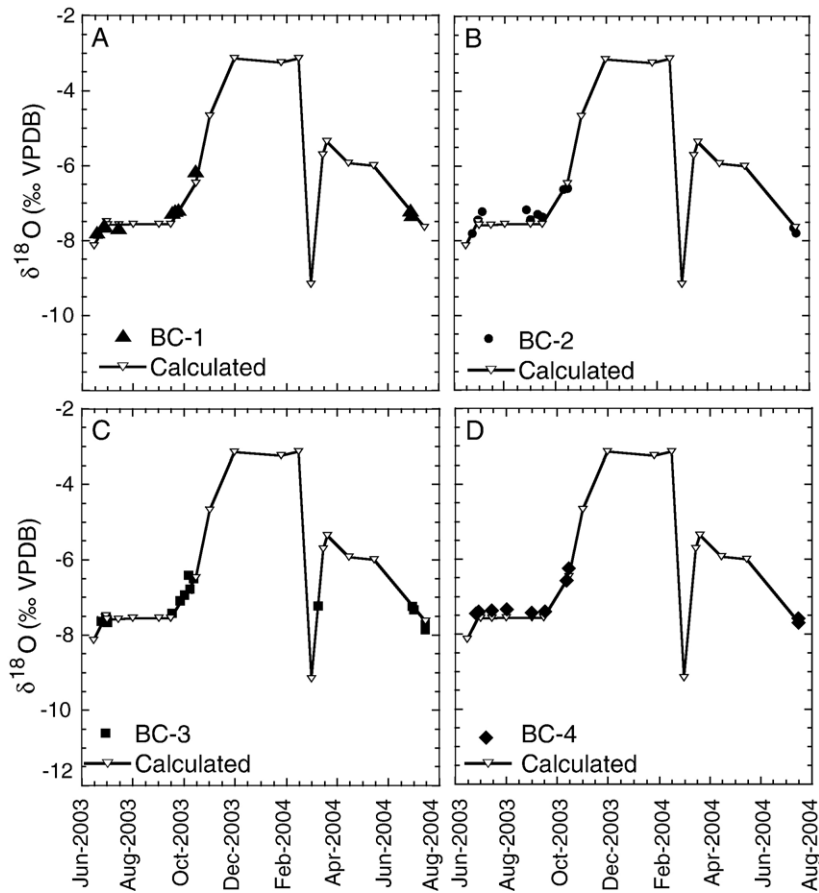


Fig. 7. Comparison of calculated to measured $\delta^{18}\text{O}_{\text{SHELL}}$ of *L. cardium* from Buffalo Creek from June 2003 to July 2004. Black line represents the calculated $\delta^{18}\text{O}$. (A) BC-1: closed triangles; (B) BC-2: closed circles; (C) BC-3: closed squares; and (D) BC-4: closed diamonds.

$\sim 1\text{‰}$; and (2) a shift in amplitude of $\sim 2.5\text{‰}$ (Figs. 4 and 5). Like Dettman et al. (1999), we found that shifts in amplitude of $\sim 2.5\text{‰}$ corresponded to thick and complete, seasonal growth lines. As a corollary, these growth lines coincided with the location of the three major peaks in all of the $\delta^{18}\text{O}_{\text{SHELL}}$ time series, supporting winter growth line formation with one exception (specimen BR-2; Fig. 4A). Of the four growth lines recorded in BR-2, one line displayed an amplitude of 2.5‰ , but the line was thin and incomplete; therefore, using amplitudes alone is insufficient to identify disturbance lines and emphasizes the need to include sclerochronologic information. In most cases, disturbance growth lines displayed an amplitude of $\sim 1\text{‰}$. Isotopic geochemistry and sclerochronology by themselves do not accurately discriminate seasonal and disturbance growth lines. Therefore, we stress the need to combine these tools to distinguish seasonal growth lines from disturbance growth lines allowing for a more accurate estimation of age.

4.3. Measured $\delta^{13}\text{C}_{\text{SHELL}}$

The profiles of $\delta^{13}\text{C}$ values follow a more or less sinusoidal trend similar to the seasonal variation of $\delta^{18}\text{O}$ (Figs. 4 and 5). This pattern suggests that seasonal factors influence variation in $\delta^{13}\text{C}$. A calculated equilibrium model of $\delta^{13}\text{C}$ was not constructed because we lacked measured $\delta^{13}\text{C}$ of dissolved inorganic carbon (DIC). However, prior studies have shown that $\delta^{13}\text{C}$ can serve as a proxy for biogeochemical cycling of carbon (Mook and Tan, 1991; Hellings et al., 1999). Other studies have shown that seasonal variation of $\delta^{13}\text{C}$ corresponds to available pools of carbon, which are influenced by primary productivity and decay of organic matter (Krantz et al., 1987; Dettman et al., 1999; Schöne et al., 2005).

Although some studies have demonstrated the utility of $\delta^{13}\text{C}$ as a proxy of primary productivity, $\delta^{13}\text{C}$ can be complicated by kinetic and/or metabolic isotope effects (i.e., “vital effects”) (Dillaman and Ford, 1982; Swart,

1983; Tanaka et al., 1986; McConnaughey et al., 1997; Furla et al., 2000; Lorrain et al., 2004; Gillikin et al., 2006). Kinetic isotope effects refer to the simultaneous depletion of ^{18}O and ^{13}C associated with fractionation during CO_2 hydration and hydroxylation (McConnaughey, 1989). Greater degrees of isotopic disequilibrium in shell carbonate correspond to rapid skeletal growth (McConnaughey, 1989; Klein et al., 1996). To evaluate whether kinetic isotope effects play a role in $\delta^{13}\text{C}_{\text{SHELL}}$, we tested whether there was correlation between $\delta^{13}\text{C}_{\text{SHELL}}$ versus $\delta^{18}\text{O}_{\text{SHELL}}$. A significant but weak correlation occurred in four (BR-2, BR-3, BC-2, and BC-4) shells with r^2 values ranging from 0.31 to 0.43, whereas the three other shells demonstrated no correlation (Table 1). At best, kinetic isotope effects can explain 43% of the observed variation in one shell. We next considered whether metabolic isotope effects contributed to the observed $\delta^{13}\text{C}_{\text{SHELL}}$ pattern.

A metabolic isotope effect refers to reactions within a biological system which result in measurable fractionation from calculated equilibrium. Metabolic controls on the incorporation of various sources of carbon, such as from respiration or food, into shell carbonate can produce such isotope effects. Metabolic carbon represented a small portion ($\sim 10\%$) of the carbon deposited in molluscan shell carbonate (McConnaughey et al., 1997; Kennedy et al., 2001; Lorrain et al., 2004; Gillikin et al., 2005; Gillikin et al., 2006). However, studies show that contribution of metabolic carbon can dampen the $\delta^{13}\text{C}_{\text{DIC}}$ signal recorded in shells and explain $\delta^{13}\text{C}_{\text{SHELL}}$ disequilibrium in bivalves (Krantz et al., 1987; Klein et al., 1996; Keller et al., 2002; Lorrain et al., 2004). Moreover, the contribution of metabolic carbon can account for the observed trend toward lower $\delta^{13}\text{C}$ values through time. This trend toward more negative values indicates that greater amounts of metabolic carbon are incorporated into shell carbonate as bivalves mature. Lorrain et al. (2004) used a metabolic carbon availability index (ratio of respired to precipitated carbon) to show that as bivalves aged, oxygen demand increased and accompanied respiratory carbon increased; hence, more respiratory carbon was incorporated into shell carbonate. We observed that all the shells followed this ontogenetic trend toward lower $\delta^{13}\text{C}$ values with increasing age (Figs. 4 and 5).

Broader trends in $\delta^{13}\text{C}_{\text{SHELL}}$ variability were observed between rivers, which may be related to landscape-scale factors. Differences in vegetation types (e.g., C-3 and C-4 plants) within each watershed may provide a possible explanation for the statistically significant offset of $\delta^{13}\text{C}$ values between shells from BR and BC (Table 1). C-4 plants (e.g., corn) have an

average $\delta^{13}\text{C}$ of -12% , whereas C-3 plants (e.g., soybeans) have an average $\delta^{13}\text{C}$ of -27% (Schlesinger, 1997). Corn is the dominant crop farmed in BR watershed, and soybeans are the primary row crop farmed in the BC watershed. Decomposed row crop material may contribute a distinct carbon isotope composition to the particulate organic matter in river water influencing the $\delta^{13}\text{C}$ of DIC and, hence, of shell carbonate. Therefore, shells from rivers within these two distinct landscapes may potentially record differences in $\delta^{13}\text{C}$ relating to C-3 and C-4 plants in the surrounding watershed. Uncovering the mechanisms controlling the difference between $\delta^{13}\text{C}$ values between shells from BR and BC cannot be determined based on the data presented here; however, this hypothesis can be tested by examining the $\delta^{13}\text{C}_{\text{DIC}}$, pH, and chlorophyll *a* within each watershed and their influence on $\delta^{13}\text{C}_{\text{SHELL}}$.

5. Conclusions

Shells of *L. cardium* from BR and BC preserve environmental records as isotopic variation; therefore, they can be used as archives of climate change in mid-latitude continental regions. High-resolution oxygen isotope data and sclerochronology indicated that *L. cardium* precipitates its shell in oxygen isotope equilibrium with ambient water conditions. These data demonstrate that change in temperature reaching a temperature threshold of $12\text{ }^\circ\text{C}$ acts as the trigger for winter growth cessation and the onset of spring growth. We were unable to resolve the processes controlling $\delta^{13}\text{C}_{\text{SHELL}}$. $\delta^{13}\text{C}_{\text{SHELL}}$ time series followed a more or less sinusoidal trend suggesting that seasonal factors influence the variation in $\delta^{13}\text{C}_{\text{SHELL}}$. The overall trend suggests that $\delta^{13}\text{C}_{\text{SHELL}}$ is at least partially influenced by seasonal processes. Seasonal signals persevered as variation in $\delta^{13}\text{C}_{\text{SHELL}}$ ratios are often dampened by kinetic and metabolic isotope effects. To address this issue, we evaluated kinetic isotope effects by examining the covariation between $\delta^{18}\text{O}_{\text{SHELL}}$ and $\delta^{13}\text{C}_{\text{SHELL}}$. The weak correlation demonstrates that at best, kinetic isotope effects can explain 43% of the observed variation in one shell. Moreover, the diminished amplitude and more negative $\delta^{13}\text{C}_{\text{SHELL}}$ values in mature portions of the shells may result from kinetic and/or metabolic isotope effects. Variation in $\delta^{13}\text{C}_{\text{SHELL}}$ potentially records local differences in landscape vegetation. Further characterization of the environment is required to test this hypothesis. Regardless, shells of *L. cardium* preserve environmental information and potentially serve as a new climate archive in mid- to high-latitudes continental regions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.palaeo.2007.06.002](https://doi.org/10.1016/j.palaeo.2007.06.002).

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