Stable isotope composition of the modern freshwater bivalve *Corbicula fluminea*

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Bivalve shells have been used in a number of paleoclimate and paleoenvironment studies. In order to investigate oxygen and carbon isotope fractionation between bivalve aragonite and host water, a one year monitoring experiment was conducted. Temperature, $\delta^{18}\text{O}_{\text{WATER}}$ and $\delta^{13}\text{C}_{\text{DIC}}$ were measured every week from January 2006 to January 2007. These data were used to calculate expected $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ equilibrium values to compare to measured shell values. At the end of the monitoring experiment, living bivalves (*Corbicula fluminea*) were collected and sampled for stable isotope analysis ($\delta^{18}\text{O}_{\text{ar}}$ and $\delta^{13}\text{C}_{\text{ar}}$). 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. Cooler temperatures seem to cause cessation of growth, suggesting that a threshold temperature may control the onset and cessation of growth. Shell $\delta^{13}\text{C}$ are more negative than predicted $\delta^{13}\text{C}$ values, and we observed that all the specimens showed a trend of lower $\delta^{13}\text{C}$ values with increasing age, this suggests that the incorporation of metabolic carbon is the cause of the negative offset in shell $\delta^{13}\text{C}$. The variable offset from equilibrium $\delta^{13}\text{C}$ values precludes the direct use of *Corbicula fluminea* shell in the study of the $\delta^{13}\text{C}_{\text{DIC}}$ in ancient bodies of water.

Keywords: oxygen and carbon isotope, *Corbicula fluminea*, bivalve, metabolic effects

INTRODUCTION

Stable isotope geochemistry has become a key tool in paleoclimate and paleoenvironment reconstruction. The oxygen isotopic signatures of biogenic carbonates, including bivalve shell, have been used to reconstruct both water temperature and salinity (e.g., Jones *et al.*, 1989; Ingram *et al.*, 1996; Watanabe and Oba, 1999; Kaandorp *et al.*, 2003; Dettman *et al.*, 2004). Assuming equilibrium conditions, variations in $\delta^{18}\text{O}$ of bivalve shells ($\delta^{18}\text{O}_{\text{ar}}$) reflect oxygen isotope ratios of ambient water ($\delta^{18}\text{O}_{\text{w}}$) and temperature during biomineralization (Epstein *et al.*, 1953; Dettman *et al.*, 1999). Although many species of bivalve precipitate their shells in oxygen isotope equilibrium with the ambient environment (Dettman *et al.*, 1999; Surge *et al.*, 2001; Elliot *et al.*, 2003; Chauvaud *et al.*, 2005), some mollusk display an offset attributed to metabolic processes (e.g., vital effects) (Wefer and Berger, 1991; Fenger *et al.*, 2007). Therefore, we must examine modern and living species to verify isotopic equilibrium before interpreting past climate and environmental conditions preserved in their fossil shells (Goewert *et al.*, 2007).

Similarly, if the $\delta^{13}\text{C}$ of shell can be related to the $\delta^{13}\text{C}$ of dissolved inorganic carbon (DIC), fossil shells could be used to investigate the $\delta^{13}\text{C}_{\text{DIC}}$ of ancient rivers and thereby to draw conclusions about paleo-productivity, paleo-atmospheric CO_2 composition and concentration, and paleo-climate (Dettman *et al.*, 1999; Lorrain *et al.*, 2004; Gillikin *et al.*, 2009; Wanamaker *et al.*, 2009). Shell $\delta^{13}\text{C}$ values have been attributed to a number of controls in various studies. Some have argued that $\delta^{13}\text{C}_{\text{ar}}$ is governed by the stable carbon isotopic composition of dissolved inorganic carbon ($\delta^{13}\text{C}_{\text{DIC}}$) and therefore records changes in environmental variables such as pH, temperature, and salinity (e.g., Keith *et al.*, 1964; Mook, 1971; Fritz and Poplawski, 1974; Gillikin *et al.*, 2006). On the other hand, shell carbonates were often found not to reflect the predicted equilibrium fractionation, being in general less enriched than predicted in ^{13}C (e.g., Klein *et al.*, 1996; McConnaughey *et al.*, 1997; McConnaughey, 2003; Kaandorp *et al.*, 2003 and many others). Most authors explain this offset by a contribution of metabolic effects (Tanaka *et al.*, 1986; Klein *et al.*, 1996; Veinott and Cornett, 1998; Dettman *et al.*, 1999; Gillikin *et al.*, 2007, 2009) and kinetic effects (McConnaughey *et al.*, 1997; McConnaughey, 2003; Lorrain *et al.*, 2005).

In the present study we monitored a population of *Corbicula fluminea* over a 13-month period in the HuaXi River of Guizhou Province, China. *Corbicula fluminea*

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is native to China, Japan and Korea, but now spread in many parts of the world (Asia, Europe, North and South America) and that could therefore provide almost “world-wide” paleoecological archives (Aucour *et al.*, 2003). The life span of *Corbicula* is about 1–5 years and adult individual shell height about 35 mm, normally *Corbicula* is dioecism, but some hermaphrodites, *Corbicula* are filter feeder and its primary food is phytoplankton. The spread of *Corbicula* is related to its rapid growth rate, early onset of maturity, high fecundity, and its ability to tolerate a wide range of environmental conditions (Morton, 1991; McMahon, 2000; Cataldo *et al.*, 2001).

To determine whether the growth increments of the aragonite shell of *Corbicula fluminea* were precipitated in isotope equilibrium with its host water, we analysed the isotopic composition of both aragonite ($\delta^{18}\text{O}_{\text{ar}}$ and $\delta^{13}\text{C}_{\text{ar}}$) and host water ($\delta^{18}\text{O}_{\text{w}}$ and $\delta^{13}\text{C}_{\text{DIC}}$). With this experiment we aim to test the applicability of *Corbicula fluminea* growth incremental stable isotope profiles for the reconstruction of past HuaXi aquatic environments.

EXPERIMENT, MATERIALS AND METHODS

Monitoring project

The studied HuaXi River, at 26°26′07.99″ N, 106°39′37.42″ E, about 10 km south of the city of Guiyang, in Guizhou Province, China (Fig. 1). The elevation of study site is about 1200 m, annual precipitation is 900 to 1400 mm (mean value is 1100 mm), annual air temperature is 5 to 30°C (mean value is 20°C), river depth varied seasonally, with an average winter depth about 1.0 m and the summer depth approximately ranging from 1.5 to 2.0 m. In HuaXi River confluence basin, karst landform widespread growth, the primary type of carbonate is Late Paleozoic and Early Mesozoic limestone.

We monitored a population of living molluscan bivalves (*Corbicula fluminea* müller, 1774) during a complete season cycle (from 10 January 2006 to 9 January 2007). For the duration of the experiment, water temperatures and pH were measured by Radiometer pIONneer 65 Portable Multi-parameter instrument every week, filtered water samples (0.45- μm mesh) were taken for isotope analysis ($\delta^{18}\text{O}_{\text{w}}$ and $\delta^{13}\text{C}_{\text{DIC}}$).

Shell preparation and isotope analysis method

Like other bivalves, *Corbicula fluminea* shell is made up of multiple shell layers with differing microstructures: the periostracum, crossed lamellar and complex crossed lamellar layers (Fig. 2). Complex crossed lamellar layers only appear in shell behind and possibly secretes in the shell accumulation process, therefore not suitable to achievement time series research object. While the crossed lamellar layers cover the entire shell, so we select the

Fig. 1. Location map of the study site.

crossed lamellar layers as the analysis object. The growth of bivalve shell will slow down or cease during winters which water temperature below certain level, and winter lines can be finding on the shell, the part of shell between two winter lines is the annual growth increment (Jones *et al.*, 1989; Goodwin *et al.*, 2001; Schöne *et al.*, 2002). *Corbicula fluminea*'s winter lines and annual growth increment was showed on Fig. 2.

On 9 Jan. 2007, two live bivalves (HX01 and HX02) were collected and immediately sacrificed and their flesh removed. Shells were cleaned, the periostracum was removed with a 50°C solution of hydrogen peroxide, followed by a demineralised water rinse and air-drying. To acquire high-resolution stable isotope profiles of growth increments of *Corbicula fluminea*, we used a scalpel blade (Geist *et al.*, 2005); samples were about 300 to 500 μm in width, 100 μm in depth and 5 to 7 mm in length.

Shell samples were not equidistant. Because we only have one year (2006) water monitor information, so we take more samples near the ventral, about 0.5 mm per sample in 7 mm increment from ventral for HX01 and 5 mm for HX02, 1 mm per sample in the rest increment that was sampled both for HX01 and HX02. Each powdered CaCO_3 samples weighing approximately 0.2 mg. Sampling began at the ventral of the shell (portion of latest growth), and along the axis of maximum growth along, perpendicular to growth increment and progressively removed a series of samples. The sampling trajectory were parallel to the growth banding, the aragonite powders recovered from each line were used to obtain stable isotope data ($\delta^{18}\text{O}_{\text{ar}}$ and $\delta^{13}\text{C}_{\text{ar}}$).

The stable isotope analysis of shell aragonite and water samples was performed at the State Key Laboratory of Environmental Geochemistry, The Institute of Geochemistry of Chinese Academy of Sciences. $\delta^{18}\text{O}_{\text{ar}}$ and $\delta^{13}\text{C}_{\text{ar}}$ were performed on Continuous Flow Isotope

Fig. 2. Schematic of the cross section of *Corbicula fluminea* shell. The bold line between the two winter lines represents the annual increment.

Ratio Mass Spectrometer. Samples were reacted with 100% orthophosphoric acid at 70°C. Results are reported relative to V-PDB by calibration to the reference standard GBW04405 ($\delta^{13}\text{C} = 0.57\text{‰}$, $\delta^{18}\text{O} = -8.49\text{‰}$), GBW04406 ($\delta^{13}\text{C} = -10.85\text{‰}$, $\delta^{18}\text{O} = -12.40\text{‰}$) and GBW04416 ($\delta^{13}\text{C} = 1.613\text{‰}$, $\delta^{18}\text{O} = -11.59\text{‰}$). $\delta^{18}\text{O}_w$ was measured on Continuous Flow Isotope Ratio Mass Spectrometer, Standardization is based on international reference materials V-SMOW. The $\delta^{13}\text{C}_{\text{DIC}}$ was measured on the Finnigan MAT-252 Mass Spectrometer after the DIC extraction procedure. Water samples are introduced into the vacutainer tubes, which was pre-loaded with 100% phosphoric acid and magnetic stir bars, using a syringe and the evolved CO_2 was extracted (Atekwana and Krishnamurthy, 1998). At 1σ precision of $\sim 0.1\text{‰}$ for $\delta^{18}\text{O}_{\text{ar}}$, $\delta^{13}\text{C}_{\text{ar}}$ and $\delta^{13}\text{C}_{\text{DIC}}$, and $\sim 0.2\text{‰}$ for $\delta^{18}\text{O}_w$.

RESULTS

Water temperature and chemistry

During the monitoring period, water temperature in the HuaXi River ranged from 6°C to 25°C, with an average summer (June to August) temperature of $21 \pm 2.42^\circ\text{C}$ and an average winter temperature (December to February) of $9 \pm 1.87^\circ\text{C}$, and pH from 7.73 to 8.44 with a mean value of 8.09. The oxygen isotope composition of water ($\delta^{18}\text{O}_w$) varied from -5.78‰ to -7.78‰ (V-SMOW). Although it does not show an obvious seasonal variation, the most negative $\delta^{18}\text{O}$ values occur from late summer and throughout the fall of 2006 (Fig. 3). $\delta^{13}\text{C}_{\text{DIC}}$ ranged from -3.60‰ to -9.58‰ (V-PDB), which is more positive when compared with other rivers (Mook and de Vries, 1998), reflecting the dominate controls of carbonate rocks in this region (Fig. 3).

Fig. 3. Isotope composition of HuaXi River water samples during the period of 10 January 2006 to 9 January 2007. $\delta^{18}\text{O}$ values are in ‰ vs. V-SMOW and $\delta^{13}\text{C}$ values are in ‰ vs. V-PDB.

Isotope composition of aragonite shells

Temporal variation in $\delta^{18}\text{O}_{\text{ar}}$ and $\delta^{13}\text{C}_{\text{ar}}$ follows a sinusoidal pattern with truncated peaks (Fig. 4). As shells mature, the amplitude of shell values decrease, and shell values show less seasonal variation. A significant correlation was observed between $\delta^{18}\text{O}_{\text{ar}}$ and $\delta^{13}\text{C}_{\text{ar}}$ of the two

Fig. 4. $\delta^{18}\text{O}_{\text{ar}}$ and $\delta^{13}\text{C}_{\text{ar}}$ of two specimens of *Corbicula fluminea*. R^2 is coefficient of $\delta^{18}\text{O}_{\text{ar}}$ and $\delta^{13}\text{C}_{\text{ar}}$ of the two individual shells.

individual shells (HX01, HX02), R^2 values were 0.53 and 0.32 respectively (Fig. 4). Measured $\delta^{18}\text{O}_{\text{ar}}$ of specimens HX01 ranged from -10.87‰ to -5.24‰ with a mean value of $-7.18 \pm 1.40\text{‰}$ ($n = 17$), and HX02 ranged from -9.11‰ to -5.28‰ with an average value of $-7.06 \pm 1.08\text{‰}$ ($n = 18$). Measured $\delta^{13}\text{C}_{\text{ar}}$ of specimens HX01 ranged from -11.12 to -7.45‰ with an average value of $-9.19 \pm 1.12\text{‰}$ ($n = 17$) and HX02 from -11.36 to -8.42‰ with a mean value of $-9.73 \pm 0.99\text{‰}$ ($n = 18$). There was no obvious difference between $\delta^{18}\text{O}_{\text{ar}}$ of HX01 and HX02, which were living in the same environment.

Calculated isotopic composition

To investigate whether *Corbicula fluminea* precipitates its aragonitic shell in equilibrium with ambient water, we compared $\delta^{18}\text{O}_{\text{ar}}$ and $\delta^{13}\text{C}_{\text{ar}}$ with calculated host water isotopic equilibrium values. Oxygen isotope fractionation is temperature dependent. Several temperature equations exist (e.g., Epstein *et al.*, 1953; Emiliani, 1955 and many others) with which the oxygen isotopic composition of shells can be predicted, when the $\delta^{18}\text{O}$ of host water and ambient temperature are known. We applied the temperature equations of Grossman and Ku (1986):

Fig. 5. Calculated $\delta^{18}\text{O}$ values that was applied the relationship of Grossman and Ku (1986), $\delta^{18}\text{O}_{\text{w}}$ and water temperature of HuaXi River.

$$T (^{\circ}\text{C}) = 20.6 - 4.34(\delta^{18}\text{O}_{\text{ar}} - \delta^{18}\text{O}_{\text{w}})$$

where $\delta^{18}\text{O}_{\text{ar}}$ is the $\delta^{18}\text{O}$ (PDB) of aragonitic shell and $\delta^{18}\text{O}_{\text{w}}$ (SMOW) of the water minus 0.2‰ .

Calculated $\delta^{18}\text{O}$ values followed a seasonal trend simi-

Fig. 6. The influence of precipitation on oxygen isotopic composition of water. The date of precipitation from the website: <http://www.wunderground.com>

lar to a sinusoidal curve, representing the full range of possible values given the local environmental conditions throughout the water monitoring period (Fig. 5). Lower values correspond to warmer months and higher values occur in colder months. Calculated $\delta^{18}\text{O}$ values from HuaXi ranged from -8.20‰ to -2.88‰ with an average value of $-5.39 \pm 1.51\text{‰}$ ($n = 52$).

DISCUSSION

This study was performed in order to evaluate how accurate seasonal/climatological signals are recorded in the isotopic geochemistry of *Corbicula fluminea* shells. First, we consider the changes in oxygen and carbon isotopes of HuaXi water. Secondly, we compared $\delta^{18}\text{O}_{\text{ar}}$ with calculated isotopic equilibrium values to investigate whether *Corbicula fluminea* precipitates its aragonitic shell in oxygen isotope equilibrium with ambient water. Finally, we discuss the variation in $\delta^{13}\text{C}_{\text{ar}}$ of *Corbicula fluminea*.

Isotope composition of water

The $\delta^{18}\text{O}$ profile of water is not demonstrated the obvious seasonal variation, but the negative excursion also exhibit during 2006 summer and fall (Fig. 3). The departures likely reflect the influence of rainfall with a differ-

ent $\delta^{18}\text{O}$ (Fig. 6). For example, on 8 Sep. 2006 (No. 251) a heavy rain dropped between 40 and 50 mm of rain in the HuaXi River drainage; $\delta^{18}\text{O}$ of the Sep. 12 water sample of HuaXi River was 0.62‰ more negative than water collected 2 weeks earlier. Steady and heavy rains fell in Oct. 2006, depositing between 120 and 130 mm of rain in the HuaXi River drainage. This rainstorm also resulted in a negative excursion of $\delta^{18}\text{O}_{\text{w}}$ which measured -7.42‰ on 24 Oct. 2006 and was 1.31‰ more negative than -6.11‰ on 19 Sep. 2006. Figure 6 show that during the monitoring period, there have three concentrative rainfalls, monitoring day No. 40–79, 181–235 and 274–352, a large decreased of $\delta^{18}\text{O}_{\text{w}}$ was appeared after every concentrative rainfalls. Although the impact of rain is unexpected because the $\delta^{18}\text{O}$ of rainfall varies widely within and between storms (Gat, 1987; Dettman *et al.*, 1999), in general the negative effect was showed in the HuaXi River drainage.

The $\delta^{13}\text{C}_{\text{DIC}}$ in Rivers is controlled by the balance of at least 3 sources and 2 sinks for carbon (McKenzie, 1985; Salomons and Mook, 1986). Sources of carbon are atmospheric CO_2 , the breakdown of organic compounds, and the dissolution of sedimentary carbonates. DIC is removed from the river by photosynthesizing algae and diatoms and by the precipitation of carbonates.

Dissolved atmospheric CO_2 ($\delta^{13}\text{C} = -7.4\text{‰}$ PDB) con-

Fig. 7. Calculated and measured $\delta^{18}\text{O}_{\text{ar}}$ for *Corbicula fluminea* from HuaXi River. The measured shell $\delta^{18}\text{O}$ profile from each specimen was matched to the predicted $\delta^{18}\text{O}_{\text{equil}}$ by comparing both absolute values and the shape of the profiles (Freitas *et al.*, 2006) and ignoring the most negative excursion value of HX01, which was probably contaminated. The dashed line represents the 2005 and 2004 shell $\delta^{18}\text{O}$ calculated values which was estimated by the 2006 calculated $\delta^{18}\text{O}_{\text{equil}}$.

tributes DIC with a $\delta^{13}\text{C}$ ranging from approximately 2.5‰ at 10°C to 0.2‰ at 28°C (using fractionations in Romanek *et al.*, 1992). In HuaXi, where the C-3 photosynthetic pathway is dominant, the breakdown of organic carbon contributes DIC with very negative $\delta^{13}\text{C}$ values, -14‰ to -26‰ PDB (Salomons and Mook, 1986). This plant material may be broken down in the river itself, or may oxidize in the soils of the drainage and periodically be added to the river during storms (Kendall, 1991). The primary type of carbonate in the Huaxi River drainage is Late Paleozoic and Early Mesozoic limestone. Dissolution of Paleozoic carbonate contributes DIC ranging from -1‰ to 4‰ (Lohmann and Walker, 1989). Finally, organic productivity and photosynthesis preferentially removes ^{12}C from the water column in the construction of organic tissues that average -28‰. This increases the $\delta^{13}\text{C}$ of remaining DIC in the river, although the amplitude of this increase is not quantifiable here (McKenzie, 1985).

Because the $\delta^{13}\text{C}_{\text{DIC}}$ of HuaXi water is more negative than dissolved atmospheric CO_2 and dissolved carbonate (Fig. 3), the decay of organic material in the river may be contribute a significant portion of the DIC. In the early spring of 2006, $\delta^{13}\text{C}_{\text{DIC}}$ has a positive excursion and may reflect the construction of organic tissues by algae and diatoms (Dettman *et al.*, 1999).

Comparison of measured and calculated $\delta^{18}\text{O}$

The development of environmental proxy data provides a means to reconstruct paleoclimate and paleoenvironmental conditions (Schöne *et al.*, 2004; Goewert *et al.*, 2007). We examined whether *Corbicula fluminea* precipitated its shell in isotopic equilibrium with the ambient environment by comparing measured $\delta^{18}\text{O}_{\text{ar}}$ to calculated values ($\delta^{18}\text{O}_{\text{equil}}$) (Fig. 6).

Fig. 8. Calculated and measured $\delta^{13}\text{C}_{\text{ar}}$ for *Corbicula fluminea* from HuaXi River. The measured shell $\delta^{13}\text{C}$ profile from each specimen was matched to the predicted $\delta^{13}\text{C}_{\text{equil}}$ by calendar dates which were assigned by oxygen isotope.

When daily growth increments are present, they can easily be used to date the incremental samples (Schöne *et al.*, 2002; Lorrain *et al.*, 2004). However, when daily increments are not easily discernible, the usual method used to assign a time scale to the shell $\delta^{18}\text{O}$ record is to compare measured and predicted $\delta^{18}\text{O}$ (Klein *et al.*, 1996; Auclair *et al.*, 2003; Elliot *et al.*, 2003; Gillikin *et al.*, 2005).

We align measured $\delta^{18}\text{O}_{\text{ar}}$ to calculated values ($\delta^{18}\text{O}_{\text{equil}}$):

(1) Dettman and Lohmann (1993) identified two growth patterns by examining variation in oxygen isotope ratios of shell: (a) continuous; and (b) seasonal. Continuous growth throughout the year was exemplified by a sinusoidal curve, while, seasonal growth corresponded to a sinusoidal curve with truncated peaks, representing winter months or growth cessation.

(2) There was no other time marker than the date of collection available for our specimens, the date of collection was 9 Jan. 2007, and therefore, ventral margin to umbo, the first negative excursion in the measured $\delta^{18}\text{O}$ profile must have precipitated during summer of 2006.

(3) The measured shell $\delta^{18}\text{O}$ profiles from each specimen was matched to the calculated $\delta^{18}\text{O}_{\text{equil}}$ by comparing both absolute values and the shape of the profiles (Klein *et al.*, 1996; Freitas *et al.*, 2006).

Firstly, comparison of measured and calculated shell $\delta^{18}\text{O}$ values revealed that the most positive calculated values are not recorded in shell $\delta^{18}\text{O}$ profiles; Variation of the measured $\delta^{18}\text{O}$ values followed a truncated sinusoidal pattern suggesting seasonal growth (Fig. 7). This observation suggests that *Corbicula fluminea* halted their growth during winter months, agreeing with published seasonal growth studies on freshwater bivalves (Dettman *et al.*, 1999; Anthony *et al.*, 2001; Goewert *et al.*, 2007). Secondly, we fit the first trough of measured $\delta^{18}\text{O}_{\text{ar}}$ profiles to the trough of calculated $\delta^{18}\text{O}$ profiles, and then

compared both absolute values and the shape of the profiles (Fig. 7). The values of $(\delta^{18}\text{O}_{\text{ar}} - \delta^{18}\text{O}_{\text{equil}})$ of HX01 and HX02 are ranged from 0 to 0.2‰, so we conclude that *Corbicula fluminea* precipitates its shell in oxygen isotope equilibrium with the ambient water and that winter values are not represented due to growth cessation. Therefore, shells of *Corbicula fluminea* provide reliable archives of paleoclimate and paleoenvironmental conditions.

Comparison of the measured $\delta^{18}\text{O}_{\text{ar}}$ against the calculated values indicates that shell growth occurred from late May to early November. To evaluate potential cessation triggers, we compared the water temperature records of shell growth cessation and onset points. Temperatures in late May (May 23, 2006) were approximately 18°C, very similar to the 17°C in early November (November 7, 2006). Therefore, growth cessation and the onset of shell growth are most likely controlled by a water temperature threshold. For *Corbicula fluminea* in this watershed, the temperature threshold is 17°C, which is higher than the temperature threshold of 12°C for unionidae (Dettman *et al.*, 1999; Goewert *et al.*, 2007).

Shell $\delta^{13}\text{C}$

Calculated $\delta^{13}\text{C}$ values are much more positive than shell $\delta^{13}\text{C}$ values throughout the year (Fig. 8). The inorganic aragonite– HCO_3^- carbon fractionation of Romanek *et al.* (1992) was used to predict aragonite $\delta^{13}\text{C}$. This fractionation is 1.0027 (± 0.0006) and is independent of temperature. Average pH of the HuaXi River is 8.0, therefore at least 98% of the DIC in the river is present as HCO_3^- . The change in the predicted $\delta^{13}\text{C}$ associated with these concentrations of H_2CO_3 is less than 0.2‰ (Salomons and Mook, 1986; Dettman *et al.*, 1999).

Some studies have argued that $\delta^{13}\text{C}_{\text{ar}}$ is controlled by the $\delta^{13}\text{C}_{\text{DIC}}$ in water, (e.g., Mook, 1971; Fritz and Poplawski, 1974), many others have suggested that both kinetic and metabolic effects play an important role in determining $\delta^{13}\text{C}_{\text{ar}}$ (Keith *et al.*, 1964; Tanaka *et al.*, 1986; Klein *et al.*, 1996; McConnaughey *et al.*, 1997; Dettman *et al.*, 1999; Lorrain *et al.*, 2004; Gillikin *et al.*, 2006, 2007, 2009). 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). Many studies evaluate whether kinetic isotope effects play a role in $\delta^{13}\text{C}_{\text{ar}}$, by checking for a correlation between $\delta^{13}\text{C}_{\text{ar}}$ versus $\delta^{18}\text{O}_{\text{ar}}$ on the assumption that this correlation is driven by kinetic processes. Though a significant correlation occurred in HX01 and HX02, R^2 values were 0.53 and 0.32 respectively. We would argue that because *Corbicula fluminea* precipitates its shell in oxygen isotope equilibrium with the ambient water, kinetic isotope effects on $\delta^{13}\text{C}$ values are probably minimal. We next considered whether metabolic

isotope effects contributed to the observed $\delta^{13}\text{C}_{\text{ar}}$ pattern.

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 (~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) and more recently up to 37% (Gillikin *et al.*, 2007). Studies show that contribution of metabolic carbon can dampen the $\delta^{13}\text{C}_{\text{DIC}}$ signal recorded in shells and explain $\delta^{13}\text{C}_{\text{ar}}$ disequilibrium in bivalves (Krantz *et al.*, 1987; Klein *et al.*, 1996; Keller *et al.*, 2002; Lorrain *et al.*, 2004; Gillikin *et al.*, 2007). 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 8), therefore the incorporation of metabolic carbon is the most likely cause of the negative offset in shell $\delta^{13}\text{C}$ of *Corbicula fluminea*.

CONCLUSIONS

We conclude that *Corbicula fluminea* precipitates its shell in oxygen isotope equilibrium with the ambient water and that winter values are not represented due to growth cessation. Therefore, shells of *Corbicula fluminea* provide reliable archives of paleoclimate and paleoenvironmental conditions.

Shell $\delta^{13}\text{C}$ values are more negative than predicted $\delta^{13}\text{C}$ values, and we observed that all shells followed a trend to lower $\delta^{13}\text{C}$ values with increasing age, therefore the incorporation of metabolic carbon is the most likely cause of the negative offset in shell $\delta^{13}\text{C}$. The variable offset from equilibrium $\delta^{13}\text{C}$ values precludes the direct use of *Corbicula fluminea* shell in the study of the $\delta^{13}\text{C}_{\text{DIC}}$ in ancient bodies of water.

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