Ontogenic increase of metabolic carbon in freshwater mussel shells (Pyganodon cataracta)

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[1] Incorporation of isotopically light metabolic carbon (C_M) can obscure carbon isotope records of dissolved inorganic carbon from biogenic carbonates. Ontogenic increases of C_M are common in marine bivalved mollusks, likely reflecting increasing absolute metabolism with increasing body size. Here we use shell aragonite from four specimens of Pyganodon cataracta to test if the same is true in freshwater bivalves. Annual δ13Oaragonite values were not different between individuals suggesting that the aragonite samples represent the same time interval. δ13Caragonite, however, showed an ontogenic decrease, with a strong linear relationship between shell height (H in mm) and %C_M (R² = 0.96; %C_M = 0.23*H + 13.33), indicating that more C_M is incorporated into larger shells. We estimate that between 15 and 35% of shell carbon is derived from metabolic CO2. Nevertheless, this vital effect does not exclude the use of δ13C_aragonite records from freshwater shells as environmental proxies.


1. Introduction

[2] The carbon isotopic signature of dissolved inorganic carbon (DIC) (δ13CDIC) is a powerful tool for understanding biogeochemical cycling in aquatic systems. For example, δ13CDIC gives an indication of changes in stream respiration, primary production, stream nutrient loading, land use change (e.g., urbanization, deforestation), pH, bedrock geology, and atmospheric CO2 levels [see Mook, 2000]. However, continuous records of δ13CDIC are usually not available and are costly to produce. An alternative to a time series of δ13CDIC measurements would be to find a proxy of δ13CDIC, such as sequentially deposited carbonates precipitated in equilibrium with the water. Fortunately, many freshwater streams have abundant populations of freshwater mussels (e.g., Unionidae) which produce carbonate shells that could potentially be used as a proxy of δ13CDIC.

[3] There have been several studies on the isotope geochemistry of shells of marine and estuarine mollusks, but comparatively fewer on freshwater mussels. Freshwater mussels are not typically well preserved due to the acidic nature of freshwater streams [Carroll and Romanek, 2006]; however, they are usually well cataloged in museums and some species can live to be well over 100 years [e.g., Mutvei and Westermark, 2001]. Indeed, studies have obtained multicentury climate records from freshwater mussel shells [e.g., Schöne et al., 2004].

[4] There are two carbon sources for shell construction: environmental dissolved inorganic carbon (DIC) and respired carbon, the latter which is ultimately derived from carbon in the diet [see McConnaughey et al., 1997]. These end-members can potentially have very different δ13C values, with freshwater δ13CDIC typically around −10 to −15% [e.g., Mook and Tan, 1991; Hellings et al., 1999], and respired carbon typically around −28% in streams draining areas with C3 vegetation dominating [see Mook and Tan, 1991; Dettman et al., 1999]. However, these values are both highly variable. Ideally, in order to reconstruct the ambient carbon cycle, shells should record stream δ13CDIC with minimal respired (metabolic) carbon being incorporated (i.e., no “vital effects”).

[5] Several authors have found that metabolic carbon (C_M) can significantly contribute to marine and freshwater bivalve shell carbonate (both [Keith et al., 1964]; marine [Tanaka et al., 1986; Klein et al., 1996; McConnaughey et al., 1997; Lorrain et al., 2004; Gillikin et al., 2006, 2007]; freshwater [Veinot and Cornett, 1998; Dettman et al., 1999; Aucour et al., 2003; Kaandorp et al., 2003]). While in most marine bivalves the amount of C_M in the shell is typically less than 10% [McConnaughey et al., 1997], more recently up to 37% C_M has been reported [Gillikin et al., 2007]. Gillikin et al. [2007] also observed a strong ontogenic increase of C_M, with a 4% decrease in δ13C in one shell.

Lorrain et al. [2004] proposed that the ratio of the total amount of respired carbon produced by the bivalve to the total carbon demand for shell construction dictated the amount of C_M in the shell. Freshwater bivalve physiology is rather different than marine bivalve physiology considering that freshwater bivalves live in dilute media and must
actively pump ions and regulate their acid-base balance [see Raikow and Hamilton, 2001]. Therefore, the ontogenic effects of metabolic carbon incorporation seen in marine bivalves are not necessarily expected in freshwater bivalve shells.

[6] There are relatively few stable carbon isotope studies on freshwater bivalves. However, a few authors have suggested 5–80% C_M in freshwater bivalve shells [Veinott and Cornett, 1998; Aucour et al., 2003; Gajurel et al., 2006]. The higher values were reported by Veinott and Cornett [1998], but they did not analyze respired δ13C (soft tissues) in their specimens and calculated C_M using the equation of Tanaka et al. [1986], which tends to overestimate C_M (see discussion). An ontogenetic increase of C_M incorporation has also been suggested [Aucour et al., 2003; Goewert et al., 2007], but has not been adequately tested. While shell δ13C time series analyses can provide a good indication of ontogenic effects, they may also reflect real environmental changes through time which can then be mistaken as ontogenic. For example, pollutants or increased chloride concentrations may possibly affect how mollusks fractionate carbon isotopes [cf. Gillikin et al., 2006].

[7] Here we present the first study to control for all environmental variations by collecting freshwater mussels of different ontogenic ages that grew at the same time and under the same environmental conditions; thereby allowing ontogenic effects to be easily separated from environmental effects. The aim of this study was to determine if the freshwater bivalve Pyganodon cataracta incorporates more metabolic carbon through ontogeny as marine bivalves do and to determine if metabolic carbon potentially obscures environmental δ13C values.

2. Methods

[8] On 7 December 2006, four Pyganodon cataracta at various growth stages (shell sizes 88.30, 86.15, 43.35 and 20.75 mm) were collected from the Casperkill, on the campus of Vassar College in Poughkeepsie, NY, USA (41°44′N, 73°35′W). P. cataracta is one of the most common and widespread unionoids in central and eastern New York State [Strayer and Jirka, 1997]. The Casperkill has a catchment of 31 km² and drains into the Hudson River. Discharge in the Casperkill varies between 0.1 and 4 m³ s⁻¹, with an average of 0.4 m³ s⁻¹ [Minder, 2004]. All shells were collected within the same 0.25 m² area of streambed. From each shell, foot, muscle and mantle tissues were collected and the remaining tissues were discarded. Shells were then air-dried. Each soft tissue sample was prepared for isotopic analysis by drying at 60°C and grinding with an agate mortar and pestle. The periostracum was removed from the ventral margin of each P. cataracta shell using sandpaper and carbonate samples were removed from the shell surface using a dental drill and a rasper pattern. Four samples were taken between the last growth line and the end of the shell so that the entire carbonate deposited between these two points was sampled (i.e., the entire last growth increment was sampled). Growth lines were assumed to be annual (but see Kesler and Van Tol [2000]). Shells were then embedded in epoxy and thinned-sectioned to visualize internal growth lines. Water samples were collected from the same location on 1 December 2006, 26 December 2006, 26 March 2007, and 27 September 2007 for oxygen isotopes (δ18O_w) and δ13C_DIC following the protocol outlined by Gillikin and Bouillon [2007]. Water temperature was measured monthly throughout the year prior to shell collection using a YSI handheld meter. Samples were collected near the bottom of the water column (at base flow water depth is ~40 cm).

[9] δ13C of soft tissues were measured on a continuous-flow isotope-ratio mass spectrometer (IRMS; Finnigan Delta Plus XP). Samples were wrapped in tin cups and were then combusted using an elemental analyzer (Costech) coupled to the IRMS. Standardization was based on NBS-22 and USGS-24 for δ13C and precision was better than ±0.06‰ (1σ), based on repeated internal standards (reference values for standards taken from Gonfiantini et al. [1995]). All carbonate isotopic analyses were performed on a Finnigan MAT 252 IRMS equipped with a Kiel III automated sampling device. Samples were reacted with >100% orthophosphoric acid at 70°C. Results are reported relative to VPDB by calibration to the NBS-19 reference standard (δ13C = +1.95‰ and δ18O = −2.20‰ VPDB). δ18O of water was measured on a Finnigan Delta S IRMS, samples were equilibrated with CO₂ gas at approximately 15°C in an automated equilibration device coupled to the IRMS. Standardization is based on international reference materials VSMOW and SLAP. Precision is 0.08‰ or better on the basis of repeated internal standards. The δ13C of DIC was measured on the continuous-flow IRMS coupled with a Gasbench automated sampler (also manufactured by Finnigan). Samples were reacted for >1 h with phosphoric acid at room temperature in Exetainer (Labco) vials previously flushed with He gas. Standardization was based on NBS-19 and NBS-18 and precision was ±0.3‰ or better (1σ). All isotopic analyses were carried out by the Environmental Isotope Laboratory, Department of Geosciences, University of Arizona.

3. Results

[10] Soft tissue δ13C values are presented in Table 1. All tissues were within 2.3‰ of each other. There was no significant difference between tissue types overall (one-way ANOVA, p = 0.34), but within individuals, mantle tissues were generally more negative than other tissues, followed by foot tissues, then muscle tissues. There was a significant strong positive correlation between shell height (in mm) and muscle tissues (R² = 0.98; p < 0.01; slope = 0.007), but not for foot versus height (p = 0.57) nor mantle versus height (p = 0.28).

[11] Aragonite oxygen isotope signatures (δ18O_ar) were similar between individuals with averages having an overall range of 1.1‰; with a similar variability within individual shells (Figure 1). There was no correlation between average δ18O_ar and shell height (p = 0.13). Aragonite carbon isotopes (δ13C_ar) were different between individuals with a strong significant negative correlation between δ13C_ar and shell height (R² = 0.95, p < 0.05; Figure 1). Shells were about 4 to 7‰ more negative than expected based on the equilibrium fractionation of Romanek et al. [1992] (Figure 1).

[12] Internal growth lines did not always correspond with external growth lines on all shells. Some prominent external growth lines toward the growing tip of the shell were
represented internally (Figure 2a). However, we feel that the external growth lines (Figure 2b) are approximately annual and that internal growth lines in younger shell sections are not highly visible.

The four water samples collected in fall, winter and spring had an average $\delta^{18}O_{ar}$ value of $-8.2 \pm 1.2\%o$ and an average $\delta^{13}C_{DIC}$ of $-9.1 \pm 0.6\%o$. Water temperature ranged from 1.7°C (January) to 26.8°C (July) and was above 12°C from April to September. Continuously recorded pH (every 20 min between 7 June and 8 April after shell collection) by a YSI model 6920-S sonde ranged between 7.2 and 8.5 approximately 1 km downstream of the collection site (K. Menking, unpublished data, 2008), but averaged about 7.7 for most of the recorded interval except in the spring where it reached values as high as 8.5.

4. Discussion

Our data clearly illustrate a trend of more negative $\delta^{13}C_{ar}$ through ontogeny in *Pyganodon cataracta* shells (Figure 1). Considering that these shells were all collected at the same time and that the carbonate analyzed should represent the same time period (~1 growth season), the ontogenic trends observed are not due to a change in environmental conditions. As $\delta^{13}C_{DIC}$ changes over the year and shell $\delta^{13}C_{ar}$ is also expected to change throughout the year [e.g., Dettman et al., 1999; Wurster and Patterson, 2001; Goewert et al., 2007] it is imperative that the average shell signal is representative of the whole year [see Krantz et al., 1989]. The $\delta^{18}O_{ar}$ of shell carbonate is dependent on both the $\delta^{18}O$ of water as well as water temperature. We expect $\delta^{18}O_{ar}$ to change sinusoidally over the year, reflecting the variations in seasonal temperature and $\delta^{18}O_{precipitation}$ in New York State (similar to Wurster and Patterson [2001]). Therefore, if some shells represent only part of the growth season, we would expect $\delta^{18}O_{ar}$ values to differ between shells, which they do not. Moreover, shell $\delta^{18}O_{ar}$ is close to what is expected using the paleo-temperature equation of Böhm et al. [2000] when averaging stream temperatures above 12°C (unionids have been shown to stop precipitating shell below 12°C [Dettman et al., 1999]), suggesting both that *P. cataracta* precipitates its shell in oxygen isotope equilibrium with its environment similar to most mollusks and that we have captured the same time intervals in all shells.

There are several hypotheses that can be put forth to explain why this ontogenic effect is present. Kinetic fractionation effects can probably be ruled out as these have never been recorded in bivalves (including freshwater bivalves), most likely due to the role of carbonic anhydrase in calcification physiology [see McConnaughey and Gillikin, 2008]. The remaining hypotheses involve either environmental or biological effects.

Considering these mussels were collected within the same 0.25 m² area, microsite conditions are very unlikely and can be ruled out.
effect would be differences in burial depth with age. Because of high biological activity in sediments, pore water δ13C of DIC can be expected to be more negative than overlying waters. If this 13C depleted pore water seeped into the extrapallial fluid (EPF; the site of calcification in bivalves) it could potentially influence shell δ13C [see Krantz et al., 1987; McConnaughey and Gillikin, 2008]. However, freshwater mussels in the Casperkill generally sit with much of the ventral margin of their shell above the sediment (personal observation) and smaller unionids have been shown to actually burrow deeper in the sediments than larger unionids [Schwabl and Pusch, 2007]. Therefore, if pore water was affecting these shells, we would expect an ontogenic increase of δ13Cw, which is not the case, so we consider burial depth to be an unlikely factor.

Since environmental factors are unlikely to cause the observed ontogenic effect, the most plausible hypotheses are biological effects. The similarity in δ13C tissue values between individuals and organs (Table 1), suggests that the diet of these mussels is similar across different age classes A decrease in δ13Cw would also result if the percent Cm being sequestered to the shell remained constant, but diet switched to a source with more negative δ13C values, however this is clearly not the case here. We actually see the opposite effect with muscle tissues becoming slightly more positive through ontogeny (see Table 1). Therefore, the hypothesis put forth by Lorrain et al. [2004] is the most parsimonious. They suggest that the amount of metabolic carbon incorporated into bivalve shells is dependent on the carbon demand during calcification. When the bivalve is young the total amount of respiring tissues between the valves is small and therefore less metabolic CO2 is produced in total (absolute metabolism is low). In addition, shell growth is fast (shell carbon demand is high). Therefore, most of the shell carbon comes from the DIC and only little metabolic carbon is incorporated in the shell. When the bivalves age, shell growth slows (shell carbon demand is low), the amount of tissues increases, and thus total respired CO2 increases (absolute metabolism is high), thereby increasing the amount of metabolic carbon incorporated in the shell. Shell carbon demand and absolute metabolism both increase through ontogeny, of course, but shell carbon demand does not increase as fast as absolute metabolism [Lorrain et al., 2004]. We contend that this is the phenomenon responsible for the ontogenic decrease in δ13Cw seen in these shells.

Although we have only limited environmental data, a rough estimate of the percentage of metabolic carbon in these shells can be calculated (a 1% change in δ13C of DIC changes Cm by ~5%). For this, a simple mixing model is used where respired carbon and environmental DIC are the two end-members. Even though the δ13C of DIC data were not collected while the mussels were growing, the data should still characterize the stream (large interannual changes are not expected). Soft tissue δ13C can be used as an approximation of respired carbon δ13C [McConnaughey et al., 1997]. Muscle tissues in bivalves typically have the slowest turnover time [Raikow and Hamilton, 2001; Lorrain et al., 2002], so we used muscle tissue δ13C as a proxy for respired carbon for the shell’s growth season. In freshwater systems, δ13C of DIC can vary dramatically throughout the year, with intraannual changes up to 5% being common in temperate regions [Mook and Tan, 1991; Dettman et al., 1999].

Therefore, our average of four samples certainly does not capture the full variability in this stream. Using the average of these four δ13C of DIC samples taken throughout the year does however allow us to calculate a rough estimate of Cm. We use the mixing equation rewritten from McConnaughey et al. [1997]:

\[
C_m = (\delta^{13}C_{ar} - \delta^{13}C_{DIC}) / (\delta^{13}C_{DIC} - \delta^{13}C_{m})
\]

where \(\delta^{13}C_{ar}\) is the fractionation factor between aragonite and HCO3- (+2.7‰ according to Romanek et al. [1992]) and \(\delta^{13}C_{m}\) is the δ13C of metabolic carbon. Tanaka et al. [1986] also proposed a model to calculate Cm which included the fractionation factor of aqueous CO2 and CaCO3 and typically results in a much higher Cm estimation. In addition to other problems with their model [see McConnaughey and Gillikin, 2008], at the pH of the Casperkill (~7.5 to 8) nearly all of the DIC is present as HCO3-, making the fractionation factor of aqueous CO2 and carbonate in the Tanaka et al. [1986] model insignificant. Using equation (1), we calculate Cm to be between 21 and 34% with a strong relationship between shell height (H in mm) and Cm (Figure 3; %Cm = 0.23 (±0.15) * H + 13.3 (±9.5); R² = 0.96).

These values are higher than the typical 10% Cm recorded for most aquatic mollusks [see McConnaughey and Gillikin, 2008], but are within the values reported for at least one marine species (Mercenaria sp.; see Gillikin et al. [2007] and Figure 3). Interestingly, the slopes between %Cm and shell height are not statistically different between the marine Mercenaria shells and the freshwater P. cataracta shells (p > 0.05 for both; Figure 3). The similarity in the slopes suggests that similar mechanisms may be responsible for the ontogenic effect seen here, despite the vast difference between the water chemistries in which these animals make their shells.

It can be argued that equation (1) does not necessarily account for all fractionation processes. Respired carbon is produced in the form of CO2, which then must convert to HCO3-. This conversion results in a ~−10‰ fractionation at 15°C [Mook, 2000], unless all CO2 converts to HCO3-. Considering freshwater mussels typically have a hemolymph (an internal body fluid) pH of around 7.5 to 8 [e.g., Byrne and Dietz, 1997], nearly all (~97%) respired CO2 should convert to HCO3- resulting in little to no fractionation. Therefore, the fractionation between aragonite and HCO3- should be used (see equation (1)). However, CO2 passes biological membranes much more easily than HCO3-[Gutknecht et al., 1977], so perhaps some of the small amount of CO2 that is present in the water makes it to the EPF disproportionate and contributes to the negative δ13Cw disequilibrium recorded in bivalve shells (see also McConnaughey and Gillikin [2008] for more detailed discussion on this). This could in part explain why Gillikin et al. [2007] found that hemolymph in marine clams contained only about 10% Cm, but shells contained much more (up to 37% Cm).

5. Conclusion

This study demonstrates that Cm increases through ontogeny in this freshwater mussel species and that the
Figure 3. Estimated percent metabolic carbon (C_M) for the four shells calculated using the equation of McConnaughey et al. [1997] showing a strong relationship between shell height (H in mm) and C_M (%C_M = 0.23 ±0.15) * H + 13.3 (+9.5); R^2 = 0.96) (black symbols and soil black line). Regressions from Mercenaria sp. shells are also plotted as dashed gray lines (data from Gillikin et al. [2007]). The slopes of the gray lines are 0.190 ±0.035 (n = 129, R^2 = 0.48) for the lower line and 0.187 ±0.092 (n = 13, R^2 = 0.64) for the upper line.

amount of C_M can be considerable (up to 34%). While our data set is small, %C_M increases from approximately 15–35% over shell heights of 20–90 mm. This ontogenetic effect, however, does not exclude δ13C_ar in freshwater mussel shells as useful environmental proxies. For example, although Goewert et al. [2007] reported an ontogenetic decrease in δ13C_ar shells had clearly different δ13C_ar values between a corn (C4) dominated and soybean (C3) dominated watershed. Therefore, as long as the ontogenetic effect is accounted for, these shells can still provide useful information. Even if δ13C_ar data cannot provide absolute δ13C_DIC records, they can still be used as indicators of environmental processes that occurred during shell growth.

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