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A large metabolic carbon contribution to the $\delta^{13}C$ record in marine aragonitic bivalve shells

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Abstract

It is well known that the incorporation of isotopically light metabolic carbon (C_M) significantly affects the stable carbon isotope ($\delta^{13}C$) signal recorded in biogenic carbonates. This can obscure the record of $\delta^{13}C$ of seawater dissolved inorganic carbon ($\delta^{13}C_{DIC}$) potentially archived in the shell carbonate. To assess the C_M contribution to *Mercenaria mercenaria* shells collected in North Carolina, USA, we sampled seawater $\delta^{13}C_{DIC}$, tissue, hemolymph and shell $\delta^{13}C$. All shells showed an ontogenic decrease in shell $\delta^{13}C$, with as much as a 4‰ decrease over the lifespan of the clam. There was no apparent ontogenic change in food source indicated by soft tissue $\delta^{13}C$ values, therefore a change in the respired $\delta^{13}C$ value cannot be the cause of this decrease. Hemolymph $\delta^{13}C$, on the other hand, did exhibit a negative relationship with shell height indicating that respired CO_2 does influence the $\delta^{13}C$ value of internal fluids and that the amount of respired CO_2 is related to the size or age of the bivalve. The percent metabolic C incorporated into the shell ($\%C_M$) was significantly higher (up to 37%, with a range from 5% to 37%) than has been found in other bivalve shells, which usually contain less than 10%C_M. Interestingly, the hemolymph did contain less than 10%C_M, suggesting that complex fractionation might occur between hemolymph and calcifying fluids. Simple shell biometrics explained nearly 60% of the observed variability in $\%C_M$, however, this is not robust enough to predict $\%C_M$ for fossil shells. Thus, the metabolic effect on shell $\delta^{13}C$ cannot easily be accounted for to allow reliable $\delta^{13}C_{DIC}$ reconstructions. However, there does seem to be a common effect of size, as all sites had indistinguishable slopes between the $\%C_M$ and shell height (+0.19% per mm of shell height).

1. INTRODUCTION

Stable isotope geochemistry has become a key tool in paleo-climate and paleo-oceanographic reconstruction. The oxygen isotopic ($\delta^{18}O_S$) signatures of different biogenic carbonates have been used to reconstruct both sea surface temperature and salinity (e.g., Jones et al., 1989; Weidman et al., 1994; Ingram et al., 1996; Dettman et al., 2004). In inorganic carbonates, stable carbon isotope enrichment factors for aragonite–bicarbonate and calcite–bicarbonate are independent of temperature and show no effect of precipitation rate (Romanek et al., 1992). On the other hand, the stable carbon isotopic composition of biogenic carbonate ($\delta^{13}C_S$) varies in a more complex manner. Although some works suggested that $\delta^{13}C_S$ reflected the $\delta^{13}C$ of dissolved inorganic carbon in seawater ($\delta^{13}C_{DIC}$) (e.g., Mook and Vogel, 1968); many others have suggested that both kinetic and metabolic effects play an important role in determining $\delta^{13}C_S$ (Keith et al., 1964; Swart, 1983; Tanaka et al., 1986; Klein et al., 1996; McConnaughey et al., 1997; Dettman et al., 1999; Lorrain et al., 2004; Gillikin et al., 2006). Kinetic effects generally affect both $\delta^{18}O_S$ and $\delta^{13}C_S$ and result in a good correlation between them (McConnaughey, 1989). As bivalves generally precipitate in oxygen isotope equilibrium with their surroundings (Epstein et al., 1953; Wefer and Berger, 1991; Chauvaud et al., 2005), kinetic effects should be minimal and disequilibrium should be

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mainly due to metabolic effects. Metabolic effects result from changes in the internal DIC pool, which is a combination of both seawater DIC and internally produced metabolic DIC (McConnaughey et al., 1997). Although, to our knowledge, the isotopic composition of this internal DIC pool has never been measured in an invertebrate, it is widely assumed that respiration, composed of ^{12}C-enriched CO_2, decreases the $\delta^{13}C$ value of the internal DIC pool. The $\delta^{13}C$ value of respired CO_2 can be assumed to approximately match the $\delta^{13}C$ of the respiring tissue ($\delta^{13}C_R$) (McConnaughey et al., 1997), but a recent study showed that coral $\delta^{13}C_R$ was up to 3‰ different from tissues (Swart et al., 2005). However, this offset would only change the percentage of respired carbon in the skeleton by about 3% when using the equation of McConnaughey et al. (1997, see Discussion). An earlier study suggested that up to 85% of mollusk-shell carbonate is composed of metabolic C (C_M) (Tanaka et al., 1986), but more recently McConnaughey et al. (1997) have shown that the former study overestimated the metabolic contribution partly because it erroneously included the enrichment factor between carbonate and aqueous CO_2. The $\delta^{13}C_{DIC}$ decrease in the internal DIC pool is now generally considered to result in small (<2‰) changes in $\delta^{13}C_S$, or approximately a 10% contribution from respired CO_2 (McConnaughey et al., 1997).

In bivalves there are varying degrees of $\delta^{13}C_S$ disequilibrium from $\delta^{13}C_{DIC}$. In some species, strong ontogenic decreases in $\delta^{13}C_S$ have been noted (Krantz et al., 1987; Kennedy et al., 2001; Keller et al., 2002; Elliot et al., 2003; Lorrain et al., 2004), whereas in others there is no discernable decrease (Buick and Ivany, 2004; Gillikin et al., 2005a, 2006; Surge and Walker, 2006). Lorrain et al. (2004) showed that the ratio of respired to precipitated carbon, which represents the amount of metabolic carbon available relative to the carbon requirements for calcification, increases through ontogeny in scallops. This suggests that the decrease of $\delta^{13}C_S$ through ontogeny is actually caused by increased utilization of this metabolic carbon to satisfy carbon requirements for calcification. Furthermore, they propose that seawater $\delta^{13}C_{DIC}$ could perhaps be reconstructed from bivalve shells if the metabolic contribution could be accounted for.

In this study, we sampled seawater DIC, tissue, hemolymph (circulatory fluid equivalent to blood) and shell $\delta^{13}C$ from *Mercenaria mercenaria* collected in North Carolina, USA to assess the contribution of metabolic carbon to the shell. *M. mercenaria* is a large aragonitic clam (Elliot et al., 2003), which can obtain ages of nearly 50 years (Peterson, 1986) and is therefore suitable to detect long-term ontogenic effects in shell geochemistry. Our aim was to (1) determine if *M. mercenaria* exhibits an ontogenic decrease in $\delta^{13}C_S$; (2) determine what causes the decrease in $\delta^{13}C_S$; and (3) assess if vital effects can be accounted for in order to estimate $\delta^{13}C_{DIC}$.

2. METHODOLOGY

Mercenaria mercenaria specimens were collected alive from the Cape Lookout region of North Carolina, USA, from three sites: Jarrett Bay (34°45′47″N, 76°29′08″W), Johnson Creek (34°45′06″N, 76°26′10″W), and Back Sound (34°39′27″N, 76°33′25″W) (Fig. 1). Salinity at Jarrett Bay ranges from 23 to 37 and from 28 to 34 at Johnson Creek and Back Sound, but lower salinities are usually short lived events after storms; temperature typically ranges from 1 to 35 °C (Peterson et al., 1983; Gillikin et al., 2005b). Sediments were muddy at the Jarrett Bay and Johnson Creek sites and were more sandy at Back Sound (Gillikin et al., 2005b). Precipitation varies between a high of 170 mm in July and minima of 76 and 88 mm in April and November, respectively, with an annual average of 113 ± 27 mm (based on average data from nearby Morehead City from 1896 to 1987; Lieth et al., 1999). Major rain storms typically occur from July to September (Lieth et al., 1999). Specimens were

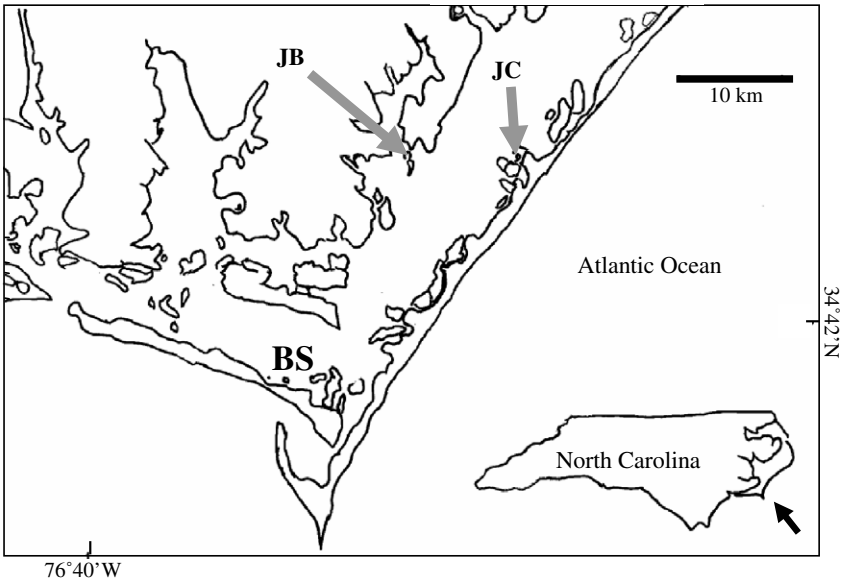

Fig. 1. Shell collection sites in eastern North Carolina, near Cape Lookout (BS: Back Sound, JC: Johnson Creek, JB: Jarrett Bay).

collected by hand from shallow (<1 m) muddy sediments below mean low water spring tide (MLWS). These clams are typically buried in the sediment from 0 to 25 mm deep, but all siphon water from the water column (Roberts et al., 1989). Additionally, to test if any pattern found in these shells is the result of modern changes in the environment, a Pliocene (~3.2 million years old) shell was collected from the Duplin Formation in South Carolina (1.5 km northwest of Timmonsville) and analyzed.

In Jarrett Bay, an extensive sampling was conducted where the $\delta^{13}C$ of shells of various sizes (12.4–99.2 mm), different tissues (gill, mantle, muscle, and foot), hemolymph DIC, water DIC, particulate matter and sediment were sampled (all on 17 Aug. 2004, when water depth was about 0.6 m). As $\delta^{13}C_{DIC}$ may differ between the water column and the sediment–water interface, DIC was collected by drawing water just above the sediment surface into a clean 25-ml syringe and gently transferring it to a glass headspace vial, adding 30 μl of $HgCl_2$ and capping with a butyl septum. To sample hemolymph, first the mantle cavity fluid was drained by prying the valves apart with a knife, then a sterile syringe and needle (fitted with a filter; 0.2 μm, cellulose acetate) was inserted into the adductor muscle and hemolymph was gently drawn into the syringe. The hemolymph sample was then quickly transferred to a sealed He-flushed headspace vial containing $HgCl_2$ (see Gillikin, 2005 for more details). Soft tissues were dissected in the field using a scalpel and were stored in microcentrifuge tubes in a cool box on ice until they were frozen (−20 °C) later in the day. Suspended particulate matter was sampled by filtering 150 ml of seawater through precombusted 47 mm glass fiber filters (Whatman GF/F, 0.7 μm), which were later dried. The top 1 cm of sediment was scraped off an area of ca. 10 cm diameter and was later dried.

Shells were collected from the other sites (Johnson Creek and Back Sound) in 1980, 1982, and 2002. Water samples and muscle tissues from various sized clams (29.3–88.8 mm shell height) were collected as described above at Johnson Creek (Aug. 2003), but not at Back Sound.

Shells were sectioned along the axis of maximal growth and were sampled in the middle shell layer (see Elliot et al., 2003 or Gillikin et al., 2005c). Only the most recently formed shell carbonate was sampled from the Jarrett Bay specimens by milling a 300 μm spot at the growing edge (in cross-section) using a Merchantek MicroMill. Shells from Johnson Creek and Back Sound were sampled at an annual resolution using the annual growth lines on the shell, which form in late summer/early winter in this region (Peterson et al., 1983). To accomplish this, lines were milled between annual growth lines using the MicroMill and a 300-μm drill bit. The Pliocene shell was sampled by continuously milling holes every 300 μm. Similarly, one of the Back Sound shells was also sampled at a high resolution (BS1). Carbonate powders (~75 μg) were reacted in an automated carbonate preparation device (ThermoFinnigan Kiel III) coupled to a ThermoFinnigan Delta^{plus}XL dual inlet IRMS with a long-term precision of 0.039‰ for $\delta^{13}C$ and 0.085‰ for $\delta^{18}O$ on the NBS-19 standard ($n = 292$; $\delta^{13}C = +1.95$‰, $\delta^{18}O = -2.20$‰), and 0.068‰ for $\delta^{13}C$ and 0.111‰ for $\delta^{18}O$ on the NBS-18 standard ($n = 22$; $\delta^{13}C = -5.04$‰, $\delta^{18}O = -23.05$‰). Results are reported relative to VPDB by calibration to the NBS-19 reference standard ($\delta^{13}C = +1.95$‰ and $\delta^{18}O = -2.20$‰VPDB).

For the analysis of $\delta^{13}C_{DIC}$ from both water and hemolymph, the method of Gillikin and Bouillon (2007) was followed. Briefly, a He headspace was created (in the case of water samples), and ~300 ml of H_3PO_4 was added to convert all inorganic carbon species to CO_2. After overnight equilibration, part of the headspace was injected into the He stream of an elemental analyzer–isotope ratio mass spectrometer (EA-IRMS, ThermoFinnigan Flash 1112 and ThermoFinnigan Delta+XL) for $\delta^{13}C$ measurements. The obtained $\delta^{13}C$ data were corrected for the isotopic equilibration between gaseous and dissolved CO_2 by using the algorithm presented in Miyajima et al. (1995).

Soft tissues were freeze-dried, homogenized with a mortar and pestle, and about 1 mg was placed into a silver cup. Two to three drops of 5% HCl were added to decarbonate the sample, and the cups were allowed to dry in an oven overnight, after which they were folded closed. Tissue $\delta^{13}C$ was measured on the EA-IRMS described above. Using this same instrument and method, Verheyden et al. (2005) reported a long-term analytical precision for $\delta^{13}C$ of 0.08‰ on 214 analyses of the IAEA-CH-6 standard (1σ). Particulate matter and sediment $\delta^{13}C$ were analyzed following standard procedures similar to that described above (see also Lorrain et al., 2003; Bouillon et al., 2004; Gillikin et al., 2006).

3. RESULTS

All shells, regardless of collection site or time of collection exhibit a large ontogenic decrease in $\delta^{13}C_S$, up to 4‰ (Fig. 2), including the Pliocene shell (Figs. 3 and 4). The Pliocene shell did not appear to have undergone isotopic diagenesis, as indicated by both $\delta^{18}O_S$ and $\delta^{13}C_S$ being similar to modern shells and not being well correlated ($R^2 = 0.11$) and because pure aragonite (i.e., no calcite) was detected by XRD analysis (see Elorza and Garcia-Garmilla, 1996, 1998; and Labonne and Hillaire-Marcel, 2000 for discussions of diagenetic indicators). The high-resolution profile of shell BS1 is shown in Fig. 5. All $\delta^{13}C_S$ data are within the range found in *M. mercenaria* shells by Elliot et al. (2003).

The different tissues from Jarrett Bay clams had significantly different $\delta^{13}C$ values ($p < 0.01$ for all), except for mantle (−19.1 ± 0.3‰) and muscle (−19.1 ± 0.2‰) tissues ($p = 0.74$), with gills being the least negative (−18.4 ± 0.3‰) and the foot the most negative (−19.5 ± 0.3‰) (see Table 1). None of the tissues from the Jarrett Bay samples exhibited a correlation with shell height. In contrast, there was a significant strong positive correlation between muscle $\delta^{13}C$ values and height in the Johnson Creek clams (slope = 0.048 ± 0.012, $R^2 = 0.98$, $p = 0.0011$, $n = 5$; see Electronic Annex 1). Three replicate $\delta^{13}C_{DIC}$ samples taken at Jarrett Bay (Aug. 2004) gave a mean of −0.77 ± 0.2‰, which is similar to the average of 10 samples taken in Aug. 2002 (−0.3 ± 0.6‰; collected on 10 different days between 11 Aug and 28 Aug. 2002; see

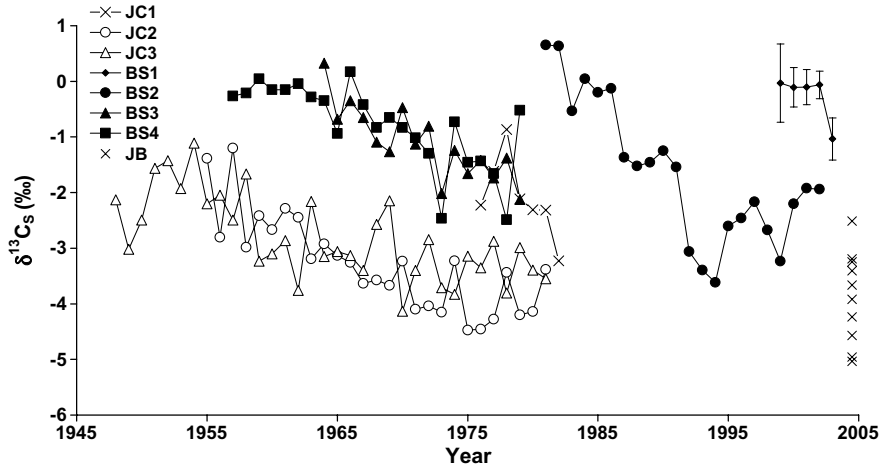

Fig. 2. Annual shell $\delta^{13}C$ from *Mercenaria mercenaria* shells collected at two sites (JC = Johnson Creek; BS = Back Sound) plotted versus year showing the clear ontogenic decrease. The samples from Jarrett Bay (each 'x' represents the most recent shell material from a different clam) are also shown here for comparison.

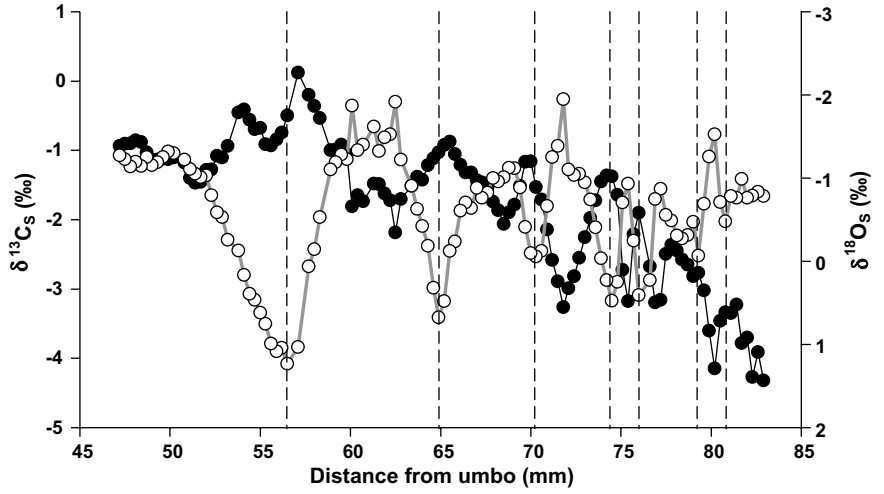

Fig. 3. High resolution $\delta^{13}C_S$ (solid circles) and $\delta^{18}O_S$ (open circles) data from a Pliocene *Mercenaria mercenaria* shell plotted versus distance from the umbo. The dashed lines illustrate the separation between different growth years. The $\delta^{13}C_S$ data was averaged between these marks (see Fig. 4).

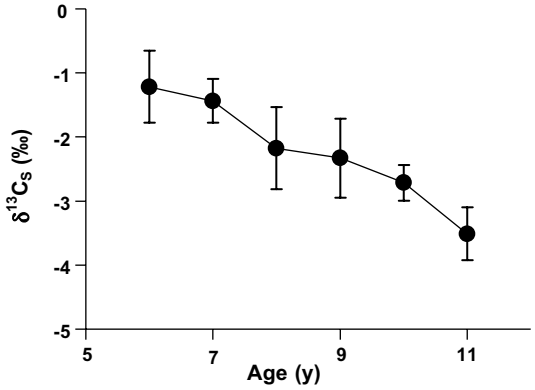

Fig. 4. Average and standard deviation of annual $\delta^{13}C_S$ data from the Pliocene *Mercenaria mercenaria* shell (data in Fig. 3). Age of the samples were estimated form growth lines on the shell and $\delta^{18}O_S$ (see Fig. 3).

Electronic Annex 2). The $\delta^{13}C$ of organic carbon in both sediments (-20.3 ± 0.14‰) and suspended matter (-21.5‰) were within 2.5‰ of tissues (~ -19‰) at Jarrett Bay. Hemolymph $\delta^{13}C$ at Jarrett Bay was negatively correlated with shell height (Fig. 6; $R^2 = 0.94$, $p = 0.007$, $n = 5$), but not with tissue or shell $\delta^{13}C$ (Table 1). The $\delta^{13}C_{DIC}$ samples from Johnson Creek (-2.4 ± 0.26‰; $n = 3$) were more negative than Jarrett Bay; however, this difference was not reflected in the muscle tissues of Johnson Creek clams (-18.2 ± 1.2‰; $n = 5$; see Electronic Annex 1).

4. DISCUSSION

4.1. $\delta^{13}C_S$ through ontogeny

All *M. mercenaria* shells investigated showed a clear ontogenic decrease in $\delta^{13}C_S$ (Figs. 2–5 and 7). The high

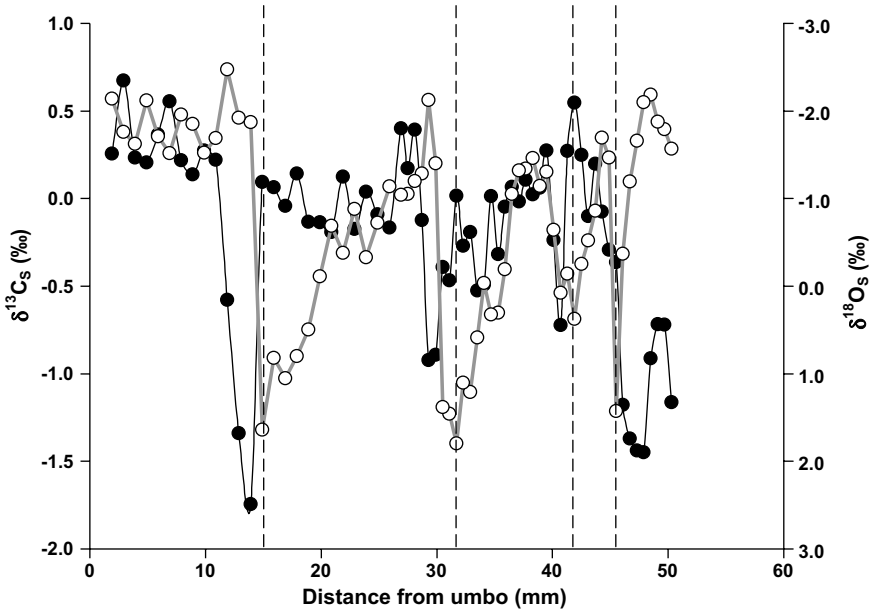

Fig. 5. High resolution $\delta^{13}C_S$ (solid circles) and $\delta^{18}O_S$ (open circles) data from shell BS1 (Back Sound). The dashed lines illustrate the separation between different growth years. The $\delta^{13}C_S$ data was averaged between these marks (see Fig. 2).

resolution profiles show the intra-annual variability of $\delta^{13}C_S$ can be large, up to 2.4‰, but is generally about 1.5‰. The standard deviation on annual $\delta^{13}C_S$ is shown in Fig. 4, which clearly illustrates that the differences in inter-annual $\delta^{13}C_S$ values are more important than the intra-annual variability. This is also evident in data presented in Elliot et al. (2003; see their Fig. 4; maximum annual $\delta^{13}C_S$ standard deviation = 0.8‰). Therefore the $\delta^{13}C_S$ decrease through time is not an artifact of the sampling methodology.

4.2. Causes of $\delta^{13}C_S$ decrease through ontogeny

There are several potential causes for the decrease in $\delta^{13}C_S$; however, kinetic effects can most definitely be ruled out. Kinetic effects result in a good, positive correlation between $\delta^{18}O_S$ and $\delta^{13}C_S$ (McConnaughey, 1989), which has not been observed in *M. mercenaria* shells (Elliot et al., 2003; Gillikin, 2005; this study). Other possible causes for the ontogenic decrease in $\delta^{13}C_S$ can be separated into two main categories: changes in environmental $\delta^{13}C_{DIC}$, and biological changes resulting in a change in the internal DIC pool. Environmental changes include the Suess effect, caused by increasing amounts of anthropogenic ^{13}C-depleted CO_2 in the atmosphere, which leads to more negative $\delta^{13}C_{DIC}$ in seawater. This phenomenon has been recorded in sclerosponge skeletons (e.g., Druffel and Benavides, 1986; Lazareth et al., 2000), but the change in seawater $\delta^{13}C_{DIC}$ over the past 50 years is on the order of 0.8‰ (Quay et al., 2003), far less than the changes observed in

Table 1
Carbon isotope data ($\delta^{13}C$) from hemolymph DIC and various tissues from13 *M. mercenaria* collected in Jarrett Bay on 17 Aug 2004

Shell height (mm)	Hemolymph (‰)	Gill (‰)	Mantle (‰)	Muscle (‰)	Foot (‰)	Shell edge (‰)	$\%C_M$ shell	$\%C_M$ hemolymph
99.2	−2.5	−18.5	−19.2	−19.0	−19.0	−5.0	37.8	9.8
96.0	−2.7	−18.5	−18.5	−19.0	−19.2	−4.6	35.7	10.7
85.1	−2.2	−18.8	−19.3	−19.3	−19.6	−3.4	28.8	7.9
68.0	−1.1	−18.8	−19.1	−19.3	−19.7	−4.2	33.3	2.0
66.5	−0.7	−18.5	−19.3	−19.3	−19.5	−5.0	37.5	0.0
65.0		−18.2	−18.9	−18.9	−19.1	−3.7	30.9	
48.1		−18.4	−19.4	−19.1	−19.6	−2.5	24.2	
48.0		−18.4	−18.8	−19.0	−19.6	−3.9	32.1	
39.0		−18.0	−18.8	−18.8	−19.6	−3.2	28.7	
35.5		−18.3	−19.5	−19.3	−19.7	−3.2	27.7	
30.3				−19.3*		−3.0	26.8	
22.1				−19.9*		−1.1	15.8	
12.4				−19.9*		−2.0	20.6	
	Average	−18.4 ± 0.3	−19.1 ± 0.3	−19.1 ± 0.2	−19.5 ± 0.3			

$\delta^{13}C_{DIC}$ of water collected near the sediment–water interface was −0.77 ± 0.2‰ at the time of sampling.
* Note that the three smallest samples were not dissected and represent whole body tissues and are not included in the average.

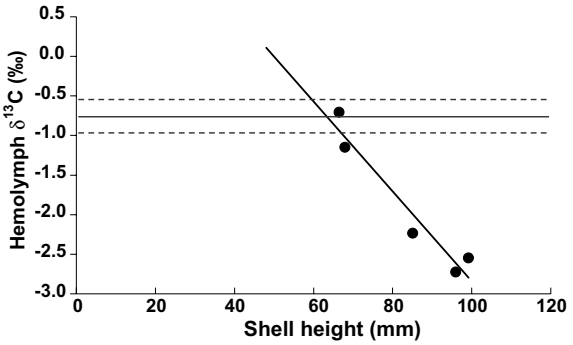

Fig. 6. $\delta^{13}C$ values of filtered *Mercenaria mercenaria* hemolymph samples from Jarrett Bay plotted versus shell height (H in mm) (closed circles) with the linear relationship: hemolymph $\delta^{13}C = -0.055(\pm 0.027) * H + 2.35(\pm 2.28)$ ($R^2 = 0.93, p < 0.01, n =$ 5 clams). The mean $\delta^{13}C_{DIC}$ value of the water where the clams were collected is also given (horizontal solid line) with the standard deviation (horizontal dashed lines). The complete dataset is available in Table 1.

these shells (up to 4‰). Additionally, similar decreases in $\delta^{13}C_S$ are noted regardless of whether the clam was collected in 1980 or 2003 (Fig. 2), and the ontogenic decrease is also evident in the Pliocene shell (Fig. 3), which grew well before anthropogenic CO_2 inputs were present. Thus, a secular change of seawater $\delta^{13}C_{DIC}$ is obviously not the dominant cause. Another possibility is that the clams may live deeper in the sediment as they age and utilize a more negative environmental $\delta^{13}C_{DIC}$ source, as suggested by Keller et al. (2002) and Elliot et al. (2003). Indeed, strong gradients in pore water $\delta^{13}C_{DIC}$ have been observed within the initial 5 cm of sediment due to the remineralization of organic matter (up to $-1‰\ cm^{-1}$; McCorkle et al., 1985). However, this is probably not a cause, as Roberts et al. (1989) found that the depth of *M. mercenaria* in the sediment was independent of clam size (they are all just below the surface when feeding and calcifying). Thus different size classes can be considered to use similar water sources as all size classes siphon water at the sediment–water interface. Moreover, as *M. mercenaria* have high pumping rates (Hamwi and Haskin, 1969), it can be assumed that they utilize water well above the pore water and sediment–water interface and therefore their shells should not be greatly influenced by processes affecting $\delta^{13}C_{DIC}$ in the sediments. Some authors have suggested that infaunal bivalves are more depleted in ^{13}C relative to epifaunal bivalves (Krantz et al., 1987). However, other species of both epifaunal (e.g., Lorrain et al., 2004; Gillikin et al., 2006) and infaunal bivalves (e.g., Gillikin et al., 2005a) have been shown to incorporate less than 10% C_M. Thus, the most probable cause is a change in the internal DIC pool, which is strongly supported by the negative relationship between shell height and hemolymph $\delta^{13}C$ (Fig. 6).

A change in the internal DIC pool could be due to differences in $\delta^{13}C_R$ caused by food sources with different $\delta^{13}C$ signatures. However, in this study, tissue $\delta^{13}C$ and shell height (or age) were generally not correlated. Although some tissue $\delta^{13}C$ data had a positive relationship with shell height, this is opposite to what is observed in the shells (i.e., a negative correlation between shell $\delta^{13}C$ and shell height). Thus, a change in food as the animal ages is not likely the cause of the $\delta^{13}C$ trend in the shells. Since lipids have a lighter $\delta^{13}C$ signal than other biochemical components (Tieszen et al., 1983), changes in lipid metabolism can also result in changes in $\delta^{13}C_R$, but this would be expected to be reflected in the tissue $\delta^{13}C$, which it is not. Moreover, lipid content has been shown to be low in *M. mercenaria* tissues, only changing the $\delta^{13}C$ value of tissues by ~0.5‰ (O'Donnell et al., 2003). pH can also affect $\delta^{13}C_S$, with increasing pH resulting in decreasing $\delta^{13}C_S$, as has been observed in foraminifera (Spero et al., 1997). However, internal pH has been shown to decrease in older bivalves (Sukhotin and Pörtner, 2001), which would lead to an increase in $\delta^{13}C_S$.

Lorrain et al. (2004) proposed that the increase of absolute metabolic rate in bivalves as shell-growth rate slows with age, leads to a larger availability of metabolic C for $CaCO_3$ precipitation through ontogeny. In other words, as the animal ages and the carbon availability from seawater remains constant, there is an increase in metabolic CO_2 production which is larger than the carbon demand for calcification, resulting in a larger amount of metabolic C available in the internal DIC pool. This can be simply expressed by a ratio of respired to precipitated carbon (see Lorrain et al., 2004) which increases through age. We therefore expect this ratio of respired to precipitated carbon also to increase through ontogeny in *M. mercenaria*. *M. mercenaria* has been shown to have a high metabolic rate compared to other bivalves (Hamwi and Haskin, 1969). This high metabolic rate, coupled with slow shell growth at older ages, would result in a relatively high ratio of respired to precipitated carbon and would account for the unusually large ontogenetic $\delta^{13}C_S$ decrease of up to 4‰. Larger metabolic carbon availability with age should lead to a decrease in the $\delta^{13}C$ value of calcifying fluids because the metabolic carbon is more depleted in ^{13}C compared to DIC. The negative relationship observed between shell height and hemolymph $\delta^{13}C$ in this study (Fig. 6) proves for the first time that there is effectively a decrease in $\delta^{13}C$ of internal fluids with age (or shell height), which seems to confirm the findings of Lorrain et al. (2004).

However, a simple mixture between seawater $\delta^{13}C_{DIC}$ and $\delta^{13}C_R$ might not be occurring. The hemolymph data presented here are similar to seawater $\delta^{13}C_{DIC}$ in an individual 66 mm in height (Fig. 6), whereas $\delta^{13}C_S$ already decreases with height in individuals with smaller shell sizes (Fig. 5). Furthermore, although carbon species seem to be freely exchangeable between the extrapallial fluid (EPF; where calcification occurs) and hemolymph (Greenaway, 1971; Wilbur and Saleuddin, 1983), hemolymph $\delta^{13}C$ may not be a good proxy of the EPF $\delta^{13}C$. The hemolymph is separated from the EPF by a membrane, where enzymatic reactions (e.g., carbonic anhydrase activity) facilitate CO_2 diffusion into the EPF (see Crenshaw, 1980; McConnaughey, 1989; Cohen and McConnaughey, 2003), which may be associated with kinetic fractionations, and thus change the $\delta^{13}C$ value. Hemolymph $\delta^{13}C$ probably can be carried over to the EPF, just not necessarily in absolute terms.

Indeed, in an experimental study on a rainbow trout, Solomon et al. (2006) found a large difference between blood and endolymph $\delta^{13}C$ values—which are internal fluids in fish equivalent to hemolymph and EPF in bivalves—suggesting that fractionation might occur between these internal fluids. In any case, our hemolymph $\delta^{13}C$ data generally agree with the respiratory gas-exchange model of McConnaughey et al. (1997), where they state that ~90% of the CO_2 inside aquatic invertebrates is derived from the water and ~10% from respiration (our data range from ~0% to ~10% CO_2 in hemolymph derived from respiration (Table 1); calculated using: $[\delta^{13}C_{DIC\text{-}hemolymph} - \delta^{13}C_{DIC\text{–}water}]/[\delta^{13}C_R - \delta^{13}C_{DIC\text{–}water}]$). It is possible that during calcification the metabolic CO_2 is preferentially used over bicarbonate from seawater because it more easily can cross biological membranes. Moreover, we cannot rule out the possibility that the EPF may actually have more contact with ambient interstitial fluids than does the hemolymph. Both of these possibilities could explain why the shell is apparently precipitated from a significantly more negative fluid.

Considering that the hemolymph data cannot be directly related to the shell, the best standing model to calculate the amount of metabolic C (C_M) in the shell is given by McConnaughey et al. (1997):

$$\%C_M * (\delta^{13}C_R) + (1 - \%C_M) * \delta^{13}C_{DIC} = \delta^{13}C_s - \varepsilon_{ar-b} \quad (1)$$

where $\%C_M$ is the percent metabolic CO_2 contribution, $\varepsilon_{ar\text{-}b}$ is the enrichment factor between aragonite and bicarbonate (2.7‰ from Romanek et al., 1992), and $\delta^{13}C_R$ is approximated from tissue $\delta^{13}C$. Although we only have $\delta^{13}C_{DIC}$ from late summer, the temporal variability in $\delta^{13}C_{DIC}$ should not be too large as is evidenced by the similarity of the samples taken in Jarrett Bay on separate years. Also, August rainfall (153 mm) is between the annual average (113 ± 27 mm) and maximum rainfall (170 mm) and therefore $\delta^{13}C_{DIC}$ should not be largely impacted by freshwater runoff. Moreover, despite the fact that Elliot et al. (2003) collected their shells from sites near and far from freshwater inputs, they noted similar $\delta^{13}C_S$ values. Nevertheless, absolute $\%C_M$ values may be over or under estimated, but the general pattern of decreasing $\delta^{13}C_s$ seen in shells from all three sites and in the Pliocene shell (Fig. 7) would not be largely affected. In the future, similar studies would greatly benefit from high resolution $\delta^{13}C_{DIC}$ data.

At the Jarrett Bay site, where we had corresponding tissue, water and shell data (shells from this site do not represent an annual average, but are sampled from there most recently formed shell material), Eq. (1) gave results ranging from 15.8 to 37.8%C_M, with a linear relationship between shell height and $\%C_M$ (Fig. 8). The $\delta^{13}C$ values from the muscle tissue were used in these calculations for two reasons: (1) the muscle has the slowest turnover time, so it integrates the longest time period (see Lorrain et al., 2002); and (2) it has the same $\delta^{13}C$ value as the mantle tissue, which is closest to the calcification site and should have the largest effect on the internal DIC pool.

For the Johnson Creek and Back Sound sites, we do not have tissue or water data to match our carbonate samples for each year. In this case, we used the data recently collected from Johnson Creek and applied them to the entire Johnson Creek dataset (i.e., $\delta^{13}C_R = -18.2‰$ and $\delta^{13}C_{DIC} = -2.4‰$). Water properties for Back Sound were assumed: water at the Back Sound site exchanges with the open ocean (Peterson and Fegley, 1986), so should have a $\delta^{13}C_{DIC}$ value closer to oceanic values. Thus we assume $\delta^{13}C_{DIC} = -0.5‰$ and tissues $= -19‰$ (i.e., the mean of the tissues from the Jarrett Bay site) at the Back Sound site. A maximum error of ~1‰ can be expected from these assumptions, which would change $\%C_M$ by ~5% for a 1‰ change in $\delta^{13}C_{DIC}$ and ~1% for a 1‰ change in $\delta^{13}C_R$ (i.e., $\delta^{13}C$ of tissues). Using Eq. (1) and the assumptions listed above results in $\%C_M$ values ranging from 7.4% to 31.4% for the Back Sound and Johnson Creek shells

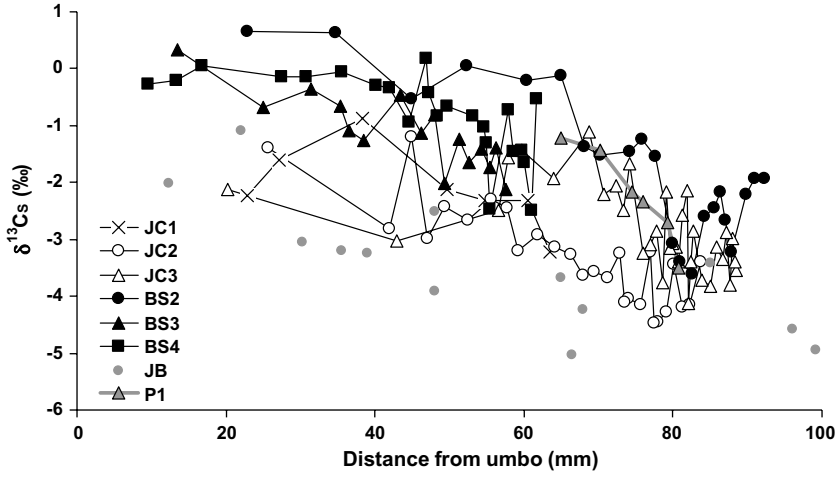

Fig. 7. Annual shell $\delta^{13}C$ from *Mercenaria mercenaria* shells collected at two sites (JC = Johnson Creek; BS = Back Sound) plotted versus shell height. Data from Jarrett Bay (JB) shells are also given, but it should be noted that these samples represent less than one year (i.e., only the most recent shell material was sampled) and thus are expected to have a higher variability than the other shells which integrate a full year of growth. Each data point from JB is a different shell. Data from the Pliocene shell (P1; annual average) are also shown.

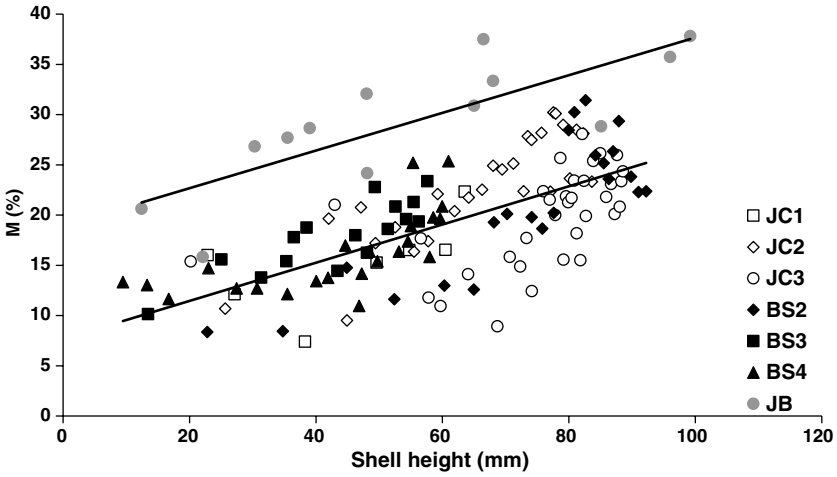

Fig. 8. Percent metabolic C ($\%C_M$) incorporated into *Mercenaria mercenaria* shells plotted versus shell height (H in mm). Data are from Jarrett Bay clams (JB) where tissue, water and the most recent shell material were sampled for each shell, and the annual $\%C_M$ incorporated into *M. mercenaria* shells collected at the other two sites (JC = Johnson Creek; BS = Back Sound). The slope and intercept were not significantly different between the JC and BS shells ($p \geqslant 0.9$). The linear relationship for the combined Johnson Creek and Back Sound datasets is $\%C_M = 0.190\ (\pm 0.035) * H + 7.65\ (\pm 2.25)$ ($p < 0.0001$, $n = 129$, $R^2 = 0.48$). The Jarrett Bay relationship is $\%C_M = 0.187\ (\pm 0.092) * H + 18.92\ (\pm 5.65)$ ($p < 0.001$, $n = 13$, $R^2 = 0.64$). The slopes between the two regressions (combined JC and BS and JB) are not statistically different ($p = 0.81$).

(Fig. 8). Correcting for the correlation between tissue $\delta^{13}C$ and shell height found in Johnson Creek clams (see results), only changes $\%C_M$ by a maximum of 2.3%. The change in tissue $\delta^{13}C$ with shell height (slope $= +0.05 \pm 0.01$) may be due to larger individuals including microphytobenthos in their diet, which have heavier $\delta^{13}C$ values ($\sim -15‰$, Herman et al., 2000; Middelburg et al., 2000) compared to phytoplankton ($\sim -20‰$, see Gillikin et al., 2006), or simply due to aging of the tissues (cf. Overman and Parrish, 2001). Nevertheless, the change in tissue $\delta^{13}C$ does not contribute greatly to the $\%C_M$ in the shell.

At all sites, the $\%C_M$ ranges are substantially higher than the proposed 10% (McConnaughey et al., 1997), even when considering possible errors. Furthermore, there is a linear relationship between $\%C_M$ and shell height (Fig. 8), with no significant difference between the slopes or intercepts of the Back Sound and Johnson Creek datasets ($p > 0.05$). The Jarrett Bay data had a similar slope to the other sites ($p = 0.81$), but the intercept is much higher (Fig. 8). This could be the result of different metabolic rates between the sites (cf. Lorrain et al., 2004; Gillikin et al., 2006) or errors in assumed environmental variables. Interestingly, the similarity in slopes suggests that the age effect between different populations with apparently different $\%C_M$ incorporation is general, with a change in $\%C_M$ of +0.19% per mm of shell height. Moreover, the shell Elliot et al. (2003) sampled from Hatteras Inlet, North Carolina ($\sim$100 km northeast of this study) had the same slope of $+0.19\%C_M$ per mm of shell height ($\%C_M$ ranged from 8% to 21% assuming similar conditions to Back Sound, but note that the slope is independent of these assumptions).

4.3. Can the metabolic effect be accounted for?

Being able to predict the contribution of metabolic carbon in the shell carbonate of mollusks would be of great value for reconstructing environmental conditions. With known $\%C_M$, $\delta^{13}C_S$, and tissue $\delta^{13}C$, Eq. (1) can be used to calculate $\delta^{13}C_{DIC}$ at the time the shell grew. Although tissue $\delta^{13}C$ would not be available for fossil or specimens collected in the past, the shell organic matter $\delta^{13}C$ could be used as a proxy of tissue $\delta^{13}C$. O'Donnell et al. (2003) found that the $\delta^{13}C$ value of organic matter extracted from *M. mercenaria* shells was indistinguishable from tissue $\delta^{13}C$. However, the predictability of $\%C_M$ from shell height is weak, with an R^2 of 0.48 for Back Sound and Johnson Creek shells and 0.64 for Jarrett Bay shells. We attempted to improve the linear model by including several biometric parameters in addition to total shell height (i.e., a multiple linear regression with annual growth-increment height, calculated annual growth increment weight, and age), but they did not improve the model by more than 4%. For example, combining age and height from the Johnson Creek and Back Sound datasets to predict $\%C_M$ resulted in the highest R^2 (0.52). Additionally, the large difference in intercepts between the two regressions shown in Fig. 8 suggests that there is no general relationship between height and $\%C_M$. Thus, unfortunately, there is too much unexplained variability in the data and apparently large differences in metabolic rates (or other factors) between sites, making $\%C_M$ predictions difficult and back calculating $\delta^{13}C_{DIC}$ highly uncertain. However, as suggested by Lorrain et al. (2004), $\delta^{13}C_S$ may provide information about metabolic rates for different populations of marine mollusks.

5. SUMMARY AND RECOMMENDATIONS FOR FUTURE RESEARCH

This study has shown that a large amount of metabolic carbon (as much as 25–40%) can be present in bivalve shells and complicate the $\delta^{13}C$ record in the shell. This is the first time such a large amount of metabolic carbon has been

reported in a bivalve shell. Moreover, the hemolymph $\delta^{13}C$ data confirm an increase in metabolic carbon availability through ontogeny and support the hypothesis of Lorrain et al. (2004)—that size of the bivalve plays an important role in the $\delta^{13}C$ record in the shell. Unfortunately, we were unable to use simple shell biometrics to account for this metabolic contribution to the shell. Our results show that using shell $\delta^{13}C$ as a proxy for $\delta^{13}C_{DIC}$ can lead to erroneous conclusions.

This work highlights the point that more research is necessary to determine fractionations at each step from carbon source to shell. The different compartments such as hemolymph and EPF need to be better characterized. Controlled laboratory experiments, where environmental parameters can be varied (such as $\delta^{13}C_{DIC}$, CO_2/O_2 ratios, $\delta^{13}C$ of food, etc.) and removed (such as a possible pore-water source) would be very beneficial.

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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.gca.2007.04.003.

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