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Carbon isotopes in mollusk shell carbonates

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Abstract Mollusk shells contain many isotopic clues about calcification physiology and environmental conditions at the time of shell formation. In this review, we use both published and unpublished data to discuss carbon isotopes in both bivalve and gastropod shell carbonates. Land snails construct their shells mainly from respired CO_2, and shell $\delta^{13}C$ reflects the local mix of C3 and C4 plants consumed. Shell $\delta^{13}C$ is typically >10‰ heavier than diet, probably because respiratory gas exchange discards CO_2, and retains the isotopically heavier HCO_3^-. Respired CO_2 contributes less to the shells of aquatic mollusks, because CO_2/O_2 ratios are usually higher in water than in air, leading to more replacement of respired CO_2 by environmental CO_2. Fluid exchange with the environment also brings additional dissolved inorganic carbon (DIC) into the calcification site. Shell $\delta^{13}C$ is typically a few ‰ lower than ambient DIC, and often decreases with age. Shell $\delta^{13}C$ retains clues about processes such as ecosystem metabolism and estuarine mixing. Ca^{2+} ATPase-based models of calcification physiology developed for corals and algae likely apply to mollusks, too, but lower pH and carbonic anhydrase at the calcification site probably suppress kinetic isotope effects. Carbon isotopes in biogenic carbonates are clearly complex, but cautious interpretation can provide a wealth of information, especially after vital effects are better understood.

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Introduction

Over the past half century, isotopic geochemists have extracted a plethora of information on paleotemperatures and hydrological processes from the oxygen isotope compositions ($\delta^{18}O$) of biogenic carbonates (e.g., Emiliani 1954, and many others since). The carbon isotopic compositions ($\delta^{13}C$) of shell and bone carbonates have also yielded useful information, but uncertainty concerning the origins of carbonate carbon has remained a problem. When does calcification draw mainly from respired CO_2, derived from food? When does inorganic carbon from ambient air or water dominate? How does carbon reach the calcification site? Does the phylogenetic position, physiology, or ecology of the calcifying animal matter? Resolving such questions will improve reconstructions of past CO_2 levels, the mixing of marine and fresh waters, animal diets, upwellings, ecological upheavals, and many other aspects that geochemists, paleontologists, ecologists, and archaeologists study using biogenic carbonate $\delta^{13}C$.

This analysis focuses on mollusks. But just as mice, nematodes and bacteria provide insights into human physiology and medicine, so too do non-mollusks provide insights into mollusks. Scientists will also want to apply insights gained from mollusks to other animals. Numerous comparisons between mollusks and other organisms are therefore included, but we always return to mollusks to discuss the strengths and weaknesses of the evidence in this group.

Mollusks make attractive environmental recorders because of their abundance in diverse environments, and their sequential skeletal deposition. Developmental or ontogenetic changes can, however, affect isotopic fractionations. This opens opportunities for monitoring the animal, but

complicates environmental monitoring. Understanding 'vital' effects is therefore important for distinguishing physiological from environmental factors. Recent insights have emerged from detailed measurements on common animals, plus examination of animals from unusual environments. Subtle differences in calcification physiology now appear to account for markedly different isotopic outcomes.

Respired CO_2 and ambient inorganic carbon both contribute to mollusk shells, and the relative importance of each source will determine whether shell $\delta^{13}C$ records mainly dietary $\delta^{13}C$, or the $\delta^{13}C$ of ambient inorganic carbon. McConnaughey et al. (1997) suggested that land snails and other air-breathing animals build their carbonates mainly from respired CO_2, while aquatic animals build their shells mainly from ambient inorganic carbon. We reexamine this issue, discuss some physiological environmental factors that affect the balance of carbon sources, and offer new insights into the observed isotopic fractionations.

This paper emphasizes generalizations that work for large parts of nature, the processes that give rise to these generalizations, and reasons why they sometimes fail. Many fundamental issues have not been settled. Highlighting these uncertainties may help geochemists, paleontologists, and archaeologists to recognize and avoid some common pitfalls, and will hopefully encourage basic research. Carbon isotopes in biogenic carbonates are clearly complex, but cautious interpretation can provide a wealth of information.

Calcification physiology

Various mollusks construct their shells of calcite, aragonite, or both (Fig. 1a). Calcification occurs from the extrapallial fluid (EPF), which is often divided into inner and outer sections (Wilbur and Saleuddin 1983; Wheeler 1992). Sampling these fluids is technically difficult due to their small volumes, and most chemical measurements have been made from the inner EPF (Crenshaw 1972; Wada and Fujinuki 1976; Lécuyer et al. 2004; Ip et al. 2006), rather than the outer EPF (Lorens 1978). The inner EPF produces the inner shell layer, and geochemical studies have concentrated on the outer shell layer for better time-resolved records (see Vander Putten et al. 2000). Outer EPF fluids have not been sampled for isotopes, although there is one report of oxygen isotopes from inner EPF (Lécuyer et al. 2004). Nevertheless, Gillikin et al. (2005a) found similar $\delta^{13}C$ and $\delta^{18}O$ in the inner and outer shell regions. Rapid calcification from the EPF indicates that it is significantly supersaturated with respect to $CaCO_3$. Hence, mollusks presumably add Ca^{2+} or $CO_3^{=}$, or both to the EPF.

Studies have observed only minor differences between inner EPF fluids, hemolymph fluids, and ambient seawater with respect to Ca^{2+} levels, and ratios of Ca^{2+} to Mg^{2+}, Sr^{2+}, and Ba^{2+} (Wada and Fujinuki 1976; Lorrain et al. 2004a; Gillikin 2005; Gillikin et al. 2006a). This seems consistent with passive conduction of ions to the calcification site, perhaps by pericellular routes through the mantle,

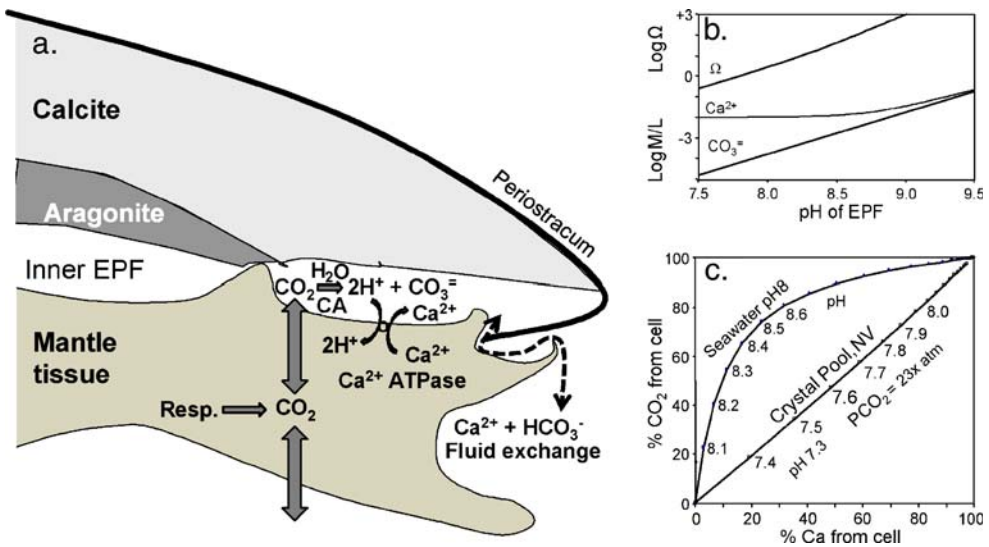

Fig. 1 **a** Cross section through a mussel shell (morphology from Vander Putten et al. 2000), showing likely transport routes for calcium and inorganic carbon. **b** Model depicting Ca^{2+} and $CO_3^{=}$ concentrations, and aragonite saturation level Ω in seawater, modified by $Ca^{2+}/2H^+$ exchange and CO_2 dissolution. $CO_3^{=}=K_1K_2[CO_2]/\{H^+\}^2$, Ca^{2+} calculated from alkalinity. **c** Fractions of skeletal carbon supplied as CO_2 from mantle, and Ca^{2+} pumped from the mantle cells, calculated for seawater (pH 8, pCO_2=atmospheric), and for freshwater Crystal Pool NV (pH 7.3, pCO_2=23×atmospheric). The balance of skeletal carbon and calcium presumably derives from fluid exchange with ambient waters, by leakage around the edge of the shell, or through mantle tissues. Based on the model of Cohen and McConnaughey (2003)

or through the periostracum (Fig. 1a). Ca^{2+} likewise seems to be only slightly elevated at the calcification site in corals (Al-Horani et al. 2003a, b), and the calcareous alga *Chara* (McConnaughey and Falk 1991). If high Ca^{2+} concentrations do not cause high $CaCO_3$ supersaturations, then $CO_3^{=}$ accumulations presumably do.

Alkalinization can elevate $CO_3^{=}$ levels. Bivalves raise pH in the EPF under calcifying conditions (e.g., Crenshaw and Neff 1969), and in the giant clam *Tridacna squamosa*, the inner EPF is more alkaline (pH~7.8) than in the clam tissues (pH~7.4–7.5; Ip et al. 2006). $CO_3^{=}$ potentially derives from several sources. HCO_3^{-} may enter the EPF by fluid exchange around the periostracum (Fig. 1a), and by transport through the mantle. It will then be deprotonated in the alkaline EPF to yield $CO_3^{=}$. CO_2 will also diffuse from the mantle tissues into the EPF, and react with H_2O and OH^{-} to produce $CO_3^{=}$. Biological membranes are highly permeable to CO_2 (Gutknecht et al. 1977), so some CO_2 contribution appears inevitable.

At chemical equilibrium, $CO_3^{=}$ increases linearly with CO_2, and inversely with the square of proton activity: $\{CO_3^{=}\}=K_1K_2\{CO_2\}/\{H\}^2$, where K_1 and K_2 are the first and second ionization constants of CO_2 (Fig. 1b). Consequently, if the EPF and adjacent tissues are in CO_2 equilibrium, $CO_3^{=}$ will concentrate in the alkaline EPF by the factor $\left\{CO_3^{=}\right\}_{EPF}\Big/\left\{CO_3^{=}\right\}_{Tissue} = 10\text{^}[2(pH_{EPF}-pH_{Tissue})]$. For the *Tridacna* pH data given above, the EPF might concentrate $CO_3^{=}$ by factors of 4–6 compared to adjacent tissues.

Several known ion transporters could theoretically extract protons from the calcification site, of which Ca^{2+} ATPase is the ion pump most frequently associated with biological calcification. Fan et al. (2007) have localized this enzyme on molluscan calcifying epithelia. Ca^{2+} ATPase comes in numerous versions. At least two mammalian isolates expel Ca^{2+} from the cell in exchange for $2H^{+}$ (Niggli et al. 1982; Dixon and Haynes 1989). ATP-driven $Ca^{2+}/2H^{+}$ exchange also appears to raise pH at the calcifying surfaces of the alga *Chara* (McConnaughey and Falk 1991). Hence, Ca^{2+} ATPase would raise the pH of the EPF, while also adding Ca^{2+}. As pH increases, CO_2 converts to HCO_3^{-}, and the reduction of CO_2 sets up a diffusion gradient that brings in more CO_2 into the EPF. With modest pH elevations, this CO_2 influx becomes the driving factor behind $CO_3^{=}$ accumulation (Fig. 1b), the major carbon input to the skeleton (Fig. 1c), and the major cause of $CaCO_3$ supersaturation (Cohen and McConnaughey 2003).

From the limited data currently available, mollusks appear to alkalinize the inner EPF by perhaps 0.5 pH units (e.g., Crenshaw and Neff 1969; Ip et al. 2006). For comparison, some foraminifers achieve >0.5 pH units alkalinization (Erez 2003), corals >1 pH units (Al-Horani et al. 2003a, b), and the calcareous alga *Chara* in low-Ca^{2+} media >2 pH units (McConnaughey and Falk 1991). The rate of CO_2 hydroxylation ($CO_2+OH^{-}\rightarrow HCO_3^{-}$) increases linearly with OH^{-}, and therefore exponentially with pH. Thus, mild alkalinization results in slow HCO_3^{-} production. Mollusks apparently accelerate the reaction using catalysis by the enzyme carbonic anhydrase (CA). Miyamoto et al. (1996, 2005) found that the mollusk shell protein nacrein, which acts as a negative regulator of calcification, contains a CA domain. Coupling CA to the calcification inhibitor nacrein may improve control over how, and where crystals grow. Bivalves often produce ordered, dense, plate-like, fracture-resistant nacreous crystals, unlike the fans of aragonite needles in corals.

The hydration, and especially hydroxylation of CO_2 at the alkaline calcification site underlies McConnaughey's (1989, 2003) 'kinetic' explanation for sub-equilibrium ^{13}C and ^{18}O levels in coral skeletons (Fig. 2a), and in many calcareous algae. Isotopic features reminiscent of kinetic effects, including positive $\delta^{13}C$–$\delta^{18}O$ correlations, and heavy isotope depletions compared to apparent equilibrium, occasionally show up mollusks, such as fossil belemnites (Spaeth et al. 1971). Nevertheless, mollusks generally do not show kinetic isotope effects. This appears to result from the relatively mild alkalinization of the calcification site and use of CA, which reduce the importance of CO_2 hydroxylation and accelerate isotopic equilibration.

Mollusks therefore appear to use fairly conventional calcification physiology: they pump protons from the calcification site, largely through $Ca^{2+}/2H^{+}$ exchange catalyzed by Ca^{2+} ATPase. The alkaline fluid then absorbs CO_2, and accumulates $CO_3^{=}$. This is compatible with additional ion transport, including leakage from the hemolymph, or fluid exchange around the periostracum. The chemical modeling in Fig. 1b, c assumes that the EPF initially contains environmental fluids. In Ca^{2+}-rich media such as seawater, fluid exchange can supply most of the Ca^{2+} to the shell, using less energy than Ca^{2+} pumping, as well as all of the other chemical constituents of ambient waters (Carré et al. 2006). But fluid exchange does not increase the $CaCO_3$ saturation state above ambient levels, and that is why additional active ion transport, particularly proton transport, is important.

Carbon isotope mixtures for the shell

Calcification physiology provides multiple routes for environmental carbon to reach the calcification site. Respired carbon, derived from dietary organic carbon, will also contribute to the shell. A simple model (Eq. 1) treats the shell as an isotopic derivative of blood DIC, containing

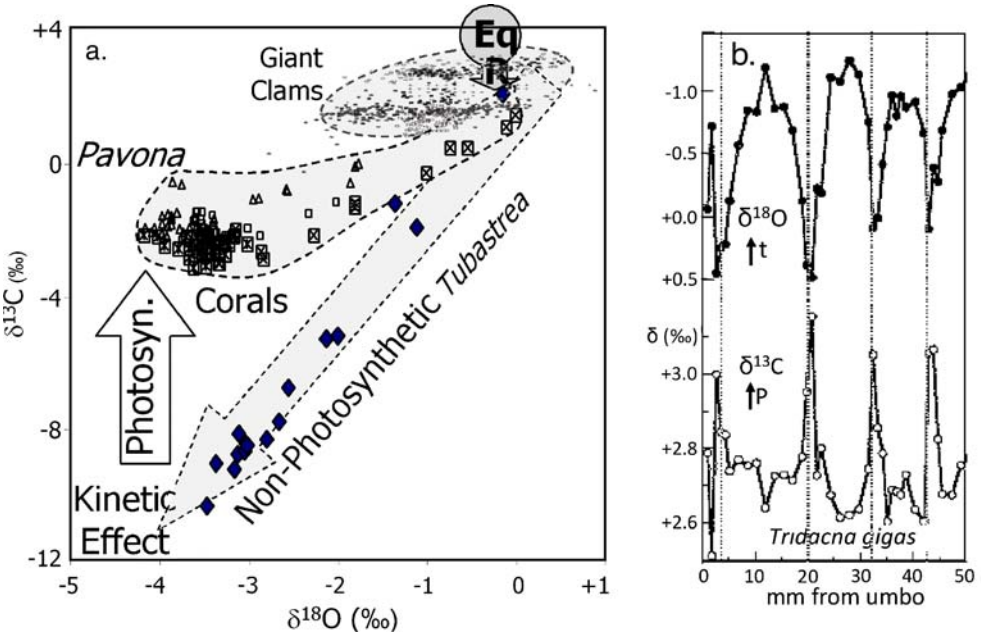

Fig. 2 Kinetic effects: strong in corals, absent in giant clams. **a** $\delta^{18}O$ and $\delta^{13}C$ in giant clams from the western Pacific (Watanabe et al. 2004), compared to photosynthetic (*Pavona*) and non-photosynthetic (*Tubastrea*) corals from Galapagos (McConnaughey 1989). Aragonite isotopic equilibrium is estimated for Galapagos; equilibrium $\delta^{18}O$ is lower for much of the western Pacific. **b** Four years of $\delta^{18}O$ and $\delta^{13}C$ for a fossil giant clam from Japan (Watanabe et al. 2004). Both isotopes approach isotopic equilibrium. Note winter $\delta^{13}C$ spikes and that $\delta^{18}O$ scale is inverted in **b** (also, t=temperature and p=possible photosynthesis)

a mixture of R parts respired carbon, and $(1-R)$ parts of environmental carbon:

$$R\delta^{13}C_{Org} + (1-R)\delta^{13}C_{Ambient} = \delta^{13}C_{Blood\ DIC} \quad (1)$$
$$= \delta^{13}C_{Shell} - \Delta$$

where Δ is the isotopic fractionation between blood DIC and the shell, and $\delta^{13}C_{Org}$ and $\delta^{13}C_{Ambient}$ the isotopic signatures of respired carbon and ambient carbon (air or DIC), respectively.

Even this simple model contains a surprising number of pitfalls. DIC speciation is deliberately unspecified. McConnaughey et al. (1997) equated $\delta^{13}C_{Ambient}$ with the $\delta^{13}C$ of ambient DIC, but CO_2 diffuses much faster than HCO_3^- across biological membranes (Gutknecht et al. 1977), and CO_2 often appears to supply much of the carbon for biological calcification (Fig. 1c; McConnaughey 2003; Cohen and McConnaughey 2003). It might then be reasonable to relate all isotopic fractionations to molecular CO_2. Nor is it clear when the final term δ should represent an equilibrium fractionation. Clearly it is not when strong 'kinetic' isotope effects are present. Finally, blood DIC may become ^{13}C enriched compared to the mixture given above. This is because CO_2–HCO_3^- equilibration in the blood will preferentially concentrate ^{12}C in the CO_2, and ^{13}C in the HCO_3^-. As the animal circulates blood through its lung or gill, the isotopically light CO_2 will preferentially escape, leaving isotopically heavy HCO_3^- in the blood.

A more complete model (Eq. 2), developed for fish otoliths (Solomon et al. 2006), examined intermediate carbon reservoirs including diet (D), ambient DIC (A), blood DIC (B), calcification site DIC (C), and finally the skeleton (S), where ^{13}C enrichment factors 'ε' are approximated by $\varepsilon_{X-Y} \approx \delta^{13}C_X - \delta^{13}C_Y$:

$$R\left(\delta^{13}C_D + \varepsilon_{B-D}\right) + (1-R)\left(\delta^{13}C_A + \varepsilon_{B-A}\right) \quad (2)$$
$$= \delta^{13}C_S - \varepsilon_{S-C} - \varepsilon_{C-B}$$

Isotopic estimates of R must ultimately agree with the basic physiology. To a first approximation, CO_2 fluxes across biological membranes are passive and proportional to CO_2 levels. The outward CO_2 flux (J_{Out}) is therefore proportional to blood pCO_2: $J_{Out} \propto pCO_{2Blood}$. Similarly, the passive inward CO_2 flux (J_{In}) is proportional to the ambient pCO_2 level: $J_{In} \propto pCO_{2Ambient}$. The difference between these fluxes equals net respiration, and the fraction R of respired CO_2 in the blood should equal (Eq. 3):

$$R = (J_{Out} - J_{In})/J_{Out} = 1 - J_{In}/J_{Out} = 1 - pCO_{2Ambient}/pCO_{2Blood} \quad (3)$$

Net CO_2 fluxes are furthermore approximately equal and opposite to O_2 fluxes, due to the net stoichiometry of respiration. For respiration of a carbohydrate, this can be simplistically represented as $CH_2O+O_2=CO_2+H_2O$. It should therefore be possible to relate CO_2 fluxes to O_2 fluxes, and ultimately to relate this to blood $\delta^{13}C$, and ultimately to shell $\delta^{13}C$.

Shell $\delta^{13}C$ values will, however, enjoy only limited use in geochemical, ecological, and archaeological studies if detailed assessments of all such factors must be undertaken in every instance. On the other hand, if broad generalizations apply to large parts of nature, then shell $\delta^{13}C$ may indeed become an informative parameter.

Land snails build their carbonates mainly from respired CO_2

Land snails generally have internal CO_2 levels considerably higher than the CO_2 levels in ambient air. The partial pressure of CO_2 (pCO_2) in the hemolymph (blood) of the land snail *Helix*, for example, is more than 30 times normal atmospheric (Michaelidis et al. 1999, 2007). By Eq. 3, the fraction of respired CO_2 in the blood of a land snail should therefore be $R=1-1/30=97\%$. Respired CO_2 should then comprise a similar fraction of skeletal carbonates. A comprehensive analysis by Balakrishnan and Yapp (2004) likewise concludes that the pCO_2 ratio between the snail and its environment affects shell incorporation of respired CO_2.

Isotopic data from the shells of land snails (Fig. 3) confirm the dominance of respired CO_2. Shell $\delta^{13}C$ more or less parallels the $\delta^{13}C$ of snail diet or shell organic matter, offset by anywhere from 8–19‰. The offsets are greatest when organics diverge most strongly from atmospheric CO_2, suggesting that atmospheric CO_2 does contribute to shell carbon. Simple graphical analysis suggests the magnitude of R. The slope of the shell vs. organic $\delta^{13}C$ regression line should equal 1 if respired CO_2 makes up 100% of shell carbon, or zero if respired CO_2 makes no contribution to the shell. Stott (2002) calculates a slope of 0.74 for *Helix* aragonite $\delta^{13}C$ plotted against diet $\delta^{13}C$, and a slope of 0.95 plotted against soft tissue $\delta^{13}C$. Accordingly, 74–95% of shell carbon may derive from respiration. Similarly, *Rabdotus* snails analyzed by Goodfriend and Ellis (2002) yield a slope of 0.78, plotted against shell organics.

The $\delta^{13}C$ enrichments in snail shells, compared to diet, result partly from the loss of isotopically light CO_2 during respiratory gas exchange. Respired CO_2 reacts internally

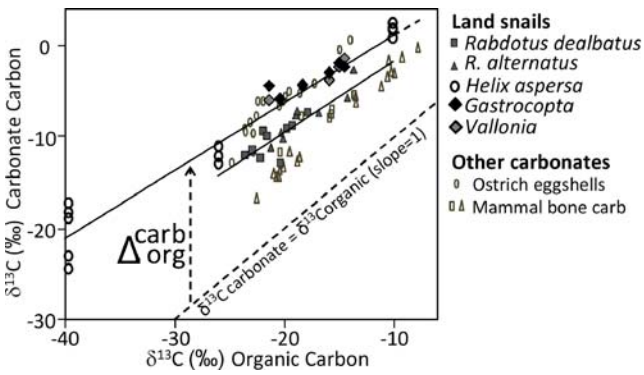

Fig. 3 $\delta^{13}C$ in shell aragonite vs. shell organic matter, or diet of land snails. *Helix aspersa* vs. food (Stott 2002). *Rabdotus* spp. vs. shell organics (Goodfriend and Ellis 2002). *Vallonia* and *Gastrocopta* vs. litter organics (Balakrishnan and Yapp 2004). Ostrich eggshells and mammal bone carbonates for comparison. *Regression lines* for *Helix* and *Rabdotus* spp

with water to form HCO_3^-, which comprises >90% of the DIC at the pH of snail hemolymph fluids (7.75 in *Helix*; Michaelidis et al. 1999, 2007). HCO_3^- is about 8‰ heavier than molecular CO_2 at isotopic equilibrium at 20°C, $\varepsilon \approx \delta HCO_3^- - \delta CO_2 = 10.78 - 0.141$ (T°C) (Zhang et al. 1995). By conservation of isotopes, DIC retains the isotopic signature of respired organic matter, while molecular CO_2 is about 7‰ lighter, and HCO_3^- about 1‰ heavier than the respired food carbon.

Blood traversing the lung or gill then loses the isotopically lighter CO_2, and retains the isotopically heavier HCO_3^-. In humans, exhaled CO_2 is ^{13}C-depleted compared to blood DIC by about 5‰ (Dangin et al. 1999; Panteleev et al. 1999). This is close to the equilibrium fractionation for a body temperature of 37°C. The full equilibrium fractionation between exhaled CO_2 and retained HCO_3^- may not be expressed if blood substantially equilibrates with air during transit through the lung, and loses much of its HCO_3^-. The ^{13}C enrichment in blood DIC therefore depends on how thoroughly the blood loses CO_2, and hence on the blood–air pCO_2 gradient (Eq. 3). Each time blood recirculates through the lung or gill, it loses more of its isotopically light CO_2, and the remaining blood DIC becomes heavier than suggested by Eqs. 1 and 2. This heavier blood DIC then provides carbon for calcification.

The snail shell appears to precipitate close to isotopic equilibrium with blood DIC. This is easiest to see when food $\delta^{13}C \approx$ air $\delta^{13}C$, as all carbon sources entering the blood then have the same value. The $\delta^{13}C$ of atmospheric CO_2 is currently about −8.2‰, and is dropping about 0.03‰ per year. At isotopic steady state, CO_2 exiting the blood must have the same $\delta^{13}C$ value as CO_2 entering the blood, and HCO_3^- that is isotopically equilibrated with blood CO_2 will be 8‰ heavier, near −0.2‰. Aragonite precipitating in isotopic equilibrium with the blood will be about 2.7‰ heavier (Romanek et al. 1992), or around +2.5‰. This is typical of observed values. When dietary $\delta^{13}C$ is much lighter than atmospheric CO_2, however, the snail shell becomes as much as 19‰ heavier than diet, indicating that atmospheric CO_2 contributes significantly to the shell.

Other air-breathing animals produce isotopically similar carbonates. Bird eggshells (Von Shirnding et al. 1982) and mammalian bone carbonates (Sullivan and Krueger 1981; Schoeninger and DeNiro 1984; Lee-Thorp 2002) approximately line up with snail shells in Fig. 3, but with slopes closer to 1. This is consistent with higher internal pCO_2 levels in birds and mammals, usually exceeding 100 times ambient, yielding values of $R>99\%$ by Eq. 3.

CO_2 exchange between an animal and its environment is then a two-way process, and the complete isotopic balance includes atmospheric CO_2 that invades the blood during respiratory gas exchange. For a land snail, bird, or mammal, in which blood pCO_2 remains well above ambient, the

invading CO_2 flux is small compared to internal CO_2 generation through respiration, and only slightly affects blood or carbonate $\delta^{13}C$.

How are such carbonate $\delta^{13}C$ values useful? For land snails, carbonate $\delta^{13}C$ reflects diet. This might depend on the local mix of C3 and C4+CAM plants, which in turn reflects climate. Similar considerations apply to bird eggshells, and the bone carbonates of mammalian grazers and browsers. Marine and terrestrial plants can also be isotopically distinct, and these isotopic clues about primary producers are transmitted up the food chain (Fry and Sherr 1984), and to the skeletal parts of higher consumers.

Carbonate and organic phases of a shell provide similar isotopic insights into diet, for air-breathing animals. What advantages might there be in analyzing one phase, the other, or both? Stott (2002) concluded that land snail shell carbonates recorded diet somewhat more accurately than did shell organics. At a minimum, carbonates confirm information gleaned from organic phases, and the quality of sample materials. Potentially, the carbonates also permit faster and easier analysis than do organics, plus simultaneous information about oxygen isotopes. And with aquatic animals, as discussed below, the isotopic composition of the carbonate phase may provide information on the isotopic content of ambient DIC, while dietary information can be obtained from organics (O'Donnell et al. 2003).

Aquatic mollusks build their carbonates largely from ambient DIC

Aquatic mollusks tend to have lower internal pCO_2 levels than do land snails, so by Eq. 3, they should incorporate less respired CO_2 in their shells. Measured hemolymph CO_2 values for bivalves, expressed as the ratio of internal to external pCO_2 during periods of immersion in water, include 5.2 for the oyster *Crassostrea gigas* (Michaelidis et al. 2005), 6.6–6.9 for the freshwater clam *Corbicula fluminea* and the unionid *Carunculina texasensis* (Byrne and Dietz 1997), as low as ~2 in the unionid *Anodonta grandis* (Byrne and McMahon 1991), and 5.2–8.6 in the zebra mussel *Dreissena polymorpha* (Byrne and Dietz 2006). These values were usually obtained in non-feeding bivalves, and since bivalves feed with their gills, it is possible that feeding would increase gas exchange. Ambient pCO_2 levels were taken as atmospheric. With such caveats, Eq. 3 estimates of the fraction of respired CO_2 in the blood (and shell) as 40–90%. While still considerable, this is less than in air-breathing land snails, birds, and mammals, where Eq. 3 always yields values of R approaching 100%.

Equation 3 assumes that respiration elevates tissue CO_2 levels, but other causes such as acidification might contribute. Tissue or hemolymph pH are around 7.4–7.6 in oysters (Littlewood and Young 1994; Michaelidis et al. 2005) and giant clams (Ip et al. 2006). This is about half a pH unit below ambient seawater. If a bivalve internalized and acidified seawater by half a pH unit, it would triple the pCO_2 levels. Acidification may account for nearly as much of the elevation of blood pCO_2 as does respiratory CO_2 generation in marine bivalves.

Nor is CO_2 the only vehicle for inorganic carbon exchange in aquatic settings. CO_2 diffuses across biological membranes far faster than HCO_3^- or $CO_3^=$ (Gutknecht et al. 1977), but HCO_3^- might be >100 times more abundant. Membranes often transport anions, too, and probably more significantly, fluid pathways between the EPF and ambient waters probably exist, both around the periostracum (Fig. 1a), and between cells of the mantle tissues. Such fluid pathways likely account for the chemical resemblance between inner EPF fluids and ambient waters. Such pathways will increase the amount of DIC exchange between the EPF and the ambient environment, and decrease the respired CO_2 fraction of shell carbon.

The multiple forms of DIC in water (CO_2, HCO_3^-, and $CO_3^=$), and their distinct isotopic compositions complicate isotopic calculations of R. The following discussion compares shell $\delta^{13}C$ with total DIC. This choice produces consistent results when R is calculated from ^{13}C (by Eq. 1) and ^{14}C datasets, while using molecular CO_2 would sometimes produce negative values of R. Internal DIC will also approach isotopic equilibrium with external DIC in aquatic animals due to the addition of HCO_3^- exchange, and the reduced CO_2 gradient.

The isotopic evidence regarding shell incorporation of respired CO_2 in aquatic mollusks is mixed and controversial. Early observational studies generally considered ambient DIC as the primary carbon source for shell formation (e.g., Craig 1953; Keith et al. 1964; Mook 1971). Fritz and Poplawski (1974) raised freshwater aquatic snails in water of which the DIC was isotopically modified to produce a range of $\delta^{13}C$ values. The resulting snail shells isotopically resembled DIC (Fig. 4 inset), not snail foods. Paull et al. (1989) found that shells of abyssal methanotrophic mussels had ^{13}C and ^{14}C contents similar to ambient DIC, rather than clam tissues or the methane (Fig. 4). McConnaughey et al. (1997) estimated R from both ^{13}C and ^{14}C data at around 10%. Gillikin et al. (2006b) also estimated R at 10% or less for the estuarine mussel, *Mytilus edulis*, as did Lorrain et al. (2004b) in the scallop *Pecten maximus*.

Other marine invertebrates yield similar results. Griffin et al. (1989) found that abyssal corals produce skeletons of which the $\Delta^{14}C$ content reflects abyssal DIC, not the $\Delta^{14}C$ content of coral tissues or likely foods. Adkins et al. (2003) confirmed this, and suggested that abyssal corals might incorporate 4–10% respired CO_2 into their skeletons. Spero

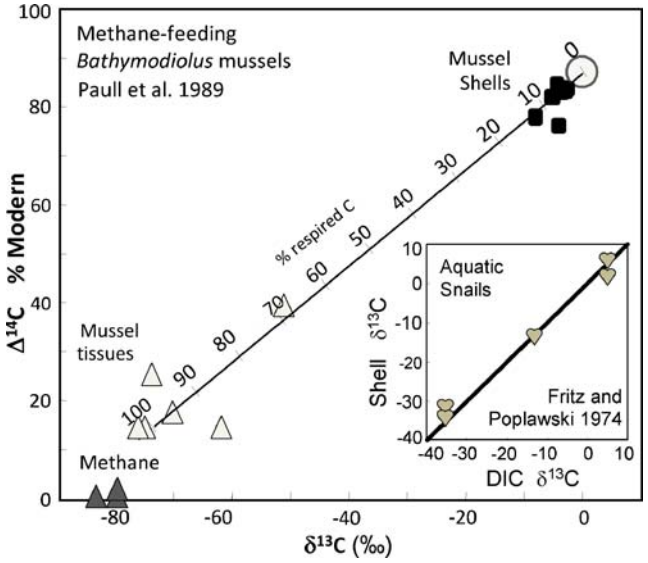

Fig. 4 $\delta^{13}C$ and ^{14}C in *Bathymodiolus* mussels from an abyssal methane seep, analyzed by Paull et al. (1989). Mussel shells appear to be built mainly from ambient DIC, while tissue carbon derives mainly from methane. *Inset*: shell $\delta^{13}C \approx$ DIC $\delta^{13}C$ in aquatic snails; experiment by Fritz and Poplawski (1974)

and Lea (1993) concluded that foraminifer shells contain about 8% respired CO_2. For the estuarine crab *Callinectes sapidus*, carapace $\delta^{13}C$ was ca. −3.5‰ in seawater, where $\delta^{13}C_{DIC}$ was ~0‰, but in the isotopically lighter Hudson River, carapace $\delta^{13}C$ was ca. −7‰ (Gillikin, unpublished data). Environmental DIC therefore appears to control shell $\delta^{13}C$, and *R* appears to be around 30%.

Several studies have, however, suggested higher values of *R* (e.g., Dillaman and Ford 1982). For example, Tanaka et al. (1986) estimated $R>50\%$ for several species, based on ^{13}C and ^{14}C data. Nevertheless, there was little consistency between $\delta^{13}C$ and ^{14}C estimates, and little consistency between animals. Heterogeneous ^{14}C pollution of the estuary most likely jeopardized the ^{14}C estimates. Furthermore, different ways of calculating *R* from $\delta^{13}C$ data, and subsequent examinations of specimens of the same species have yielded estimates of *R* less than 10% (Gillikin et al. 2006b). Yet, Dettman et al. (1999) also found offsets from −1.3‰ up to −9‰ from equilibrium in freshwater mussels. However, they suggested that the largest disequilibrium may be associated with the hatching and brooding of young in the marsupial of the mussel.

Gillikin et al. (2007a) compared the $\delta^{13}C$ content of shells and hemolymph DIC for the estuarine clam *Mercenaria* (Fig. 5). Equation 1, with equilibrium fractionations compared to ambient DIC, estimates *R* at 8–37% in clam shells, with larger (older) clams yielding the highest *R* values. Hemolymph DIC yielded lower *R* values (0–10%), calculated as $R = \left(\delta^{13}C_{\text{Hemolymph DIC}} - \delta^{13}C_{\text{Ambient DIC}}\right)/\left(\delta^{13}C_{\text{Organic}} - \delta^{13}C_{\text{Ambient DIC}}\right)$. Larger clams again yielded larger values of hemolymph *R* than did smaller clams, but less than a third of the *R* values calculated from the shells.

Contrary to equilibrium fractionations, *Mercenaria* shells are then ^{13}C depleted compared to hemolymph DIC. This might suggest a 'kinetic' effect, but that seems improbable—among other reasons, the shells are close to $\delta^{18}O$ equilibrium with ambient water. Or perhaps sampling hemolymph DIC from the abductor muscle missed higher contributions from respired CO_2 in the mantle tissues. Although unlikely, it is also possible that sediment interstitial waters, with high concentrations of isotopically light DIC due to sediment respiration (McCorkle et al. 1985), may contribute significantly to the EPF and to the shell, but not much to the hemolymph. Fluid exchange around the edge of the shell, as shown in Fig. 1b, might produce this outcome. Decreased shell $\delta^{13}C$ in larger clams might then result from deeper burial in the sediments, or greater conductivity between the EPF and ambient waters. Various arguments can be raised for, and against the interstitial water scenario. For example, infaunal bivalves are sometimes isotopically lighter than are epifaunal bivalves (Krantz et al. 1987). However, an isotopic transect along a growth line of a single clam shell showed little variability (Kingston et al. 2008), suggesting that depth of burial is not relevant here (i.e., the part of the shell buried deepest in the sediment is similar to the section buried less deep). Only further research will settle the matter.

In summary, the hemolymph $\delta^{13}C$ data of Gillikin et al. (2007a) suggest that the clam shell does not necessarily precipitate in isotopic equilibrium with hemolymph DIC, and may be several ‰ lighter. If the $\delta^{13}C$ of hemolymph DIC reflects its contribution from respired CO_2, then respired CO_2 contributes less than 10% to hemolymph DIC, and therefore to the shell. For larger clams in particular, alternative isotopically light carbon sources and/or isotopic fractionations must then contribute significantly to the shells.

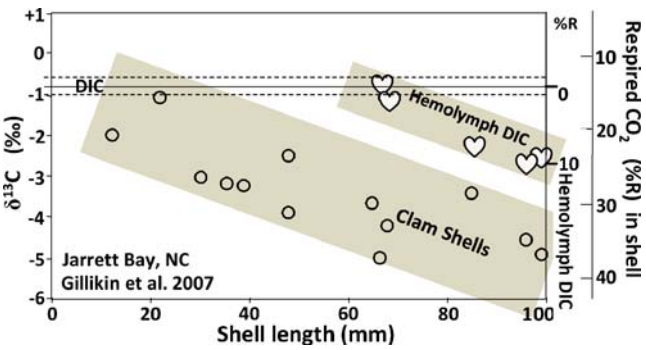

Fig. 5 $\delta^{13}C$ in clam shells (*circles*) compared to hemolymph DIC (*hearts*) and ambient DIC (−0.77‰) for *Mercenaria mercenaria*, analyzed by Gillikin et al. (2007a). Separate %R scales on the *right* for clam shells (Eq. 1), and for hemolymph DIC, $R=(\delta^{13}C_H-\delta^{13}C_A)/(\delta^{13}C_O-\delta^{13}C_A)$, where H, A, and O refer to hemolymph DIC, ambient DIC, and organics, respectively

Respiratory gas exchange model

McConnaughey et al. (1997) constructed a simple gas exchange model to accommodate both the high levels of respired CO_2 in the shells of terrestrial animals, and lower levels in aquatic animals. This model deals with respiratory gas exchange, but not the importation of ambient water containing DIC into the calcification site. CO_2 fluxes across respiratory membranes occur by passive diffusion, consistent with high CO_2 solubility in lipids, and high permeability through lipid bilayer membranes (Gutknecht et al. 1977). HCO_3^- and $CO_3^=$ remain behind in solution.

The model assumes that animals ventilate only enough to obtain the O_2 they need. This might underestimate respiratory gas exchange for bivalves, which use their gills for feeding. The animal also absorbs environmental CO_2 proportionally to the environmental CO_2/O_2 ratio. High ambient CO_2 levels increase CO_2 influx, diluting respired CO_2 and reducing the shell fraction R. Low ambient O_2 levels stimulate breathing, which increases the efflux of respired CO_2, and increases the influx of environmental CO_2. The ratio of environmental (1–R) to respired (R) carbon in the skeleton therefore depends on the ambient molar CO_2/O_2 ratio (Eq. 4):

$$(1-R)\Big/R = [CO_2]_A\Big/[O_2]_A * P_C/P_O * (1-[O_2]_B/[O_2]_A)^{-1} \quad (4)$$

Respiratory physiology is roughly parameterized. The ratio of CO_2 to O_2 permeabilities (P_C/P_O) depends mainly on gas solubilities in lipid-rich biological membranes, but is also affected by stagnant boundary layers of air or water that overlie the respiratory surfaces. Oxygen-binding proteins like hemoglobin, and efficient blood circulation, speed oxygen transport to the tissues and allow animals to tolerate low ratios of blood to ambient O_2 ($[O_2]_B/[O_2]_A$). With reduced respiratory gas exchange, their bodies accumulate more respired CO_2.

Air contains relatively little CO_2 (about 0.038% in 2008, less in pre-industrial times), but a lot of O_2 (20.95%). Terrestrial animals therefore absorb relatively little ambient CO_2. Higher CO_2/O_2 ratios in water alter the balance. CO_2 is more soluble than O_2 in water, and the CO_2/O_2 ratio in warm seawater, in equilibrium with the atmosphere, is about 30 times higher than in air.

Making some broad generalizations, calculated values of R range from >90% in air-breathing vertebrates, to about 10% in aquatic invertebrates (Fig. 6). Distinguishing 'vertebrates' from 'invertebrates' clearly oversimplifies biological O_2 and CO_2 transport, just as 'air' and 'water' simplifies CO_2/O_2 ratios especially in aquatic habitats. With such caveats, land snails and other air breathers build their carbonates mostly from respired CO_2, while aquatic invertebrates use mainly environmental CO_2. Land snails actually have blood O_2 levels lower than shown (Michaelidis et al. 1999, 2007), which should increase R. Pulmonate aquatic snails and other air-breathing aquatic mollusks, possibly including bivalves that 'gape' and ventilate with air at low tide (e.g., Littlewood and Young 1994), might also produce R values higher than for typical aquatic animals (McConnaughey et al. 1997).

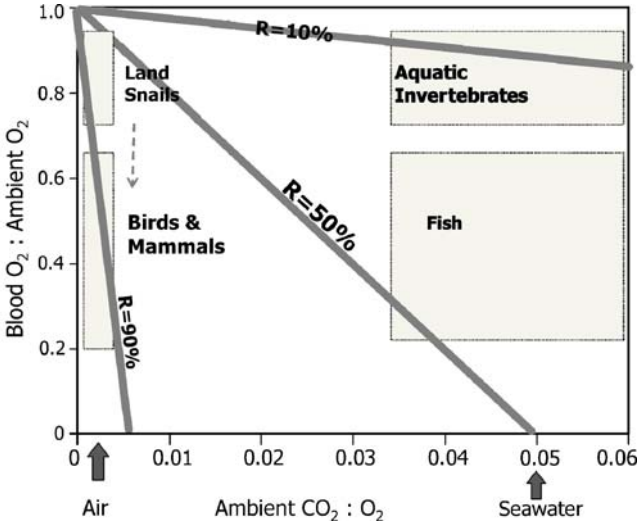

Fig. 6 Respired CO_2 in animal carbonates, based on respiratory gas exchange model

Fish occupy the ambiguous middle ground, and isotopic data suggest that their otoliths incorporate variable levels of respired CO_2 (Kalish 1991). Gauldie (1996), Schwarcz et al. (1998), Wurster and Patterson (2003), and others attribute trends in otolith $\delta^{13}C$ to metabolism. Sherwood and Rose (2003) found that morphological adaptations for active swimming correlated with decreased otolith $\delta^{13}C$. Solomon et al. (2006) used $\delta^{13}C$ data to suggest a range of 0–40% respired carbon in fish otoliths. Direct experiments yielded 17% in goldfish (Tohse and Mugiya 2004) and rainbow trout (Solomon et al. 2006). Solomon et al. (2006) conducted particularly thorough experiments in which the $\delta^{13}C$ of ambient DIC and food varied independently, and blood, endolymph, and otolith $\delta^{13}C$ were all measured. Their most consistent experiments, using food enriched to >100‰ $\delta^{13}C$ and unspiked water, suggested that respired CO_2 contributed 14%, 6%, and 12% of the carbon to the otoliths, blood, and endolymph, respectively.

Variations in ambient CO_2/O_2 ratios

The ambient CO_2/O_2 ratio is particularly important in the above model. Air has a CO_2/O_2 ratio of about 0.0018, and water equilibrated with the air has a ratio about 30 times higher. Temperature and salinity affect gas solubilities, and pH affects DIC speciation. An excess of ecosystem

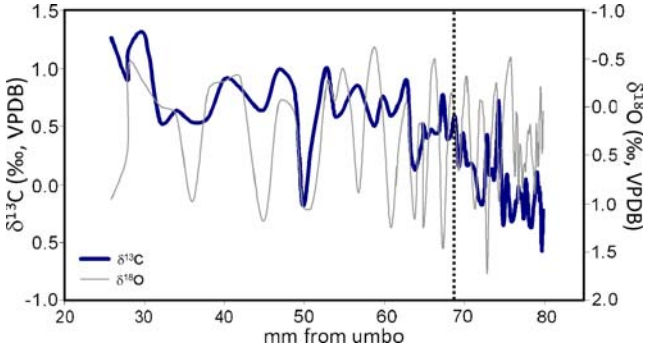

Fig. 7 An ~20 year old *Saxidomus giganteus* shell from Old Harbour, Kodiak Island, Alaska, USA. Using the inverted $\delta^{18}O$ data as a temperature proxy, it is evident that $\delta^{13}C$ values drop after the 9th year sampled (*dotted line*). $\delta^{18}O$ data are from Gillikin et al. (2005b); $\delta^{13}C$ data have not previously been published

respiration over photosynthesis can elevate aquatic CO_2/O_2 ratios to infinity, when O_2 is exhausted. Rapid breathing under low oxygen conditions should cause animals to exchange away more of their internal respired CO_2 for environmental CO_2. Conversely, intense photosynthesis reduces CO_2/O_2 ratios. Warm surface seawater (25°C, salinity=35) in equilibrium with the atmosphere contains about 10 μMol/Kg of molecular CO_2, and 206 μMol/Kg of O_2, giving it a CO_2/O_2 ratio of about 0.05. If photosynthesis consumes 25% of the DIC, pH rises to 8.6, CO_2 falls to 1.3 μMol/Kg, and O_2 rises to 620 μMol/Kg. The CO_2/O_2 ratio drops to 0.002, approaching the 0.0017 ratio of air. Aquatic invertebrates might then approach the *R* values typical of air breathers. But this requires strong photosynthesis.

The estuary where Gillikin et al. (2007a) collected *Mercenaria* (Fig. 5) had a molar CO_2/O_2 ratio of about 0.08, so $R<10\%$ for hemolymph DIC is reasonable, leaving the low $\delta^{13}C$ values in the larger clam shells enigmatic. Mussels (*Mytilus edulis*) from the Sheldt estuary in The Netherlands all produced shell *R* values less than ~10%, using Eq. 1 with equilibrium fractionations into the shell (Gillikin et al. 2006a; equilibrium fractionations according to Romanek et al. 1992). Mussels living near the marine end of the estuary, with CO_2/O_2 ratios ~0.06, yielded R~10%, while upstream mussels, with CO_2/O_2 ratios around 0.23, yielded R~3.5%. Thus, ambient CO_2/O_2 ratios may have influenced shell $\delta^{13}C$. This analysis does not, however, explain why the lowest apparent values of *R* (<2%) occurred in the mid-range of the estuary. Perhaps a combination of elevated CO_2/O_2 ratios coupled with higher metabolic rates caused by increased wave action at the marine site caused the higher *R* here (comparable to what Sherwood and Rose (2003) found in fish otoliths).

Some studies have suggested that respired CO_2 comprises the bulk of skeletal carbon even in high CO_2 environments. Kaandorp et al. (2003), for example, examined unionid bivalves from the Amazon river, where ambient pH was around 6. Mussel shells had $\delta^{13}C$ values near the $\delta^{13}C$ of ambient DIC. This appears compatible with fairly quantitative precipitation of (alkalinized) DIC, and with low values of *R*. Prosobranch aquatic snails living in CO_2-rich springs in Nevada also appear to produce low *R* values of 0–3% (Shanahan et al. 2005). However, the unionid bivalves analyzed by Dettman et al. (1999) were up to 4‰ more negative than DIC in a stream where pH levels would create low ambient CO_2 levels. This may be due to brooding of young in some unionid species. Geist et al. (2005) suggested that freshwater mussels shells were made mainly from metabolic carbon, but assumed the stream was in equilibrium with the atmosphere, an unlikely scenario (see Schöne et al. 2006 for more comments on this paper).

Isotopic variability

Isotopic fluctuations including downward trends in shell $\delta^{13}C$ with age are common in both marine and freshwater mollusks (Krantz et al. 1987; Klein et al. 1996; Dettman et al. 1999; Kennedy et al. 2001; Keller et al. 2002; Aucour et al. 2003; Elliot et al. 2003; Kaandorp et al. 2003; Lorrain et al. 2004b; Geist et al. 2005; Fenger et al. 2007; Gillikin et al. 2007a; Goewert et al. 2007; Kingston et al. 2008; Gillikin et al. 2007b). Figures 5 and 7 illustrate such features. Changing respiration may play a role (Lorrain et al. 2004b), as may changing calcification physiology, or hydrology.

Among the giant clams of the genus *Tridacna*, skeletal $\delta^{13}C$ sometimes remains fairly constant for decades, sometimes decreases gradually, sometimes decreases abruptly, and sometimes fluctuates around a stable baseline (Romanek and Grossman 1989; Aharon 1991; Watanabe et al. 2004). Figure 2b illustrates recurrent wintertime $\delta^{13}C$

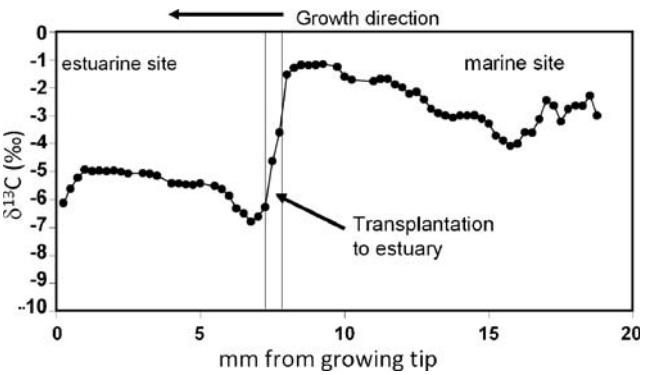

Fig. 8 Isotopic change following transplantation of a mussel, *Mytilus edulis*, from a marine site (salinity~35) to an estuarine site (salinity~20). Sampling may smooth the isotopic transition. Data are from Gillikin et al. (2006b)

spikes. Their causes remain unknown, but by analogy to photosynthetic corals (McConnaughey 2003), higher skeletal $\delta^{13}C$ may result from stronger photosynthesis by the clam's symbiotic algae. If so, then increased wintertime photosynthesis might result from increased nutrient levels. However, symbiont photosynthesis clearly raises skeletal $\delta^{13}C$ more in corals than in giant clams. This may result partly from different geometries: DIC traverses the photosynthetic tissues on its way to the skeleton to a greater degree in corals than in giant clams.

Environmental monitoring using shell $\delta^{13}C$ values

Mollusks provide many opportunities for environmental monitoring. They are common and widespread, often live for decades or longer, calcify rapidly, generate lots of material for skeletal sampling, and can be dated by sclerochronological and isotopic techniques. Their $\delta^{18}O$ thermometers often approach 1°C accuracies (e.g., Dettman et al. 1999; Chauvaud et al. 2005; Wanamaker et al. 2007). Despite many complexities, shell $\delta^{13}C$ also reflects environmental conditions.

Shell $\delta^{13}C$ can, for example, provide a proxy for salinity. Fluvial DIC is often isotopically lighter than oceanic DIC, due to the input of CO_2 derived from the decomposition of terrestrial plants. As rivers enter the ocean, mollusk shells pick up the mixture of fluvial and marine DIC, and shell $\delta^{13}C$ reflects the mixture (e.g., Mook and Vogel 1968; Gillikin et al. 2006b). Figure 8, for example, shows a shift to lower shell $\delta^{13}C$ when a mussel was transplanted from marine into estuarine conditions. Shell $\delta^{18}O$ may also reflect freshwater inputs to the ocean, especially at higher latitudes, and together the two isotopes can provide a consistent picture of the mixing regime (for example, see Andrus and Rich 2008, this issue). If the waters differ greatly in DIC content, however, then plots of $\delta^{13}C$ versus $\delta^{18}O$ or salinity would show curvature (e.g., Fry 2002). Finally, by providing an estimate of freshwater input, shell $\delta^{13}C$ potentially provides a means of correcting paleotemperature estimates derived from $\delta^{18}O$, or at least provide an indication of $\delta^{18}O_{Water}$ changes.

Because mollusks seldom show strong kinetic isotope effects, mollusk shell $\delta^{13}C$ and $\delta^{18}O$ data can also be used to estimate isotopic equilibrium, and thereby calibrate non-equilibrium isotopic systems such as calcareous algae and corals.

Conclusions

Mollusks probably use much the same calcification physiology as corals and algae. The lack of kinetic effects in mollusks likely results from milder alkalinization, and the presence of carbonic anhydrase in the calcification site.

Shell carbon in land snails derives mainly from respired CO_2. Shell $\delta^{13}C$ is generally >10‰ higher than dietary carbon, however, probably due largely to the loss of isotopically light CO_2, and retention of isotopically heavy HCO_3^- during respiratory gas exchange.

Shell carbon in aquatic mollusks derives mainly from ambient DIC. This is partly because aquatic CO_2/O_2 ratios tend to be higher than those in air, causing aquatic animals to exchange away more of their respired CO_2 for environmental CO_2 during respiratory gas exchange. Environmental DIC might possibly also reach the calcification site through fluid pathways, including pericellular pathways through the mantle tissues, and gaps between the shell and the periostracum (see Hickson et al. 1999).

Shell carbon does not necessarily precipitate in isotopic equilibrium with hemolymph DIC. The causes remain uncertain, and need to be explored further.

The proper interpretation of shell $\delta^{13}C$ records depends on context. In some situations, shell $\delta^{13}C$ may provide a fairly straightforward recorder of salinity. In others, shell $\delta^{13}C$ may respond to variables such as the environmental CO_2/O_2 ratio, DIC content, or the animals' physiology.

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References

Adkins JF, Boyle EA, Curry WB, Lutringer A (2003) Stable isotopes in deep-sea corals and a new mechanism for "vital effects". Geochim Cosmochim Acta 67:1129–1143

Aharon P (1991) Recorders of reef environment histories: stable isotopes in corals, giant clams, and calcareous algae. Coral Reefs 10:71–90

Al-Horani FA, Al-Moghrabi SM, de Beer D (2003a) The mechanism of calcification and its relation to photosynthesis and respiration in the scleractinian coral *Galaxea fascicularis*. Mar Biol 142:419–426

Al-Horani FA, Al-Moghrabi SM, de Beer D (2003b) Microsensor study of photosynthesis and calcification in the scleractinian coral, *Galaxea fascicularis*. J Exp Mar Biol Ecol 288:1–15

Andrus CFT, Rich KW (2008) A preliminary assessment of oxygen isotope fractionation and growth increment periodicity in the estuarine clam *Rangia cuneata*. In: Gröcke DR, Gillikin DP (eds) Advances in mollusc sclerochronology and sclerochemistry: tools for understanding climate and environment. Proc Pages, SSHRC Worksh, 11–13 July 2007, Parks Canada Discovery Centre, Ontario, Canada. Geo-Mar Lett SI 28 (in press)

Aucour AM, Sheppard SMF, Savoye R (2003) d^{13}C of fluvial mollusk shells (Rhône River): a proxy for dissolved inorganic carbon? Limnol Oceanogr 48:2186–2193

Balakrishnan M, Yapp CJ (2004) Flux balance models for the oxygen and carbon isotope compositions of land snail shells. Geochim Cosmochim Acta 68:2007–2024

Byrne RA, Dietz TH (1997) Ion transport and acid–base balance in freshwater bivalves. J Exp Biol 200:457–465

Byrne RA, Dietz TH (2006) Ionic and acid–base consequences of exposure to increased salinity in the Zebra Mussel, *Dreissena polymorpha*. Biol Bull 211:66–75

Byrne RA, McMahon BR (1991) Acid–base and ionic regulation, during and following emersion, in the freshwater bivalve, *Anodonta grandis simpsoniana* (Bivalvia: Unionidae). Biol Bull 181:289–297

Carré M, Bentaleb I, Bruguier O, Ordinola E, Barrett NT, Fontugne M (2006) Calcification rate influence on trace element concentrations in aragonitic bivalve shells: evidences and mechanisms. Geochim Cosmochim Acta 70:4906–4920

Chauvaud L, Lorrain A, Dunbar RB, Paulet Y-M, Thouzeau G, Jean F, Guarini J-M, Mucciarone D (2005) The shell of the Great Scallop *Pecten maximus* as a high frequency archive of paleoenvironmental change. Geochem Geophys Geosys 6:Q08001 doi:10.1029/2004GC000890

Cohen AL, McConnaughey TA (2003) Geochemical perspectives on coral mineralization. In: Dove PM, De Yoreo JJ, Weiner S (eds) Biomineralization. Rev Miner Biochem 54:151–187

Craig H (1953) The geochemistry of stable carbon isotopes. Geochim Cosmochim Acta 3:53–92

Crenshaw MA (1972) Inorganic composition of molluscan extrapallial fluid. Biol Bull 143:506–512

Crenshaw MA, Neff JM (1969) Decalcification at the mantle—shell interface in molluscs. Am Zool 9:881–885

Dangin M, Desport JC, Gachon P, Beaufrère B (1999) Rapid and accurate $^{13}CO_2$ isotopic measurement in whole blood: comparison with expired gas. Am J Physiol Endocrinol Metab 276:212–216

Dettman DL, Reische AK, Lohmann KC (1999) Controls on the stable isotope composition of seasonal growth bands in aragonitic freshwater bivalves (Unionidae). Geochim Cosmochim Acta 63:1049–1057

Dillaman RM, Ford SE (1982) Measurement of calcium-carbonate deposition in mollusks by controlled etching of radioactively labeled shells. Mar Biol 66:133–143

Dixon DA, Haynes DH (1989) Ca^{2+} pumping ATPase of cardiac sarcolemma is insensitive to membrane potential produced by K^+ and Cl^- gradients but requires a source of counter-transportable H^+. J Membr Biol 112:169–183

Elliot M, deMenocal PB, Linsley BK, Howe SS (2003) Environmental controls on the stable isotopic composition of *Mercenaria mercenaria*: potential application to paleoenvironmental studies. Geochem Geophys Geosys 4:1056 doi:10.1029/2002GC000425

Emiliani C (1954) Temperatures of Pacific bottom waters and polar superficial waters during the Tertiary. Science 119:853–855

Erez J (2003) The source of ions for biomineralization in foraminifera and their implications for paleoceanographic proxies. In: Dove PM, De Yoreo JJ, Weiner S (eds) Biomineralization. Rev Miner Biochem 54:151–187

Fan W, Li C, Li S, Feng Q, Xie L, Zhang R (2007) Cloning, characterization, and expression patterns of three sarco/endoplasmic reticulum Ca^{2+}-ATPase isoforms from pearl oyster (*Pinctada fucata*). Acta Biochim Biophys Sin 39:722–730

Fenger T, Surge D, Schöne BR, Milner N (2007) Sclerochronology and geochemical variation in limpet shells (*Patella vulgata*): a new archive to reconstruct coastal sea surface temperature. Geochem Geophys Geosys 8:Q07001 doi:10.1029/2006GC001488

Fritz P, Poplawski S (1974) ^{18}O and ^{13}C in the shells of freshwater molluscs and their environments. Earth Planet Sci Lett 24:91–98

Fry B (2002) Conservative mixing of stable isotopes across estuarine salinity gradients: a conceptual framework for monitoring watershed influences on downstream fisheries production. Estuaries 25:264–271

Fry B, Sherr EB (1984) $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Cont Mar Sci 27:13–47

Gauldie RW (1996) Biological factors controlling the carbon isotope record in fish otoliths: principles and evidence. Comp Biochem Physiol B 115:201–208

Geist J, Auerswald K, Boom A (2005) Stable carbon isotopes in freshwater mussel shells: environmental record or marker for metabolic activity? Geochim Cosmochim Acta 69:3545–3554

Gillikin DP (2005) Geochemistry of marine bivalve shells: the potential for paleoenvironmental reconstruction. Ph.D. thesis, Vrije Universiteit Brussel, Belgium

Gillikin DP, De Ridder F, Ulens H, Elskens M, Keppens E, Baeyens W, Dehairs F (2005a) Assessing the reproducibility and reliability of estuarine bivalve shells (*Saxidomus giganteus*) for sea surface temperature reconstruction: implications for paleoclimate studies. Palaeogeogr Palaeoclimatol Palaeoecol 228:70–85

Gillikin DP, Lorrain A, Navez J, Taylor JW, André L, Keppens E, Baeyens W, Dehairs F (2005b) Strong biological controls on Sr/Ca ratios in aragonitic marine bivalve shells. Geochem Geophys Geosys 6:Q05009 doi:10.1029/2004GC000874

Gillikin DP, Dehairs F, Lorrain A, Steenmans D, Baeyens W, André L (2006a) Barium uptake into the shells of the common mussel (*Mytilus edulis*) and the potential for estuarine paleo-chemistry reconstruction. Geochim Cosmochim Acta 70:395–407

Gillikin DP, Lorrain A, Bouillon S, Willenz P, Dehairs F (2006b) Shell carbon isotopic composition of *Mytilus edulis* shells: relation to metabolism, salinity, $\delta^{13}C_{DIC}$ and phytoplankton. Org Geochem 37:1371–1382

Gillikin DP, Lorrain A, Meng L, Dehairs F (2007a) A large metabolic carbon contribution to the $\delta^{13}C$ record in marine aragonitic bivalve shells. Geochim Cosmochim Acta 71:2936–2946

Gillikin DP, Hutchinson K, Kumai Y (2007b) Ontogenic increase of metabolic carbon in freshwater mussel shells. Eos Trans. AGU, 88(52), Fall Meet. Suppl., Abstract B31D-0615

Goewert A, Surge D, Carpenter SJ, Downing J (2007) Oxygen and carbon isotope composition of *Lampsilis cardium* (Unionidae) from two streams in agricultural watersheds, Iowa. Palaeogeogr Palaeoclimatol Palaeoecol 252:637–648

Goodfriend GA, Ellis GL (2002) Stable carbon and oxygen isotopic variations in modern *Rabdotus* land snail shells in the southern Great Plains, USA, and their relation to environment. Geochim Cosmochim Acta 66:1987–2002

Griffin S, Griffin E, Druffel RM (1989) Sources of carbon to deep-sea corals. Radiocarbon 31:533–543

Gutknecht J, Bisson MJ, Tosteson FC (1977) Diffusion of carbon dioxide through lipid bilayer membranes: effects of carbonic anhydrase, bicarbonate, and unstirred layers. J Gen Physiol 55:1–17

Hickson JA, Johnson ALA, Heaton THE, Balson PS (1999) The shell of the Queen Scallop *Aequipecten opercularisis* (L.) as a promising tool for palaeoenvironmental reconstruction: evidence and reasons for equilibrium stable-isotope incorporation. Palaeogeogr Palaeoclimatol Palaeoecol 154:325–337

Ip YK, Loong AM, Kiong KC, Wong WP, Chew SF, Reddy K, Sivalonganathan B, Ballantyne JS (2006) Light induces an increase in the pH of and a decrease in the ammonia concentration in the extrapallial fluid of the giant clam *Tridacna squamosa*. Physiol Biochem Zool 79:656–664

Kaandorp RJG, Vonhof HB, Del Busto C, Wesselingh FP, Ganssen GM, Marmól AE, Pittman LR, van Hinte JE (2003) Seasonal

stable isotope variations of the modern Amazonian freshwater bivalve *Anodontites trapesialis*. Palaeogeogr Palaeoclimatol Palaeoecol 194:339–354

Kalish JM (1991) ^{13}C and ^{18}O isotopic disequilibria in fish otoliths: metabolic and kinetic effects. Mar Ecol Prog Ser 75:191–203

Keith ML, Anderson GM, Eichler R (1964) Carbon and oxygen isotopic composition of mollusk shells from marine and freshwater environments. Geochim Cosmochim Acta 28:1757–1786

Keller N, Del Piero D, Longinelli A (2002) Isotopic composition, growth rates and biological behaviour of *Chamelea gallina* and *Callista chione* from the Gulf of Trieste (Italy). Mar Biol 140:9–15

Kennedy H, Richardson CA, Duarte CM, Kennedy DP (2001) Oxygen and carbon stable isotopic profiles of the fan mussel, *Pinna nobilis*, and reconstruction of sea surface temperatures in the Mediterranean. Mar Biol 139:1115–1124

Kingston A, Gröcke DR, Burchell M (2008) A multi-axial growth analysis of stable isotopes in the modern shell of *Saxidomus gigantea*: implications for sclerochronology studies. Geochem Geophys Geosyst 9:Q01007 doi:10.1029/2007GC001807

Klein RT, Lohmann KC, Thayer CW (1996) Sr/Ca and $^{13}C/^{12}C$ ratios in skeletal calcite of *Mytilus trossulus*: covariation with metabolic rate, salinity, and carbon isotopic composition of seawater. Geochim Cosmochim Acta 60:4207–4221

Krantz DE, Williams DF, Jones DS (1987) Ecological and paleoenvironmental information using stable isotope profiles from living and fossil mollusks. Palaeogeogr Palaeoclimatol Palaeoecol 58:249–266

Lécuyer C, Reynard B, Martineau F (2004) Stable isotope fractionation between mollusc shells and marine waters from Martinique Island. Chem Geol 213:293–305

Lee-Thorp J (2002) Two decades of progress towards understanding fossilization processes and isotopic signals in calcified mineral tissues. Archaeometry 44:435–446

Littlewood DTJ, Young RE (1994) The effect of air-gaping behavior on extrapallial fluid pH in the tropical oyster *Crassostrea rhizophorae*. Comp Biochem Physiol A Physiol 107:1–6

Lorens RB (1978) A study of biological and physiological controls on the trace metal content of calcite and aragonite. Ph.D. thesis, University of Rhode Island

Lorrain A, Gillikin DP, Paulet Y-M, Paillard C, Navez J, André L, Dehairs F, Baeyens W, CALMARs group (2004a) Toward a mechanistic understanding of trace element proxy incorporation in bivalve shells. In: Abstr Vol Int Paleo-environments Symp QRA2004, R Belgian Inst Nat Sci, Brussels, Belgium

Lorrain A, Paulet Y-M, Chauvaud L, Dunbar R, Mucciarone D, Fontugne M (2004b) $\delta^{13}C$ variation in scallop shells: increasing metabolic carbon contribution with body size? Geochim Cosmochim Acta 68:3509–3519

McConnaughey TA (1989) ^{13}C and ^{18}O isotopic disequilibrium in biological carbonates: II. In vitro simulation of kinetic isotope effects. Geochim Cosmochim Acta 53:163–171

McConnaughey TA (2003) Sub-equilibrium oxygen-18 and carbon-13 levels in biological carbonates: carbonate and kinetic models. Coral Reefs 22:316–327

McConnaughey TA, Falk RH (1991) Calcium-proton exchange during algal calcification. Biol Bull 180:185–195

McConnaughey TA, Burdett J, Whelan JF, Paull CK (1997) Carbon isotopes in biological carbonates: respiration and photosynthesis. Geochim Cosmochim Acta 61:611–622

McCorkle DC, Emerson SR, Quay PD (1985) Stable carbon isotopes in marine porewaters. Earth Planet Sci Lett 74:13–26

Michaelidis B, Rofalikou E, Grieshaber MK (1999) The effects of hypercapnia on force and rate of contraction and intracellular pH of perfused ventricles from the land snail *Helix lucorum* (L.). J Exp Biol 202:2993–3001

Michaelidis B, Haas D, Grieshaber MK (2005) Extracellular and intracellular acid-base status with regard to the energy metabolism in the oyster *Crassostrea gigas* during exposure to air. Physiol Biochem Zool 78:373–383

Michaelidis B, Vavoulidou D, Rousou J, Pörtner HO (2007) The potential role of CO_2 in the initiation and maintenance of estivation in the land snail *Helix lucorum*. Physiol Biochem Zool 80:113–124

Miyamoto H, Miyashita T, Okushima M, Nakano S, Morita T, Matsushiro A (1996) A carbonic anhydrase from the nacreous layer in oyster pearls. Proc Natl Acad Sci U S A 93:9657–9660

Miyamoto H, Miyoshi F, Kohno J (2005) The carbonic anhydrase domain protein nacrein is expressed in the epithelial cells of the mantle and acts as a negative regulator in calcification in the mollusc *Pinctada fucata*. Zool Sci 22:311–315

Mook WG (1971) Paleotemperatures and chlorinities from stable carbon and oxygen isotopes in shell carbonate. Palaeogeogr Palaeoclimatol Palaeoecol 9:245–264

Mook WG, Vogel JC (1968) Isotopic equilibrium between shells and their environment. Science 159:874–875

Niggli VE, Sigel E, Carafoli E (1982) The purified Ca^{2+} pump of the human erythrocyte membrane catalyzes an electroneutral $Ca^{2+}/2H^{+}$ exchange in reconstituted liposomal systems. J Biol Chem 257:2350–2356

O'Donnell TH, Macko SA, Chou J, Davis-Hartten KL, Wehmiller JF (2003) Analysis of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in organic matter from the biominerals of modern and fossil *Mercenaria* spp. Org Geochem 34:165–183

Panteleev N, Péronnet F, Hillaire-Marcel C, Lavoie C, Massicotte D (1999) Carbon isotope fractionation between blood and expired CO_2 at rest and exercise. Respir Physiol 116:77–83

Paull CK, Martens CS, Chanton JP, Neumann AC, Coston J, Jull AJT, Toolin LJ (1989) Old carbon in living organisms and young $CaCO_3$ cements from abyssal brine seeps. Nature 342:166–168

Romanek CS, Grossman EL (1989) Stable isotope profiles of *Tridacna maxima* as environmental indicators. Palaios 4:402–413

Romanek CS, Grossman EL, Morse JW (1992) Carbon isotopic fractionation in synthetic aragonite and calcite: effects of temperature and precipitation rate. Geochim Cosmochim Acta 56:419–430

Schoeninger MJ, DeNiro MJ (1984) Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochim Cosmochim Acta 48:625–639

Schöne BR, Rodland DL, Surge DM, Fiebig J, Gillikin DP, Baier SM, Goewert A (2006) Comment on "Stable carbon isotopes in freshwater mussel shells: environmental record or marker for metabolic activity?" by J. Geist et al. (2005). Geochim Cosmochim Acta 70:2658–2661

Schwarcz HP, Gao Y, Campana S, Browne D, Knyf M, Brand U (1998) Stable carbon isotope variations in otoliths of Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 55:1798–1806

Shanahan TM, Pigati JS, Dettman DL, Quade J (2005) Isotopic variability in the aragonite shells of freshwater gastropods living in springs with nearly constant temperature and isotopic composition. Geochim Cosmochim Acta 69:3949–3966

Sherwood GD, Rose GA (2003) Influence of swimming form on otolith $\delta^{13}C$ in marine fish. Mar Ecol Prog Ser 258:283–289

Solomon CT, Weber PK, Cech JJ Jr, Ingram BL, Conrad ME, Machavaram MV, Pogodina AR, Franklin RL (2006) Experimental determination of the sources of otolith carbon and associated isotopic fractionation. Can J Fish Aquat Sci 63:79–89

Spaeth C, Hoefs J, Vetter U (1971) Some aspects of isotopic composition of belemnites and related paleotemperatures. Geol Soc Am Bull 82:3139–3150

Spero HJ, Lea DW (1993) Does the carbon isotopic composition of planktonic foraminifera prey affect shell $\delta^{13}C$ values? Eos Trans AGU 74:183

Stott LD (2002) The influence of diet on the $\delta^{13}C$ of shell carbon in the pulmonate snail *Helix aspersa*. Earth Planet Sci Lett 195:249–259

Sullivan CH, Krueger HW (1981) Carbon isotope analysis of separate chemical phases in modem and fossil bone. Nature 292:333–335

Tanaka N, Monaghan MC, Rye DM (1986) Contribution of metabolic carbon to mollusk and barnacle shell carbonate. Nature 320:520–523

Tohse H, Mugiya Y (2004) Sources of carbonate in fish otoliths: incorporation from bicarbonate and glucose. In: Kobayashi I, Ozawa H (eds) Biomineralization: formation, diversity, evolution and application. Tokai University Press, Tokyo, pp 190–193

Vander Putten E, Dehairs F, Keppens E, Baeyens W (2000) High resolution distribution of trace elements in the calcite shell layer of modern *Mytilus edulis*: environmental and biological controls. Geochim Cosmochim Acta 64:997–1011

Von Shirnding Y, van der Merwe NJ, Vogel JC (1982) Influence of diet and age on carbon isotope ratios of ostrich eggshell. Archaeometry 24:3–20

Wada K, Fujinuki T (1976) Biomineralization in bivalve molluscs with emphasis on the chemical composition of the extrapallial fluid. In: Watabe N, Wilbur KM (eds) The mechanisms of mineralization in the invertebrates and plants. University of South Carolina Press, Columbia, SC, pp 175–190

Wanamaker AD, Kreutz KJ, Borns HW, Introne DS, Feindel S, Funder S, Rawson PD, Barber BJ (2007) Experimental determination of salinity, temperature, growth, and metabolic effects on shell isotope chemistry of *Mytilus edulis* collected from Maine and Greenland. Paleoceanography 22:PA2217 doi:10.1029/2006PA001352

Watanabe T, Suzuki A, Kawahata H, Kan H, Ogawa S (2004) A 60-year isotopic record from a mid-Holocene fossil giant clam (*Tridacna gigas*) in the Ryukyu Islands: physiological and paleoclimatic implications. Palaeogeogr Palaeoclimatol Palaeoecol 212:343–354

Wheeler AP (1992) Mechanisms of molluscan shell formation. In: Bonucci E (ed) Calcification in biological systems. CRC, Boca Raton, FL, pp 179–216

Wilbur KM, Saleuddin ASM (1983) Shell formation. In: Saleuddin ASM, Wilbur KM (eds) The Mollusca. Academic, New York, pp 235–287

Wurster CM, Patterson WP (2003) Metabolic rate of late Holocene freshwater fish: evidence from $\delta^{13}C$ values of otoliths. Paleobiology 29:492–505

Zhang J, Quay PD, Wilbur DO (1995) Carbon isotope fraction during gas-water exchange and dissolution of CO_2. Geochim Cosmochim Acta 59:107–114