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Isotopic variability in the aragonite shells of freshwater gastropods living in springs with nearly constant temperature and isotopic composition

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Abstract—We conducted a year-long, intensive monitoring program of live aquatic gastropods (*Helisoma duryi, Melanoides tuberculata, Physa virgata, Pyrgulopsis* sp., and *Tyronia* sp.) and their host springs in the Ash Meadows National Wildlife Refuge of southern Nevada. Our purpose was to constrain the degree of natural variation in the isotopic values of shell aragonite for gastropods living in near-constant conditions. Inter- and intraspecies variations, as well as within-shell variations, of $\delta^{18}O$ and $\delta^{13}C$ values for all taxa were larger than predicted based on variations in environmental conditions alone. This result suggests that different organisms growing in identical or nearly identical environmental conditions may not produce shells with equilibrium isotopic compositions and that these offsets from equilibrium may differ by small, but statistically significant amounts. For the gill-breathing, fully aquatic gastropods *M. tuberculata, Pyrgulopsis* sp., and *Tyronia* sp., the deviation of measured isotopic values compared to predicted values based on average environmental conditions were consistent with differences between taxa in the seasonal timing of shell growth. Measured values for the lung-breathing gastropods *H. duryi* and *P. virgata* were higher for $\delta^{18}O$ and lower for $\delta^{13}C$ than predicted at isotopic equilibrium, even when accounting for seasonality effects. We suggest that explaining the differences between the shell isotopic composition of lung- and gill-breathing snails requires a combination of both behavioral and physiologic factors. Our results illustrate the potential complexities of interpreting stable isotopic data from fossil gastropod shells even when environmental conditions are nearly constant, and place limitations on the paleoenvironmental deductions that can be made from the isotopic measurements on fossil gastropods. *Copyright © 2005 Elsevier Ltd*

1. INTRODUCTION

Stable isotopic analysis of microsampled biogenic carbonate, such as from corals, mollusks, and otoliths, has become an important tool for reconstructing past environmental and climatic conditions (e.g., Aharon and Chappell, 1983; Patterson et al., 1993; Boehm et al., 2000; Cole et al., 2000; Guilderson et al., 2001; Wurster and Patterson, 2001; Gasse, 2002; Brand et al., 2003). These biologic archives are particularly appealing because shell or skeletal aragonite is often deposited continually over the lifetime of the organisms, and isotopic and/or chemical compositions recorded in the carbonate can potentially be used to reconstruct past conditions at high resolution. Moreover, biogenic carbonates can survive thousands to millions of years of burial, and therefore data can be obtained for a variety of temporal and spatial scales (e.g., Dettman and Lohmann, 2000). The development of high-resolution proxy records is essential for understanding how climate has changed on annual to decadal timescales in the recent past.

Aquatic mollusks (marine and freshwater) are a potentially important source of paleoenvironmental information, but interpretation of their stable isotope composition requires an understanding of the impact of environmental variables on isotopic fractionation. For oxygen isotopes, the relationship between $\delta^{18}O_{water}$, $\delta^{18}O_{shell}$, and the temperature of carbonate precipitation must be well constrained. Similarly, the relationship between the $\delta^{13}C_{shell}$ and the $\delta^{13}C_{DIC}$ values of ambient waters must be known to use biogenic $\delta^{13}C$ values as indicators of paleoproductivity, paleo-pCO_2, or paleoecology. A number of previous researchers have attempted to quantify these relationships using both inorganically and biologically precipitated carbonates (Table 1). There remain, however, large discrepancies between estimates, and it is unclear which, if any, are applicable to aquatic mollusks. Furthermore, these organisms typically live in environments with highly variable environmental conditions, and it is uncertain how well this variability is recorded in the isotopic composition of their shells.

This study investigates the $\delta^{18}O$ and $\delta^{13}C$ values of biogenic aragonite for five species of freshwater gastropods collected live from two extant springs with nearly constant water temperature and $\delta^{18}O_{water}$ values. Measured isotopic values are compared with predicted isotopic equilibrium values using the empiric fractionation relationships for $\delta^{18}O$ in Table 1, and with the inorganic equilibrium relationship of Romanek et al., (1992) for $\delta^{13}C$. The results provide new insights into the magnitude of isotopic variation between and within mollusk species and their relationship to predicted equilibrium values. They yield constraints on the future use and interpretation of isotopic data from mollusks for reconstruction of past environmental conditions.

2. BACKGROUND

2.1. Mechanisms of Shell Construction in Gastropods

Before discussing the isotopic character of biologically precipitated carbonate, it is useful to review how they are formed.

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Table 1. Empirical estimates of the effect of temperature on oxygen isotope fractionation in aragonite and predicted values for Ash Meadows.

Reference	Experiment	Temp. [°C]	Relationship	Predicted equilibrium values mean $\delta^{18}O$ ‰*	
				Crystal pool	Point of rocks
McCrea (1950)	Inorganic synthesis	−1.2 to 31.8	$10^3 \ln \alpha = 16.26 \times 10^3 T^{-1} - 26.01$	−16.3 (−16.1, −16.5)	−16.4 (−16.2, −16.6)
Grossman and Ku (1986)	Stream unionids	2.6 to 22	$10^3 \ln \alpha = 18.07 \times 10^3 T^{-1} - 31.08$	−15.4 (−15.3, −15.6)	−15.6 (−15.4, −15.7)
Patterson (1993)	Lake fish otoliths	3.2 to 30.3	$10^3 \ln \alpha = 18.56 \times 10^3 T^{-1} - 33.49$	−16.2 (−16.0, −16.4)	−16.4 (−16.1, −16.5)
Thorrold et al., (1997)	Tank fish otoliths	18.2 to 25	$10^3 \ln \alpha = 18.56 \times 10^3 T^{-1} - 32.54$	−15.2 (−15.0, −15.5)	−15.4 (−15.2, −15.6)
White et al., (1999)	Tank mollusc (*L. peregra*)	8 to 24	$10^3 \ln \alpha = 16.74 \times 10^3 T^{-1} - 26.39$	−15.1 (−15.0, −15.3)	−15.2 (−15.0, −15.4)
Boehm et al., (2000)	Sclerosponges, forams, molluscs	3 to 28	$10^3 \ln \alpha = 18.45 \times 10^3 T^{-1} - 32.54$	−15.6 (−15.5, −15.8)	−15.8 (−15.6, −15.9)
Zhou and Zheng (2003)	Inorganic synthesis	0 to 70	$10^3 \ln \alpha = 20.44 \times 10^3 T^{-1} - 41.48$	−17.9 (−17.8, −18.2)	−18.2 (−17.9, −18.3)

* reported as: mean (minimum value, maximum value).

Shell carbonate is formed in the mantle tissue, a thin organ lining the inside of the shell (Wilbur and Saleuddin, 1983). Carbonate precipitation and crystalline formation occur within a thin zone of fluid located between the mantle and existing shell called the extrapallial fluid (EPF), which is isolated from the surrounding environment by the outer epithelium. Ca and other ions are supplied by direct uptake at the external body surface, through the gills and in some cases (*Lymnaea stagnalis*) by digestion (Wilbur and Saleuddin, 1983). Because of the low concentrations of Ca in freshwater environments, Ca may be provided by active transport, presumably via Ca-activated ATPases (Wilbur, 1964). Carbonate in the shell is derived from the mantle CO_2-bicarbonate pool, which in turn originates from either metabolic carbon or from the surrounding environment. Most carbon isotopic studies of aquatic mollusks have concluded that the metabolic source is less than 6%–10% of the total (McConnaughey et al., 1997). In contrast, the proportion is believed to be much higher for terrestrial snails (Goodfriend, 1992). The conversion of CO_2 to bicarbonate and then carbonate within the EPF is believed to be catalyzed by carbonic anhydrase (Wilbur, 1964). Carbonate crystals are bound into the shell structure by an organic matrix, which is formed by CO_2 fixation within the EPF (Wilbur, 1964).

The resultant shell material is composed of a thin outer periostracum that is organic, and 2–3 calcareous layers: the outer prismatic layer, the middle lamellate layer and the inner nacreous layer (the hypostracum). Both the prismatic and lamellate layers are composed almost entirely from calcium carbonate (either aragonite or calcite), but can be distinguished by differences in growth structure (i.e., in the prismatic layer the carbonate is deposited as vertical crystals bound by organic proteins). In the nacreous layer, the carbonate is deposited as thin lamellae interleaved with conchin (Brusca and Brusca, 1990).

2.2. $\delta^{18}O$ Fractionation in Carbonates

The theoretical basis for the $^{18}O/^{16}O$ thermometer was first established (Urey, 1947) and confirmed in the laboratory precipitation of inorganic carbonates (McCrea, 1950), and biologic carbonates (Epstein et al., 1953). Since then, oxygen isotopes have been used in numerous studies to reconstruct past climatic conditions. To do so, the relationship between the equilibrium $\delta^{18}O$ value of carbonate and the temperature of precipitation must be well constrained. However, estimated fractionation factors differ significantly from experimental measurements (McCrea, 1950; O'Neil et al., 1969; Tarutani et al., 1969; Kim and O'Neil, 1997; Zheng, 1999; Zhou and Zheng, 2002; Zhou and Zheng, 2003), theoretical calculations (Bottinga, 1968; Tarutani et al., 1969; O'Neil, 1986; Chacko et al., 1991; Zheng, 1999) and biologic specimens in natural settings (Grossman and Ku, 1986; Patterson et al., 1993; Thorrold et al., 1997; Dettman et al., 1999; White et al., 1999; Boehm et al., 2000), especially for aragonite (Table 1). These differences complicate the interpretation of $\delta^{18}O$ data because it is often unclear which temperature-$\delta^{18}O$ relationship is appropriate for a particular setting or organism.

There are several possible causes of the observed differences in the proposed equilibrium fractionation relationships. Well-controlled laboratory conditions are the optimum setting for

quantifying isotopic variability solely due to changes in temperature, pH, and the $\delta^{18}O$ value of host water. Laboratory settings, however, may fail to account for complexities experienced or displayed by organisms only in natural settings, such as seasonal migration, diurnal environmental change, microhabitat preferences, and response to stress. Moreover, biogenic carbonate may be subject to disequilibrium fractionation (or vital effects), which depends on a number of physical, chemical and biologic factors. The large differences between the inorganic experiments (McCrea, 1950; O'Neil et al., 1969; Tarutani et al., 1969; Kim and O'Neil, 1997; Zheng, 1999; Zhou and Zheng, 2002; Zhou and Zheng, 2003) and existing studies on biologic carbonates are particularly puzzling, and may indicate that such inorganic synthesis experiments are subject to some substantial, but as yet unidentified, fractionation mechanism.

2.3. $\delta^{13}C$ Fractionation in Carbonates

Carbon isotopes have also been used extensively in paleoclimatic studies, although they are typically more complicated to interpret than oxygen isotopes. Changes in the $\delta^{13}C$ values of carbonate are generally viewed to result from changes in metabolism (Wurster and Patterson, 2003), productivity and pCO_2 (Maslin et al., 1997), ocean circulation (Elliot et al., 2002) and exchange between terrestrial and oceanic carbon pools (Ikeda and Tajika, 2003; Mueller-Lupp et al., 2003) because under typical environmental conditions, fractionation is temperature independent.

The most commonly used equilibrium fractionation factors for carbon isotopes were empirically derived by Romanek et al. (1992) for calcite and aragonite, based on laboratory experiments. According to this work, the fractionation factor between shell carbonate and aqueous HCO_3^-, or $\alpha_{CaCO3\text{-}HCO3}$, is 1.0027 $\pm$ 0.0006, and is only weakly dependent on temperature. More recent laboratory experiments support these results, and suggest that precipitation rate effects observed in earlier studies are related to mineralogy rather than kinetic effects (Jimenez-Lopez et al., 2001). As with oxygen isotopes, however, there are also strong indications that equilibrium conditions may not always be achieved in biologic carbonates because of vital effects, the causes of which we consider in the next section.

2.4. Causes of Vital Effects in Biologic Carbonates

Evidence for vital effect offsets has been suggested from both carbon and oxygen isotope data in studies of biogenic carbonate (Grossman, 1987; von Grafenstein et al., 1999; Lee and Carpenter, 2001; Curry and Fallick, 2002; McConnaughey, 2002; Brand et al., 2003; Adkins et al., 2003). A detailed description of the proposed mechanisms for these offsets can be found in McConnaughey (2003). The two leading hypothesis are the "kinetic" model (McConnaughey, 1989a; McConnaughey, 1989b) and the "carbonate" model (Adkins et al., 2003).

The "kinetic" model assumes that the isotopic composition of biogenic carbonates derives from molecular CO_2 contained in the extracellular calcifying fluid (ECF). This fluid, in which calcification occurs, is separated from the surrounding host water by a thin membrane which is permeable to CO_2 but impermeable to HCO_3^-. CO_2 diffuses across this membrane and, depending on the pH of the ECF, undergoes hydration or hydroxylation to form DIC. These reactions discriminate against the heavy isotopes, producing depletions of ^{13}C and ^{18}O in DIC. This fractionation effect is preserved in the carbonate material (McConnaughey, 2003) because precipitation of carbonate from DIC is much more rapid than equilibration with the surrounding fluid. As pH increases, a greater proportion of the DIC is produced from molecular CO_2, more DIC is produced from hydroxylation and DIC residence time decreases, causing less isotopic equilibration with the solution. These factors produce increasingly depleted isotopic values, correlations between the isotopic depletions in ^{13}C and ^{18}O and positive correlations with growth rate.

An alternative "carbonate" model has also been proposed, which does not require disequilibrium between the carbonate species and the ECF (Adkins et al., 2003). In this model, enzyme-controlled transport of Ca^{2+} into the ECF sets up a large pH gradient across the membrane, the magnitude of which is related to the rate of calcification. As in the "kinetic model," CO_2 diffuses across the membrane and forms DIC species, but in this case, the "carbonate" model assumes that these species equilibrate with the surrounding solution. At isotopic equilibrium, however, CO_3^{2-} is more depleted in ^{13}C and ^{18}O than HCO_3^-, so that the equilibrium isotopic composition of the carbonates depends on the ratio of these species, which is in turn controlled by the pH of the solution (McCrea, 1950). Changes in the pH of the ECF caused by changes in the rate of calcification will also change the $\delta^{18}O$ value of the carbonate that is precipitated. Recently, the plausibility of this mechanism has been questioned because rates of precipitation may be too rapid to allow exchange between isotopes in the DIC and the surrounding water, and because this exchange is likely to also be affected by equilibration with water outside of the ECF, which is at a different pH (McConnaughey, 2003).

Metabolic CO_2 may also be an important factor in controlling the $\delta^{13}C$ values of biologically precipitated carbonates (e.g., Tanaka et al., 1986; Veinott and Cornett, 1998; Dettman et al., 1999). Metabolic carbon has its origin in ingested food, which usually has a much lower $\delta^{13}C$ value than DIC. Although the isotopic composition of metabolic carbon may be modified by biologic processes (such as tissue synthesis or excretion), it is cited as a likely source of ^{13}C-depleted carbon when explaining shell $\delta^{13}C$ values that are less than calculated equilibrium values (e.g., Tanaka et al., 1986; McConnaughey et al., 1997; Dettman et al., 1999; Furla et al., 2000). There is strong evidence for a large (60%–80%) contribution of respired carbon in vertebrates, including fish otoliths (Mulcahy et al., 1979; Radtke et al., 1987; Kalish, 1991) the eggshells of birds (Von Shirnding et al., 1982; Schaffner and Swart, 1991) and the bones of mammals (Deniro and Epstein, 1978; Sullivan and Krueger, 1981; Shoeninger and Deniro, 1982). However, the influence of respired CO_2 on the $\delta^{13}C$ value of carbonates in other organisms (e.g., mollusks, gastropods, corals, foraminifera) has been questioned (Fritz and Poplawski, 1974; Paull et al., 1989; Paull et al., 1991; Paull et al., 1992; McConnaughey et al., 1997; Spero et al., 1997; Adkins et al., 2003). Fritz and Poplowski (1974) showed that aquatic snails reared under differing DIC conditions had $\delta^{13}C$ values that closely resembled the isotopic composition of the DIC rather than the food they ingested. Spero et al. (1997) came to similar conclusions regarding foraminifera. ^{14}C data on deep-sea corals was used

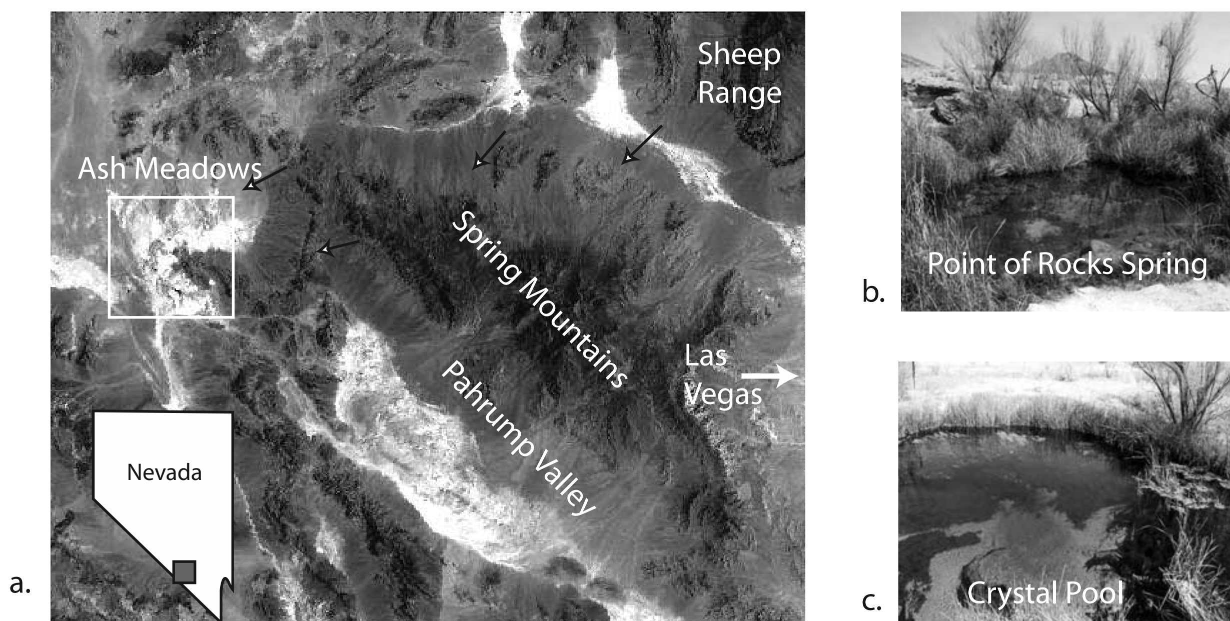

Fig. 1. (a) Location of the study area in southeastern Nevada. Landsat topographic image of the area surrounding the Ash Meadows site. Arrows indicate regional ground-water flow paths. (b) Point of Rocks Springs. (c) Crystal Pool.

by Adkins et al. (2003) to suggest that metabolic CO_2 contributes less than 10% of the carbon to coral skeletons. Paull et al. (1989, 1991, 1992) examined mollusks growing near a deep-sea methane seep and found that the tissues of these organisms had strongly depleted isotopic signatures, consistent with the isotopic composition of their food source, whereas shell $\delta^{13}C$ values more closely resembled seawater isotopic values, implying that these organisms grew their shells from ambient water with a negligible metabolic component. McConnaughey et al. (1997) noted in their study that fully aquatic, gill-breathing snails also produced shells with $\delta^{13}C$ values similar to DIC, whereas aquatic, lung-breathing snails and land snails produced shells containing significant amounts of respired carbon, thus implying a possible physiologic control on the incorporation of metabolic carbon.

To explain observed differences in $\delta^{13}C$ of carbonate in these organisms, and the apparent differences in the incorporation of respired carbon, McConnaughey (1997) developed a respiratory gas exchange model. The idea behind this model is that organisms must obtain oxygen from the environment, and in doing so they inevitably will pick up CO_2 as well, in a proportion related to the environmental ratio of CO_2:O_2. In environments with low CO_2:O_2, little CO_2 is exchanged and the proportion of respired carbon used in shell formation is larger. Conversely, environments with high CO_2:O_2 will result in the gas transfer of large amounts of CO_2 from the environment and respired carbon will make up a smaller proportion of the carbon used to build shells.

This model can explain most of the results observed for biologic carbonates. Land organisms obtain their oxygen from the air, which has a very low CO_2:O_2 (ca. 0.0017), and consequently, are expected to produce shells made mostly of respired carbon. CO_2:O_2 values are much higher in aquatic systems, resulting in greater amounts of environmental carbon being incorporated into their skeletons. Because vertebrates have highly efficient circulatory systems, they can tolerate much lower ratios of blood oxygen to ambient oxygen (around 0.4) than invertebrates (around 0.9), further reducing their need for gas exchange and the influence of environmental CO_2 on shell carbonate (McConnaughey et al., 1997). This may explain why fish otoliths appear to use greater amounts of respired carbon to make up their shell material.

Irrespective of the mechanism, it is unclear if such disequilibrium offsets occur in all biogenic carbonates, if the offsets are similar between different species or organisms, and whether they can be predicted or accounted for. Isotopic records from aquatic organisms in some studies suggest that shell carbonate forms in equilibrium with the host water, with no biologic or vital effect on stable isotope fractionation (Epstein et al., 1953; Grossman and Ku, 1986). Other studies indicate differing degrees of fractionation, depending upon the species under investigation (Brand et al., 2003), the rate of growth (Owen et al., 2002) and even the portion of the shell material analyzed (Curry and Fallick, 2002).

3. SITE DESCRIPTION AND METHODS

3.1. Ash Meadows

Aquatic gastropods were collected from two active springs in the Ash Meadows National Wildlife Refuge (AMNWR) near the southern margin of the Amargosa Desert Hydrographic Area (ADHA). Ground-water recharge for the ADHA occurs over an area of ~12,000 km^2 in southern Nevada, including several mountain ranges and large tracts of the Mohave Desert (Winograd and Thordarson, 1975). $\delta^{18}O$ values from spring waters indicate that most recharge occurs via winter snowfall in the Spring Mountains and Sheep Range (Winograd et al., 1978), both of which are located in the northeast region of the ADHA and reach ~3000 m in elevation. Ground-water flow is generally from northeast to southwest across the ADHA and is modestly heated during deep circulation before discharging (Winograd and Friedman, 1972; Winograd and Thordarson, 1975; Laczniak et al., 1999).

Ground-water discharge in the ADHA occurs primarily at Ash

Meadows, which is a loosely defined area in the Amargosa Desert of southern Nevada near the California-Nevada border (Fig. 1). Ash Meadows encompasses ~200 km^2 of spring-fed wetlands and alkali desert and is at an elevation of 650–700 m above sea level. Before development of ground-water pumping in the area, the Ash Meadows spring discharge system had an average discharge of 58,300 $m^3 day^{-1}$ (Walker and Thomas, 1963). Increased groundwater pumping during the 1970s caused substantial decreases in the flow at Crystal Pool (the largest spring in Ash Meadows), but court orders restricted pumping to preserve the springs (see discussion in Laczniak et al., 1999). ~90 km^2 of Ash Meadows has since been designated as the AMNWR, which protects a number of endemic and endangered species, including desert pupfish. Following the ground-water pumping restrictions, discharge rates have returned to near prepumping levels throughout the area.

Ash Meadows is located in the north-central part of the Mohave Desert, and is therefore subject to mild winters, long hot summers, and minimal precipitation. Mean annual precipitation is between 75 to 100 mm, based on measurements at nearby National Weather Service stations (Laczniak et al., 1999). Mean annual temperature at these weather stations ranges from 15 to 25°C. Evapotranspiration determined from on-site meteorological stations between 1994 and 1997 ranges from 18 cm yr^{-1} over dry saltgrass to 262 cm yr^{-1} over open water (Laczniak et al., 1999).

3.2. Crystal Pool and Point of Rocks Spring

The AMNWR is home to several dozen active springs, including the two largest, Crystal Pool and Point of Rocks Spring (hereafter CP and POR) (Fig. 1). The temperature and isotopic composition of discharging waters at CP and POR remain nearly constant throughout the year (Winograd and Friedman, 1972; Winograd and Thordarson, 1975) because ground-water flow paths are long and deep, and because discharge is high enough (15,800 and 11,400 $m^3 day^{-1}$ at CP and POR, respectively (Walker and Thomas, 1963)) to minimize thermal and isotopic exchange with the atmosphere before the water leaves the spring pools. Water temperatures at CP and POR are ~31 to 32°C, which are 12 to 13°C higher than the local mean annual temperature (Winograd and Friedman, 1972; Winograd and Thordarson, 1975). Other springs in the AMNWR have cooler and more variable water temperatures, indicating a greater degree of equilibrium with the local air temperature, and were avoided for this study.

Measurements of the $\delta^{18}O$ value of spring water are highly consistent between springs in the AMNWR, ranging from −13.7 to −13.6‰ (SMOW) at CP and POR, respectively, to slightly higher values of −13.4 to −13.5‰ (SMOW) at Big Spring, which is located between CP and POR (Winograd and Pearson, 1976). The isotopic uniformity of these results reflects the thorough mixing and very high discharge rates of the springs compared to the small, calculated losses due to evaporation ($4–6 \times 10^{-4}$ m^3/d).

Reported $\delta^{13}C$ values for spring water DIC from Ash Meadows are −4.6 to −4.8‰ (PDB) at POR and −5.0‰ (PDB) at CP (Winograd and Pearson, 1976). The dominant algae in the springs (*Lyngbya* sp. and *Spirogyra* sp.) have $\delta^{13}C$ values of −28.0 ± 0.1‰ (PDB) (Riggs, 1984). Vegetation in and around the spring, which presents additional potential sources of carbon, consists primarily of saltcedar (*Tamarix aphyllia, T. Parviflora, T. ramosissma*), saltgrass (*Distichlis spicata* var *stricata*), cattails (*Typha domingensis*), reeds (*Phragmites australis*), and bulrush (*Scripus robustus*) (Laczniak et al., 1999).

Constraints on seasonal changes in the chemistry of CP and POR is provided by the spring monitoring study of (Winograd and Pearson, 1976) and data from the U.S.G.S. (2005). The pH at CP, measured 16 times between May of 1973 and September of 1998, was relatively constant, with a mean value of 7.3 ± 0.2 and no apparent seasonal trends. High values of 7.6 and 7.8 were only observed during visits in June and July of 1998 and are considered anomalous. Alkalinity at CP ranged from 4.84 to 5.15 meq l^{-1} (mean 5.0 ± 0.1) during seven visits between 1973 and 1997. Measurements of dissolved oxygen in CP during 1996 gave values ranging between 2.5 and 3.7 mg l^{-1} (mean 2.8 ± 0.4). Only data from (Winograd and Pearson, 1976) is available for POR. The pH during visits in October 1974 and November 1975 was 7.3 and 7.5. Alkalinity varied from 4.93 to 5.08 meq l^{-1} in POR over the same period.

3.3. Water Sampling and Measurements

We installed HOBO temperature loggers in CP and POR at depths of 50–75 cm during an initial visit in October 2001. Because of potential problems with vandalism, loggers were not placed in the same locations that gastropod and water collections were taken, but they were located in areas which showed similar water temperatures during our site visits (not in the outflow channels). Water temperatures were recorded at 2-h intervals over a period of 13 months. Water samples were collected on a bi-weekly or monthly basis by on-site National Park Service personnel and stored at room temperature in sealed plastic vials for oxygen isotopic analysis at the University of Arizona. The $\delta^{18}O$ values of water samples were measured using a Finnigan Delta S mass spectrometer at the University of Arizona and are reported relative to the VSMOW standard. Measurement reproducibility was ± 0.15‰ for $\delta^{18}O_{water}$ values.

3.4. Gastropod Sampling and Measurements

At least five species of aquatic gastropods (*Helisoma duryi, Melanoides tuberculatus, Pyrgulopsis* sp., *Physa virgata*, and *Tyronia* sp.) live in the spring pools and outflow channels at CP and POR. Many of the other springs in the AMNWR contain only the invasive *Melanoides tuberculata*. We collected live aquatic gastropods during site visits in January, May, and September 2002. After collection, the gastropods were culled, their bodies were removed from the shells, and the shells were soaked in dilute (6%) NaOCl overnight to remove residual organic material. XRD measurements were made on representative samples to confirm that all shell material used in the study was aragonite (only aragonite was present). Shell material was collected for isotopic analysis using a microdrill at the University of Arizona. For several individuals, samples were collected from multiple locations around the whorl, equivalent to different periods of the snail's life.

The isotopic composition of the shell aragonite was measured using a Finnigan MAT 252 mass spectrometer equipped with a Kiel III automated sampling device at the University of Arizona. Samples were reacted with dehydrated phosphoric acid at 70°C. Carbonate samples ranged in size from 10 to 30 μg with a standard precision of 0.08‰ for $\delta^{18}O$ and 0.05‰ for $\delta^{13}C$ (1σ). All carbonate results are reported in permil notation with respect to the V-PDB standard.

A subset of samples of snail flesh were taken from *Melanoides tuberculata* and *Helisoma duryi* collected from Crystal Pool during the September visit. Flesh was measured to determine the $\delta^{13}C$ of respired carbon, for comparison with the isotopic composition of the shell material. Isotopic measurements of organic material were combusted using an elemental analyzer (Costech) coupled to a continuous flow gas ratio mass spectrometer (Finnigan Delta PlusXL). Organic $\delta^{13}C$ measurements had a standard precision of 0.06‰ (1σ).

3.5. Aquatic Gastropods

3.5.1. *Helisoma duryi*

Helisoma duryi (Subclass Pulmonata) is a freshwater, lung-breathing gastropod commonly found in springs, wetlands, and lacustrine settings. Reproduction typically occurs either once or twice a year, usually in late spring and summer, depending on environmental conditions. We observed that *H. duryi* typically survives a single season of breeding (<1 year) in Ash Meadows Springs, although in laboratory studies, they may survive as long as 1.5 yrs.

The influence of temperature on survival and breeding in *H. duryi* has been studied extensively (de Kock and Joubert, 1991; El-Emam and Madsen, 1982). Optimal growth occurs between 26 and 28°C but individuals were found to survive for several weeks in temperatures as hot as 35°C. Above this temperature the survival time dropped exponentially to less than a week at 37°C (Aboul-Ela and Beddiny, 1980). Laboratory experiments at lower temperatures (8°, 10°, 18°, 33°C) showed that *H. duryi* laid significantly fewer eggs below 18°C (El-Emam and Madsen, 1982) and stopped reproducing altogether below 12°C,

although minimal effect on survivorship was noted (Aboul-Ela and Beddiny, 1980). At 10°C, shell growth slows significantly and survivorship drops by ~25% (El-Emam and Madsen, 1982).

Pulmonate snails have been shown to respire by taking up oxygen both via surface breathing and cutaneously using dissolved oxygen. The need for additional oxygen, particularly as snails increase in size can result in substantial seasonal migration of snails towards the oxygenated zone in large lakes. In shallow, highly oxygenated springs like the ones studied here, surface breathing may not be significant, and we did not observe *H. duryi* at or just below the water surface during the course of this study. Individuals collected during this study were found at depths between 20 cm and 60 cm, although they were observed at depths as great as 1 m. Most were found on the surfaces of rocks in the pool or under matted vegetation around the pool margins, whereas others were collected from the submerged parts of reeds growing in the springs.

Numerous studies have investigated the rate of shell growth in *H. duryi* (Wong and Saleuddin, 1972; Kunigelis and Saleuddin, 1978; Saleuddin et al., 1980; Kunigelis and Saleuddin, 1983). Under natural conditions, *H. duryi* precipitates shell material at ~6-h increments (Saleuddin et al., 1980) and produces banding which is believed to be daily in nature and internally controlled (Saleuddin et al., 1980). Rate of shell growth is a strong function of both temperature and photoperiod (El-Emam and Madsen, 1982; Saleuddin et al., 1980). In controlled laboratory experiments, specimens given 12 hours of light and 12 hours of dark had daily growth rates ranging from 0.18 to 0.21 mm day^{-1} whereas those held in complete darkness grew between 0.25 to 0.26 mm day^{-1} (Saleuddin et al., 1980). Controlled temperature experiments indicated that growth rates dropped from a maximum of 0.19 mm day^{-1} at 28°C to 0.10 mm day^{-1} at 18°C and 0.06 mm day^{-1} at 10°C (El-Emam and Madsen, 1982).

3.5.2. *Melanoides tuberculata*

Melanoides tuberculata (Subclass Prosobranchia) is a freshwater, gill-breathing gastropod native to Africa, southeast Asia, China and the islands of the Indo-Pacific (Berry and Kadri, 1974; Brandt, 1974; Brown, 1980; Dudgeon, 1986), and recently introduced to North America. They are viviparous and reproduce parthenogenetically throughout the year (Berry and Kadri, 1974), although it is unclear if young are released throughout the year (Dudgeon, 1986). Laboratory and field evidence indicate that release is variable and may be the result of parental response to exogeneous stimuli such as temperature (Dudgeon, 1986), the rainy season (Pointier et al., 1992; Pointier et al., 1993), or population density (Livshits and Fisgelson, 1983). In most natural settings, an increase in the abundance of juveniles is observed in mid summer.

M. tuberculata precipitate shell carbonate throughout their lives, though the increment of shell deposited per unit time decreases as the shell widens with maturation to permit larger brood sizes (Pointier et al., 1992). Growth exponentially decreases with time, reaching maximum sizes of between 25 and 30 mm after several years (Pointier et al., 1992). The rate of growth appears to depend primarily on the ambient temperature (Leveque, 1971).

The life span of *M. tuberculata* is <5 yrs though studies from laboratory (Pointier et al., 1992) and natural environments (Berry and Kadri, 1974; Dudgeon, 1986) indicate that it may vary widely, depending on environmental conditions. In Lake Chad, few *M. tuberculata* survive more than one season (Leveque, 1971) whereas individuals survived 2–2.5 yrs in Hong Kong (Dudgeon, 1986) and 3.5 yrs in Malaysia (Berry and Kadri, 1974). The widespread nature of *M. tuberculata* as an invasive species indicates that it can survive and reproduce over a relatively large range of environmental conditions.

M. tuberculata appeared to occupy the largest range of environmental conditions of any of the gastropods examined during this study. They were always found on rocky or muddy substrate, either in relatively shallow environments (depths >20 cm) associated with matted vegetation around the margins of the pools, or in deeper locations (up to depths >2 m) not associated with vegetation. During seasons when they were in association with abundant *H. duryi*, they generally occupied deeper portions of the pools.

3.5.3. *Physa virgata*

Physa virgata (Subclass Pulmonata) is also a freshwater, lung-breathing gastropod that is common in wetlands, springs, and lakes. Reproduction typically occurs once a year, in summer (Russell-Hunter, 1978), though in some settings (e.g., abundant nutrients, warmer temperatures) as many as three generations may be produced annually (McMahon, 1975). Growth continues throughout the year, but most of this occurs in the first two months, after which growth slows considerably (McMahon, 1975). Few individuals survive more than one annual cycle.

P. virgata are typically found on aquatic vegetation and hard substrates near the shallow margins of pools and streams to depths of <1 m (McMahon and Payne, 1980). In our study, they were found almost entirely in very shallow waters (<10 cm) on either live aquatic vegetation or in vegetation mats in the very shallow margins of the pools. Individuals have been observed in natural settings at temperatures ranging from 8 to 35°C (McMahon and Payne, 1980), though temperature tolerances of up to 43°C have been noted in other genera of *Physa* (McMahon and Payne, 1980). Growth rates are strongly temperature-dependent (McMahon, 1975), with individual shells reaching lengths of up to 14 mm in warm springs.

3.5.4. *Hydrobiidae (Pyrgulopsis* sp. *and Tyronia* sp.*)*

Hydrobiidae (Subclass Prosobranchia) are one of the most common types of gill-breathing, aquatic gastropods, with over 200 species known from North America alone (Mladenka and Minshall, 2001). *Hydrobiidae* are highly adaptive and particularly sensitive to their environment, and most are endemic to particular drainages or water bodies. In general, *Hydrobiidae* are found in groundwater-fed springs, streams and marshes that have stable temperatures, chemistries and stream flow conditions (Herschler, 1998). Genera of the family *Hydrobiidae* have been found to exist in natural settings with temperatures ranging from 10 to 39°C, though the largest populations occur in the temperature range 22 to 35°C (Herschler, 1998). At least some types of *Hydrobiidae* appear to be adapted to extreme conditions such as brackish waters or waters with extremely high levels of CO_2 (O'Brien and Blinn, 1999).

Pyrgulopsis makes up the largest genus of *Hydrobiidae* in North America. This genus inhabits the moist zone around the margins of pools and is found either on hard substrates or among aquatic vegetation. During our study, *Pyrgulopsis* were observed primarily in two areas: in the very shallow outflow channel where they were attached to rock surfaces (none were measured from this environment) and from depths of around 0.5 m, in regions where live aquatic vegetation was abundant. Individuals examined during this study were collected exclusively from the rocks located adjacent to the temperature probes, where they were abundant.

Shells reach a maximum length of ~2.5 mm and exhibit 3–4 whorls (Herschler and Sada, 1987). Although no data are available on growth rates of *Pyrgulopsis erythopoma* from Ash Meadows, studies of *Pyrgulopsis bruneauensis* indicate that they grow between 0.020 and 0.034 mm day^{-1} at temperatures ranging between 15 and 34°C (Mladenka and Minshall, 2001). Data on recruitment are equivocal and vary from seasonal (mostly in November-December) to year round depending on site conditions (Mladenka and Minshall, 2001).

Tyronia angulata is also abundant in the springs of Ash Meadows (Herschler and Sada, 1987). *Tyronia* is a fairly large-sized *Hydrobiidae*, ranging from 1.2 to 7.0 mm in length and is endemic to the springs at Ash Meadows (Herschler and Sada, 1987). They are abundant in the soft substrate and vegetation mats at the margins of the larger pools, where the water is relatively stagnant. During our study, *Tyronia* were collected exclusively from this environment, always at depths of <20 cm. No published information is available about either the life history or growth rates of *Tyronia*, and the low abundances of these individuals makes it difficult to accurately assess the life history of these gastropods during our study period.

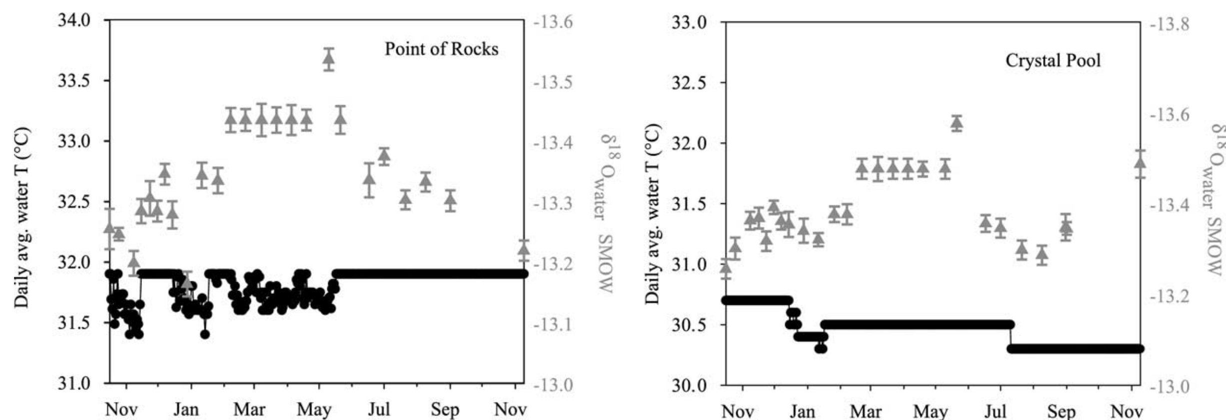

Fig. 2. $\delta^{18}O_{water}$ values (solid triangles) and temperature (solid circles) monitoring conducted over the course of this study at (a) Point of Rocks Springs and (b) Crystal Pool.

4. RESULTS

4.1. Water Temperature and $\delta^{18}O_{water}$

In agreement with prior studies, temperature and oxygen isotopic composition vary only slightly in CP and POR over the course of the year, despite large variations in air temperature and evapotranspiration (Fig. 2). At CP, the average water temperature was 30.5 ± 0.5°C (recorded temperature variations were smaller than the reported instrument uncertainty). At POR, the average water temperature was 31.8 ± 0.5°C. The error-weighted mean $\delta^{18}O$ (VSMOW) values of the spring water over the measurement period was −13.39 ± 0.28‰ and −13.33 ± 0.21‰ at CP and POR, respectively. As indicated earlier, temperatures were monitored continuously at stationary locations within the pool, at water depths of ca. 0.5 m. Water samples for $\delta^{18}O$ measurements were collected from slightly shallower depths (ca. 0.25m).

Additional measurements of temperature and water $\delta^{18}O$ values were conducted during a site visit in September 2002 to assess the spatial and diurnal variability of the spring pools and outflow channels. At that time, water samples were collected at 5 h increments from five different locations within the spring pool and water temperature measurements were made at the same locations throughout the day. The $\delta^{18}O_{water}$ value did not vary significantly in the main pools at either CP or POR, and water temperature in the upper 20–25 cm of the water column varied by only 0.3°C in both CP and POR. Diurnal temperature variations at the same depth during a visit in August 2002 were less than 0.5°C at CP and 0.7°C at POR.

In September 2002, we also collected five water samples along a ~35 m stretch of the outflow channel from POR where we expected the influence of evaporation and solar heating of the spring water to be greatest (no mollusks were collected from the outflow) to test the upper limits on the magnitude of these effects. The outflow is <20 cm deep and 1 m wide. $\delta^{18}O_{water}$ (VSMOW) varied by 0.13‰ (13.89 to 13.76‰) and water temperature varied by <1°C over this area, which indicates that spring discharge is high enough to minimize effects from evaporative enrichment even under these conditions. Even smaller changes were observed for water in the outflow channel of CP.

The combined results of this monitoring indicate that the spatial variations in temperature and $\delta^{18}O$ of water in the pools are small enough that they will not exert a significant influence on the composition of gastropod aragonite precipitated at different locations within the spring. However, there are slight seasonal changes in the $\delta^{18}O$ values of the pools, which need to be addressed when considering the conditions under which different species precipitated shell material.

4.2. Isotopic Results for Gastropod Shell Carbonate

There is significant variability in the measured isotopic ($\delta^{13}C$ and $\delta^{18}O$) composition of shell aragonite between species, between specimens, and within individual shells (Table 2). This is despite the relatively constant isotopic composition and temperature of the spring water. For the full set of $\delta^{18}O$ (VPDB) data, measured values ranged from −14.76 to −16.17‰ with a mean of −15.58 ± 0.30‰ (n = 146). The measured $\delta^{13}C$ (VPDB) values ranged from −1.92 to −6.96 ‰, with a mean of −3.19 ± 1.06‰ (n = 146).

4.2.1. Observed interspecies isotopic differences

Our results indicate that there are significant differences in the isotopic composition ($\delta^{18}O$ and $\delta^{13}C$) between different species (Fig. 3; mean values by species in Table 3). To examine the statistical significance of these differences, we performed an analysis of variance (ANOVA) test on the full suite of $\delta^{18}O$ and $\delta^{13}C$ isotopic data.

The results of the ANOVA tests show that there are statistically significant differences in the $\delta^{18}O$ composition of shells produced by different gastropod species (CP: $F_{2,56}$ = 42.05, $p < 0.001$; POR: $F_{4,67}$ = 18.31, $p < 0.001$ based on an ANOVA F-test) (Fig. 3a). Observed differences between the three species (*Melanoides, Tyronia* and *Helisoma*) collected from CP cannot be attributed to chance. Average $\delta^{18}O$ (VPDB) values for these three species are −15.51 ± 0.18, −15.82 ± 0.19 and −15.31 ± 0.19‰, respectively. $\delta^{18}O$ values for *Helisoma* are 0.55 ± 0.06‰ (95% C.I. 0.41 to 0.70) higher than those of *Tyronia* and 0.22 ± 0.06‰ (95% C.I. 0.08 to 0.37) higher than those of *Melanoides* (this and all subsequent

Table 2. Isotopic data for molluscs collected live from Ash Meadows.

Sample ID	date collect	loc. mm	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
Crystal Pool				
Melanoides				
Mel-1a	1/17/02	21	−3.02 ± 0.03	−15.72 ± 0.09
Mel-1b	1/17/02	16	−2.72 ± 0.10	−15.67 ± 0.39
Mel-1c	1/17/02	7	−2.91 ± 0.04	−15.81 ± 0.04
Mel-1d	1/17/02	2	−3.02 ± 0.04	−15.65 ± 0.03
Mel-2a	1/17/02	18	−2.34 ± 0.04	−15.32 ± 0.11
Mel-2b	1/17/02	9	−2.93 ± 0.08	−15.25 ± 0.13
Mel-2c	1/17/02	2	−3.45 ± 0.03	−15.18 ± 0.03
Mel-3a	1/17/02	22	−2.83 ± 0.04	−15.40 ± 0.08
Mel-3b	1/17/02	11	−2.99 ± 0.04	−15.57 ± 0.03
Mel-3c	1/17/02	2	−2.93 ± 0.04	−15.63 ± 0.05
Mel-4a	9/2/02	1	−3.08 ± 0.01	−15.33 ± 0.04
Mel-4b	9/2/02	8	−2.79 ± 0.03	−15.42 ± 0.04
Mel-4c	9/2/02	22	−2.73 ± 0.02	−15.61 ± 0.04
Mel-4d	9/2/02	32	−3.12 ± 0.06	−15.49 ± 0.10
Mel-4e	9/2/02	40	−3.27 ± 0.02	−15.53 ± 0.02
Mel-5	9/2/02	1	−3.06 ± 0.02	−15.52 ± 0.09
Helisoma				
Heli-1a	1/17/02	21	−4.83 ± 0.05	−15.40 ± 0.05
Heli-1b	1/17/02	15	−4.45 ± 0.04	−15.58 ± 0.04
Heli-1c	1/17/02	8	−3.95 ± 0.04	−15.44 ± 0.07
Heli-2a	1/17/02	10	−3.79 ± 0.04	−15.19 ± 0.06
Heli-2b	1/17/02	5	−4.14 ± 0.03	−15.25 ± 0.02
Heli-2c	1/17/02	1	−3.74 ± 0.15	−15.35 ± 0.29
Heli-3a	1/17/02	18	−4.32 ± 0.10	−15.15 ± 0.16
Heli-3b	1/17/02	14	−4.33 ± 0.04	−15.08 ± 0.04
Heli-3c	1/17/02	11	−4.26 ± 0.03	−15.18 ± 0.04
Heli-3d	1/17/02	7	−3.93 ± 0.11	−14.85 ± 0.15
Heli-3e	1/17/02	5	−3.96 ± 0.05	−15.18 ± 0.04
Heli-3f	1/17/02	3	−4.15 ± 0.03	−15.38 ± 0.06
Heli-3g	1/17/02	1	−3.54 ± 0.03	−15.09 ± 0.07
Heli-4	5/12/02	outer	−4.10 ± 0.01	−15.52 ± 0.10
Heli-5	5/12/02	outer	−3.69 ± 0.01	−15.56 ± 0.10
Heli-6	5/12/02	outer	−3.77 ± 0.01	−15.65 ± 0.10
Heli-7	9/2/02	outer	−3.55 ± 0.06	−15.30 ± 0.07
Heli-8	9/2/02	outer	−3.24 ± 0.03	−15.05 ± 0.07
Heli-9	9/2/02	outer	−4.43 ± 0.03	−15.29 ± 0.07
Heli-10a	9/2/02	20	−3.98 ± 0.01	−15.45 ± 0.05
Heli-10b	9/2/02	15	−4.28 ± 0.06	−15.37 ± 0.13
Heli-10c	9/2/02	12	−4.20 ± 0.04	−15.44 ± 0.08
Heli-10d	9/2/02	8	−4.05 ± 0.02	−15.29 ± 0.06
Heli-10e	9/2/02	5	−3.64 ± 0.05	−15.24 ± 0.13
Heli-10f	9/2/02	0	−4.65 ± 0.04	−15.53 ± 0.07
Tyronia				
Tyr-1	1/17/02	shell	−2.43 ± 0.09	−15.67 ± 0.06
Tyr-2	1/17/02	shell	−2.50 ± 0.02	−15.66 ± 0.04
Tyr-3	1/17/02	shell	−2.65 ± 0.04	−15.43 ± 0.03
Tyr-4	1/17/02	shell	−2.37 ± 0.05	−15.70 ± 0.06
Tyr-5	1/17/02	shell	−2.23 ± 0.09	−15.86 ± 0.09
Tyr-6	1/17/02	shell	−2.25 ± 0.02	−15.72 ± 0.05
Tyr-7	1/17/02	shell	−2.26 ± 0.01	−15.78 ± 0.09
Tyr-8	1/17/02	shell	−2.44 ± 0.01	−15.82 ± 0.10
Tyr-9	1/17/02	shell	−2.48 ± 0.01	−16.00 ± 0.10
Tyr-10	1/17/02	shell	−2.57 ± 0.01	−16.17 ± 0.10
Tyr-11	5/12/02	shell	−2.28 ± 0.02	−15.63 ± 0.10
Tyr-12	5/12/02	shell	−2.45 ± 0.01	−16.02 ± 0.10
Tyr-13	5/12/02	shell	−2.55 ± 0.01	−15.95 ± 0.10
Tyr-14	5/12/02	shell	−2.58 ± 0.01	−15.93 ± 0.10
Tyr-15	5/12/02	shell	−2.92 ± 0.02	−15.73 ± 0.09
Tyr-16	5/12/02	shell	−2.57 ± 0.01	−16.04 ± 0.09
Point of Rocks				
Melanoides				
Mel-6a	1/17/02	25	−2.29 ± 0.03	−15.41 ± 0.06
Mel-6b	1/17/02	18	−2.43 ± 0.03	−15.84 ± 0.05
Mel-6c	1/17/02	10	−2.12 ± 0.11	−15.80 ± 0.35
Point of Rocks cont.				
Melanoides cont.				
Mel-6d	1/17/02	1	−2.81 ± 0.04	−15.43 ± 0.06
Mel-7a	1/17/02	50	−1.92 ± 0.11	−15.71 ± 0.33
Mel-7b	1/17/02	43	−2.56 ± 0.02	−15.94 ± 0.05
Mel-7c	1/17/02	37	−2.74 ± 0.04	−15.77 ± 0.05
Mel-7d	1/17/02	34	−2.86 ± 0.13	−15.78 ± 0.07
Mel-7e	1/17/02	31	−2.49 ± 0.06	−15.63 ± 0.04
Mel-7f	1/17/02	28	−2.52 ± 0.03	−15.82 ± 0.06
Mel-7g	1/17/02	25	−2.99 ± 0.05	−15.76 ± 0.04
Mel-7h	1/17/02	22	−2.65 ± 0.03	−15.89 ± 0.04
Mel-7i	1/17/02	18	−2.67 ± 0.06	−15.73 ± 0.07
Mel-7j	1/17/02	15	−3.06 ± 0.02	−15.76 ± 0.07
Mel-7k	1/17/02	12	−2.88 ± 0.06	−15.90 ± 0.09
Mel-7l	1/17/02	8	−3.72 ± 0.02	−15.79 ± 0.04
Mel-7m	1/17/02	1	−4.78 ± 0.04	−14.86 ± 0.08
Mel-8a	1/17/02	2	−2.32 ± 0.04	−15.60 ± 0.06
Mel-8b	1/17/02	8	−2.67 ± 0.08	−15.02 ± 0.27
Mel-8c	1/17/02	14	−2.98 ± 0.08	−14.98 ± 0.23
Mel-8d	1/17/02	18	−3.54 ± 0.05	−15.41 ± 0.06
Mel-9a	5/12/02	6	−2.39 ± 0.02	−16.00 ± 0.03
Mel-9b	5/12/02	26	−3.12 ± 0.03	−16.01 ± 0.04
Mel-9c	5/12/02	36	−3.18 ± 0.03	−16.05 ± 0.06
Mel-12a	5/12/02	2	−2.50 ± 0.01	−15.99 ± 0.06
Mel-12b	5/12/02	8	−2.36 ± 0.04	−16.09 ± 0.07
Mel-12c	5/12/02	16	−2.59 ± 0.02	−15.89 ± 0.03
Mel-12d	5/12/02	26	−2.43 ± 0.04	−16.01 ± 0.06
Mel-12e	5/12/02	33	−2.94 ± 0.02	−15.88 ± 0.05
Mel-17	5/12/02	outer	−2.86 ± 0.01	−15.95 ± 0.10
Mel-18	5/12/02	outer	−2.92 ± 0.01	−15.90 ± 0.09
Mel-19	5/12/02	outer	−2.67 ± 0.02	−15.92 ± 0.09
Mel-20	9/2/02	outer	−1.95 ± 0.01	−15.82 ± 0.04
Mel-21	9/2/02	outer	−2.07 ± 0.02	−15.87 ± 0.03
Mel-22	9/2/02	outer	−2.12 ± 0.01	−15.82 ± 0.05
Mel-23a	9/2/02	10	−1.92 ± 0.01	−15.87 ± 0.03
Mel-23d	9/2/02	40	−2.75 ± 0.06	−15.92 ± 0.04
Mel-23e	9/2/02	46	−2.70 ± 0.04	−16.01 ± 0.03
Helisoma				
Heli-11a	1/17/02	21	−4.43 ± 0.10	−15.59 ± 0.22
Heli-11b	1/17/02	11	−3.03 ± 0.05	−15.40 ± 0.07
Heli-11c	1/17/02	2	−3.61 ± 0.06	−14.76 ± 0.13
Heli-12a	1/17/02	20	−3.85 ± 0.03	−15.39 ± 0.06
Heli-12b	1/17/02	10	−3.18 ± 0.11	−15.48 ± 0.07
Heli-12c	1/17/02	3	−3.18 ± 0.14	−15.15 ± 0.30
Heli-13a	1/17/02	19	−4.36 ± 0.05	−15.01 ± 0.08
Heli-13b	1/17/02	9	−4.47 ± 0.04	−15.41 ± 0.09
Heli-13c	1/17/02	2	−3.56 ± 0.06	−15.62 ± 0.04
Heli-14a	5/12/02	2	−4.76 ± 0.02	−15.27 ± 0.09
Heli-14b	5/12/02	9	−3.93 ± 0.05	−15.49 ± 0.05
Heli-14c	5/12/02	13	−4.22 ± 0.02	−15.54 ± 0.09
Heli-14d	5/12/02	20	−4.61 ± 0.02	−15.50 ± 0.02
Heli-15a	5/12/02	0	−4.04 ± 0.02	−15.49 ± 0.07
Heli-15b	5/12/02	5	−3.90 ± 0.03	−15.45 ± 0.03
Heli-15c	5/12/02	9	−4.26 ± 0.03	−15.64 ± 0.03
Heli-15e	5/12/02	20	−4.99 ± 0.02	−15.75 ± 0.06
Heli-23	9/2/02	outer	−3.50 ± 0.05	−15.59 ± 0.08
Heli-24	9/2/02	outer	−2.97 ± 0.11	−15.60 ± 0.08
Heli-25	9/2/02	outer	−3.16 ± 0.02	−15.24 ± 0.07
Heli-26a	9/2/02	1	−3.32 ± 0.02	−15.47 ± 0.03
Heli-26b	9/2/02	16	−3.14 ± 0.03	−15.38 ± 0.04
Heli-13c	1/17/02	2	−3.56 ± 0.06	−15.62 ± 0.04
Heli-14a	5/12/02	2	−4.76 ± 0.02	−15.27 ± 0.09
Heli-14b	5/12/02	9	−3.93 ± 0.05	−15.49 ± 0.05
Heli-14c	5/12/02	13	−4.22 ± 0.02	−15.54 ± 0.09
Heli-14d	5/12/02	20	−4.61 ± 0.02	−15.50 ± 0.02

(Continued)

Table 2. (Continued)

Sample ID	date collect	loc. mm	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
Point of Rocks cont.				
Helisoma cont.				
Heli-15a	5/12/02	0	−4.04 ± 0.02	−15.49 ± 0.07
Heli-15b	5/12/02	5	−3.90 ± 0.03	−15.45 ± 0.03
Heli-15c	5/12/02	9	−4.26 ± 0.03	−15.64 ± 0.03
Heli-15e	5/12/02	20	−4.99 ± 0.02	−15.75 ± 0.06
Heli-23	9/2/02	outer	−3.50 ± 0.05	−15.59 ± 0.08
Heli-24	9/2/02	outer	−2.97 ± 0.11	−15.60 ± 0.08
Heli-25	9/2/02	outer	−3.16 ± 0.02	−15.24 ± 0.07
Heli-26a	9/2/02	1	−3.32 ± 0.02	−15.47 ± 0.03
Heli-26b	9/2/02	16	−3.14 ± 0.03	−15.38 ± 0.04
Physa				
Phys-1	5/12/02	outer	−6.47 ± 0.04	−15.38 ± 0.02
Phys-2	5/12/02	outer	−6.64 ± 0.01	−15.25 ± 0.05
Phys-3	5/12/02	outer	−6.81 ± 0.04	−15.35 ± 0.07
Phys-5	5/12/02	outer	−6.14 ± 0.00	−15.75 ± 0.10
Phys-6	5/12/02	outer	−6.96 ± 0.01	−15.15 ± 0.10
Phys-7	5/12/02	outer	−5.51 ± 0.01	−14.77 ± 0.10
Phys-8	5/12/02	outer	−5.40 ± 0.02	−15.83 ± 0.10
Pyrgulopsis				
Pyrg-1	1/17/02	shell	−2.25 ± 0.04	−16.01 ± 0.05
Pyrg-2	1/17/02	shell	−1.96 ± 0.03	−15.69 ± 0.05
Pyrg-3	1/17/02	shell	−2.28 ± 0.03	−15.50 ± 0.03
Pyrg-5	9/2/02	shell	−2.19 ± 0.04	−15.69 ± 0.09
Pyrg-6	9/2/02	shell	−2.10 ± 0.03	−15.68 ± 0.03
Pyrg-7	9/2/02	shell	−2.37 ± 0.02	−15.78 ± 0.02
Pyrg-8	9/2/02	shell	−1.92 ± 0.05	−15.54 ± 0.05
Pyrg-9	9/2/02	shell	−2.16 ± 0.02	−15.90 ± 0.04
Pyrg-10	9/2/02	shell	−2.10 ± 0.01	−15.78 ± 0.01
Pyrg-11	9/2/02	shell	−2.06 ± 0.02	−15.79 ± 0.04
Pyrg-12	9/2/02	shell	−2.10 ± 0.02	−15.85 ± 0.03
Pyrg-13	9/2/02	shell	−2.10 ± 0.01	−15.50 ± 0.02
Pyrg-14	9/2/02	shell	−2.19 ± 0.03	−15.76 ± 0.03
Pyrg-15	9/2/02	shell	−2.03 ± 0.03	−15.79 ± 0.08
Pyrg-16	9/2/02	shell	−2.10 ± 0.02	−15.73 ± 0.03
Pyrg-17	9/2/02	shell	−2.17 ± 0.02	−15.63 ± 0.01
Tyronia				
Tyr-17	5/12/02	shell	−2.04 ± 0.02	−15.47 ± 0.03
Tyr-18	9/2/02	shell	−2.51 ± 0.01	−15.42 ± 0.04
Tyr-19	9/2/02	shell	−2.06 ± 0.04	−15.98 ± 0.03
Tyr-20	9/2/02	shell	−2.16 ± 0.01	−15.79 ± 0.05
Tyr-21	9/2/02	shell	−1.93 ± 0.03	−15.74 ± 0.05
Tyr-22	9/2/02	shell	−2.15 ± 0.01	−15.65 ± 0.02

loc: location of sample on shell.
outer: sample collected from outermost edge of shell.
shell: whole shell used for analysis.

comparisons are based on the Tukey-Kramer procedure (Rice, 1995)).

Specimens collected from POR included the same species as collected from CP, plus an additional *Hydrobiidae (Pyrgulopsis)* and a semiaquatic gastropod (*Physa*). Mean estimates of the $\delta^{18}O$ (VPDB) value for *Melanoides, Helisoma, Physa, Tyronia* and *Pyrgulopsis* were −15.94 ± 0.08, −15.42 ± 0.23, −15.35 ± 0.36, −15.67 ± 0.21 and −15.73 ± 0.14‰, respectively (Fig. 3a). There is no evidence that the $\delta^{18}O$ values of either *Helisoma* vs. *Physa* or *Tyronia* vs. *Pyrgulopsis* differ from one another. However, differences are statistically significant between *Melanoides* and all other species (F-test *p*-value <0.0001 from a linear contrast ANOVA) and between *Physa* and *Helisoma* and the two genera of *Hydrobiidae* (F-test *p*-value <0.0001 from a linear contrast ANOVA). $\delta^{18}O$ value is 0.59‰ lower for *Melanoides* than for both *Helisoma* (95% C.I. 0.37 to 0.80) and *Physa* (95% C.I. 0.30 to 0.87). The $\delta^{18}O$ value is 0.32 ‰ higher in *Tyronia* (95% C.I. 0.09 to −0.73 and 0.05 to −0.69) and 0.38‰ higher in *Pygulopsis* (95% C.I. −0.07 to −0.68 and −0.13 to −0.62) than in *Physa* and *Helisoma*.

There are also significant differences in the $\delta^{13}C$ of carbonate produced by different gastropods (CP: $F_{2,56} = 143.14$, $p < 0.001$; POR: $F_{4,66} = 79.06$, $p < 0.001$ based on an ANOVA F-test) (Fig. 3b). Average $\delta^{13}C$ (VPDB) values for *Melanoides, Tyronia* and *Helisoma* from CP were −2.95 ± 0.25, −2.47 ± 0.18 and −4.04 ± 0.37‰ PDB, respectively. As was the case for $\delta^{18}O$, differences between the species are significant and cannot be attributed to chance (F-test *p*-value <0.0001 for all comparisons based on a linear contrast ANOVA). Values for *Helisoma* were 1.61‰ (95% C.I. 1.36 to 1.87) lower than *Tyronia* and 1.12‰ (95% C.I. 0.90 to 1.35) lower than *Melanoides*. $\delta^{13}C$ values for *Tyronia* and *Melanoides* only differed by 0.49‰ (95% C.I. 0.21 to 0.77).

The results were similar for the $\delta^{13}C$ of shells from POR. Mean $\delta^{13}C$ values for species from POR (*Melanoides, Helisoma, Physa, Tyronia* and *Pyrgulopsis*) were: −2.56 ± 0.39, −3.84 ± 0.61, −6.19 ± 0.62, −2.14 ± 0.20 and −2.13 ± 0.11‰ (Fig. 3b). Differences in $\delta^{13}C$ values were significant between all species except between the two *Hydrobiidae*, which are statistically indistinguishable. Differences between *Physa* and the other species were largest, ranging from 4.3‰ (95% C.I. 3.35 to 5.27: *Hydrobiidae*), to 3.76‰ (95% C.I. 3.00 to 4.53: *Melanoides*) and 2.73‰ (95% C.I. 2.02 to 3.43: *Helisoma*). Differences were also significant between *Helisoma* and the *Hydrobiidae*: 1.58‰ (95% C.I. 0.85 to 2.31), *Helisoma* and *Melanoides*: 1.03‰ (95% C.I. 0.59 to 1.48) and *Melanoides* and the *Hydrobiidae*: 0.55‰ (95% C.I. −0.25 to 1.34).

4.2.2. *Observed intraspecies and within-shell isotopic differences*

Our results also indicate that there is a substantial intraspecies and within-shell variation in the $\delta^{18}O$ and $\delta^{13}C$ values of gastropod aragonite (Fig. 3). Combined intraspecies and within-shell variability in $\delta^{18}O$ value have a range (minimum—maximum) of 0.63‰ (CP) to 0.26 ‰ (POR) for *Melanoides*, 0.81‰ (CP) to 0.99‰ (POR) for *Helisoma*, 0.74‰ (CP) to 0.56‰ (POR) for *Tyronia*, 0.51‰ (CP) for *Pyrgulopsis* and 1.06‰ (POR) for *Physa*. Variations in $\delta^{13}C$ values have a range of 1.11‰ (CP) to 1.26‰ (POR) for *Melanoides*, 1.59‰ (CP) to 2.03‰ (POR) for *Helisoma*, 0.69‰ (CP) to 0.58‰ (POR) for *Tyronia*, 0.44 ‰ (CP) for *Pyrgulopsis* and 1.56‰ (POR) for *Physa*.

Isotopic variability of shell material taken at locations along the growth axis of individual shells of *Melanoides* ($\delta^{13}C$: range = 1.26‰, $\sigma = 0.33$‰; $\delta^{18}O$: range = 0.97‰, $\sigma = 0.11$‰) and *Helisoma* ($\delta^{13}C$: range = 1.59‰, $\sigma = 0.10$‰; $\delta^{18}O$: range = 1.06‰, $\sigma = 0.14$‰) are similar in magnitude to that observed between shells of different individuals of the same species (Fig. 4). There is no systematic pattern of stable isotope composition associated with the size of the shell, or position of sampling along the shell.

4.2.3. *Comparisons between the $\delta^{13}C$ of snail shell and flesh*

There exist large differences between the carbon isotopic composition of snail shell and flesh material in both *Helisoma*

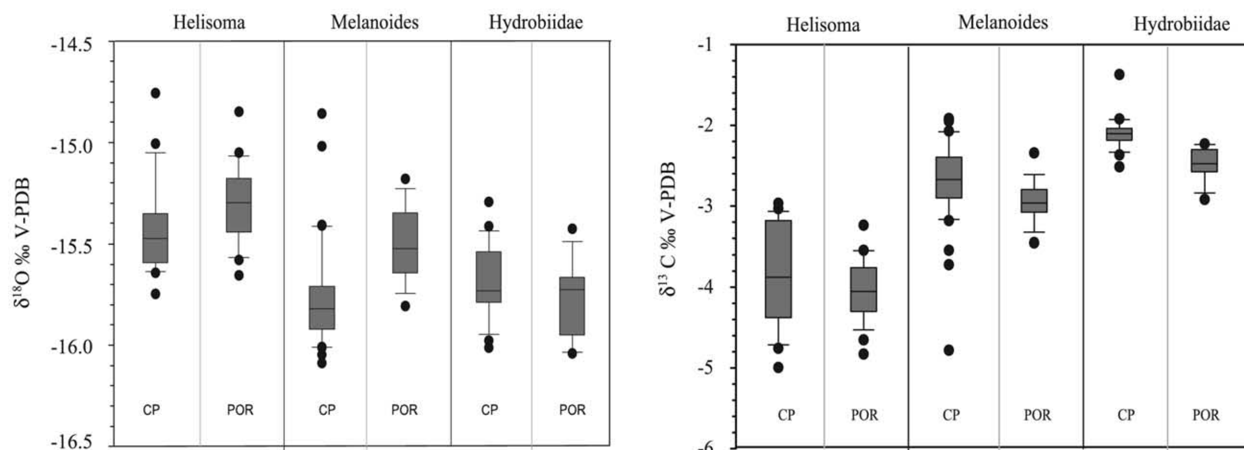

Fig. 3. Box plot comparisons of isotopic data from samples. (a) δ^{18}O data and (b) δ^{13}C data. The upper and lower margins of the grey box indicates the 75 percentile, whiskers represent 95 percentile and line through box indicates the median.

and *Melanoides* collected from Crystal Pool (Table 4). The mean δ^{13}C of flesh from *Helisoma* was −30.77‰ (range = −30.02 to −31.42) compared to a mean δ^{13}C of shell material (from the same set of organisms) of −4.07‰ (range = −3.8 to −4.28). Differences were only slightly smaller for *Melanoides* (flesh = −26.18‰: −24.58 to −27.53; shell = −2.6‰: −2.16 to −3.2). Data from both species suggest that the component of respired carbon incorporated into shell material is probably small. Flesh δ^{13}C values are very similar to reported values for the δ^{13}C of algae growing in the springs (−28.0 ± 0.01‰), suggesting that the depleted isotopic values of the snail tissues reflect the isotopic composition of food sources in the springs.

5. DISCUSSION

5.1. Sources of Isotopic Variability Within Shells and Within Species

There are several potential sources that may contribute to the observed isotopic variability in the gastropods shell carbonate, including laboratory procedures and measurement error, environmental conditions, and biologic influences. We examine each of these potential sources in detail below.

5.1.1. Error in the mass spectrometric measurement

The isotopic variability of carbonate in this study cannot be explained by errors in mass spectrometric measurements. Mean values of δ^{18}O of NBS-19 for the three analysis periods spanned by our study range from −2.17 ± 0.02 to −2.21 ± 0.11‰ and are indistinguishable within both the measurement uncertainties (0.02–0.15‰; μ = 0.06‰) and within the variability of the individual measurements (μ = 0.08‰) (Table 5). Calibration and normalization based on NBS-19 and NBS-18 force long-term means of these measurements to agree with the defined δ^{18}O of the NBS-19 standard (−2.20‰; Gonfiantini et al., 1995). Values of δ^{13}C ranged from 1.94 ± 0.08 to 1.99 ± 0.03‰. As with δ^{18}O, δ^{13}C measurements on the NBS-19 standard were indistinguishable within individual measurement uncertainties (0.01–0.17‰; μ = 0.04‰), the variability of individual measurements (μ = 0.05‰), and they matched the defined δ^{13}C of NBS-19 (1.95‰; Gonfiantini et al., 1995). Based on these data, the high degree of variability in the δ^{13}C and δ^{18}O values of gastropod shell aragonite observed in this study cannot be solely attributed to analytical error.

Table 3. Mean values for the isotopic concentrations in gastropods from Ash Meadows.

	n	δ^{18}O [‰ PDB]	δ^{13}C [‰ PDB]
Crystal Pool			
Melanoides	16	−15.51 ± 0.18	−2.95 ± 0.25
Helisoma	25	−15.31 ± 0.19	−4.04 ± 0.37
Tyronia	16	−15.82 ± 0.19	−2.47 ± 0.18
Point of Rocks			
Melanoides	17	−15.94 ± 0.08	−2.56 ± 0.39
Helisoma	22	−15.42 ± 0.23	−3.84 ± 0.61
Tyronia	6	−15.67 ± 0.21	−2.14 ± 0.2
Pyrgulopsis	16	−15.73 ± 0.14	−2.13 ± 0.11
Physa	7	−15.35 ± 0.36	−6.19 ± 0.62

5.1.2. Variable environmental conditions

An alternative explanation for the observed isotopic results is that they reflect small, but significant variations in the temperature and isotopic compositions of the springs, since both water temperature and $\delta^{18}O_{water}$ values at CP and POR are nearly but not exactly constant. Such variations could potentially account for both the within-shell and intraspecies isotopic variability observed during this study.

To assess whether the within species isotopic variability could be caused by variations in spring conditions, we used measured temperature (CP: 30.5 ± 0.5°C; POR: 31.8 ± 0.5°C) and $\delta^{18}O_{water}$ values (CP: −13.39 ± 0.28‰; POR: −13.33 ± 0.21‰) for each sampling interval, and the equations in Table 1, to compute the maximum predicted range in shell δ^{18}O values caused by these variations. Our calculations indicate that the maximum potential ranges in shell isotopic variability caused solely by temperature and water composition changes are 0.4 and 0.3‰ for POR and CP, respectively. Measured intraspecies and within shell variations in the δ^{18}O of shell aragonite are much larger in virtually all cases (see Section 4.2.2). Thus, the full range of variability in the measured isotopic composition within individual mollusks and within

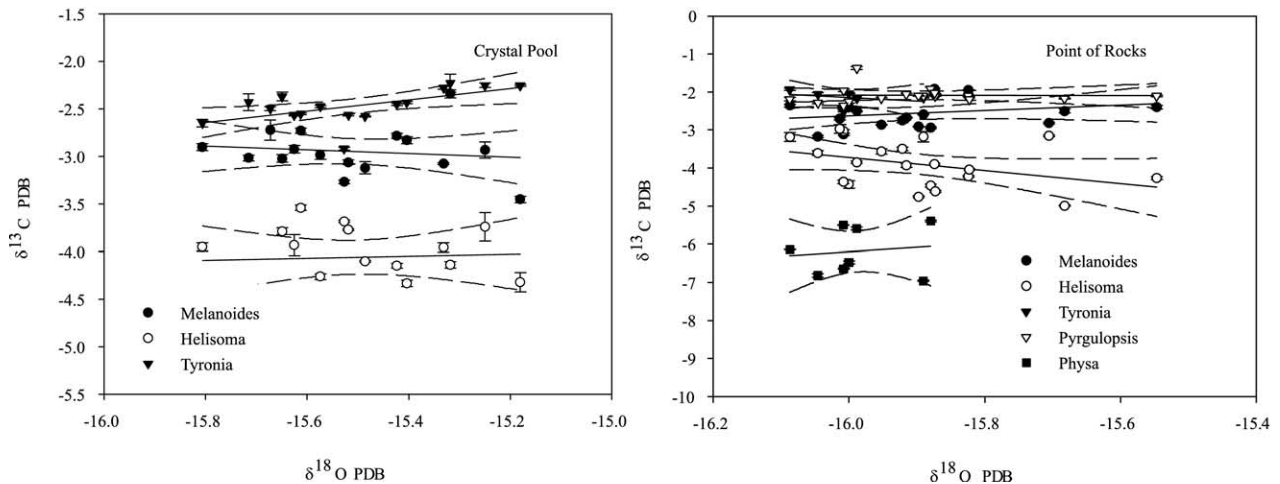

Fig. 4. $\delta^{18}O$ and $\delta^{13}C$ values along sampling transects along the growth axis for individuals of *Melanoides* and *Helisoma*. Each symbol represents the samples from a different individual.

species cannot be explained solely by seasonal variations in water temperature or $\delta^{18}O_{water}$ in the springs.

5.1.3. Biologic disequilibrium (vital effects)

Vital effects can also influence the stable isotopic composition of gastropod shell carbonate of different species, between individuals of the same species, or within the shell of a single individual, Therefore, these effects may explain both the observed variability in shell isotopic composition and the differences between species from this study.

Vital effects during carbonate precipitation should result in covariance between $\delta^{18}O$ and $\delta^{13}C$ values in biologic carbonates, according to both of the leading theories of carbonate formation (Adkins et al., 2003; McConnaughey, 1989b). However, no such relationship is observed in either the data for individual species or for the data within individual shells. Linear regression on the $\delta^{18}O$ data for gastropods from CP and POR (Fig. 5) did not indicate a significant relationship between the $\delta^{18}O$ and $\delta^{13}C$ variations in *Melanoides* (CP: $p = 0.62$; POR: $p = 0.26$), *Helisoma* (CP: $p = 0.18$; POR: $p = 0.26$), *Physa* (POR: $p = 0.54$), *Pyrgulopsis* (POR: $p = 0.20$) or *Tyronia* (CP: $p = 0.37$; POR: $p = 0.46$).

There is also no apparent relationship between the variations in isotopic composition as a function of shell size (Fig. 4). This suggests that the variations in the measured $\delta^{13}C$ and $\delta^{18}O$ values along the shells cannot be attributed to age-related or systematic changes in vital effects, whether caused by changes in growth rate, movement between unidentified microenvironments within the spring, or utilization of different resources (e.g., sources of oxygen, carbon) during different parts of the life cycle. Both of these findings suggest that the observed isotopic variability within species and within individuals cannot be explained by either of the proposed mechanisms for vital effects, though some yet unidentified mechanism cannot be ruled out.

5.2. Isotopic Differences Between Species

The summary data in Table 3 show that, irrespective of the within shell and within species variability in isotopic compositions of the gastropod shells examined during this study, there are substantial differences in the mean values obtained for different species. Two possible causes of these differences are growth under differing environmental conditions and biologic effects. These are discussed below.

5.2.1. Environmental variability

Seasonal variations in the temperature and $\delta^{18}O$ value of the springs may impart significant differences in the equilibrium isotopic composition of gastropod shell carbonate if species or individuals only precipitate their shells during certain months of the year. Table 1 lists the predicted equilibrium $\delta^{18}O$ values for biogenic aragonite produced in the two springs, computed using the various equilibrium expressions. Also listed are the range of possible equilibrium isotopic compositions for shell carbonate, given the measured variability in the spring water temperature and isotopic composition. Measured $\delta^{18}O$ values in gastropod shells are most consistent with predicted equilibrium values of Grossman and Ku (1986) and Boehm et al.

Table 4. Paired measurements of the carbon isotopic composition of snail shell and flesh from Crystal Pool.

Sample	Individual	$\delta^{13}C$ ‰ flesh	$\delta^{13}C$ ‰ shell
Helisoma			
XP1-1	1	−31.36	−3.95
XP1-2	1	−31.10	−4.10
XP2-1	2	−31.42	−4.07
XP2-2	2	−31.10	−3.80
XP2-3	2	−30.97	
XP3-1	3	−30.19	−4.20
XP3-2	3	−30.02	−4.28
XP3-3	3	−30.03	
Melanoides			
XM1-1	1	−25.17	−2.93
XM1-2	1	−26.83	−3.20
XM2-1	2	−25.47	−2.45
XM2-2	2	−24.58	
XM3-1	3	−27.48	−2.30
XM3-2	3	−27.53	−2.16

Table 5. Data on carbonate standards measured using the Kiel at U. Arizona.

Measurement period		δ^{18}O PDB	δ^{13}C PDB	Pressure
February, 2002		−2.18 ± 0.12	1.86 ± 0.02	1.78
		−2.20 ± 0.04	1.92 ± 0.07	3.20
		−2.27 ± 0.04	1.83 ± 0.02	2.51
		−2.20 ± 0.04	1.93 ± 0.04	2.95
		−2.18 ± 0.07	2.06 ± 0.05	2.95
		−2.31 ± 0.03	1.97 ± 0.07	1.68
		−2.20 ± 0.03	1.94 ± 0.03	2.44
		−2.15 ± 0.05	2.03 ± 0.05	2.52
	Avg.	−2.21 ± 0.05	1.94 ± 0.08	2.50 ± 0.55
March, 2002		−2.26 ± 0.05	1.93 ± 0.04	2.05
		−2.19 ± 0.06	1.94 ± 0.03	2.30
		−2.15 ± 0.07	1.96 ± 0.02	2.36
		−2.18 ± 0.09	2.02 ± 0.05	1.87
	Avg.	−2.19 ± 0.05	1.96 ± 0.04	2.14 ± 0.23
May, 2002		−2.17 ± 0.02	1.96 ± 0.05	3.03
		−2.18 ± 0.05	1.99 ± 0.01	2.47
		−2.15 ± 0.07	2.02 ± 0.03	2.67
	Avg.	−2.17 ± 0.02	1.99 ± 0.03	2.72 ± 0.28
January, 2003		−2.25 ± 0.12	1.94 ± 0.02	1.45
		−2.15 ± 0.02	2.00 ± 0.01	2.51
		−2.27 ± 0.07	1.98 ± 0.03	2.26
		−2.29 ± 0.06	1.93 ± 0.05	2.18
		−2.00 ± 0.07	2.06 ± 0.05	2.54
		−2.36 ± 0.13	1.93 ± 0.17	1.23
		−2.11 ± 0.06	1.87 ± 0.05	2.50
		−2.19 ± 0.09	1.90 ± 0.03	2.12
		−2.35 ± 0.05	1.92 ± 0.02	1.89
		−2.15 ± 0.06	1.95 ± 0.02	2.08
		−2.11 ± 0.04	2.01 ± 0.05	1.56
		−2.04 ± 0.03	1.98 ± 0.01	1.79
		−2.17 ± 0.03	1.98 ± 0.04	2.04
		−2.11 ± 0.07	1.94 ± 0.04	2.26
		−2.24 ± 0.15	1.99 ± 0.05	1.44
		−2.38 ± 0.03	1.90 ± 0.04	1.57
		−2.18 ± 0.12	1.99 ± 0.06	2.27
		−2.28 ± 0.07	1.95 ± 0.01	2.15
		−2.26 ± 0.09	1.89 ± 0.02	2.18
		−2.29 ± 0.02	1.95 ± 0.02	2.27
	Avg.	−2.21 ± 0.11	1.95 ± 0.05	2.01 ± 0.39

Standard measured NBS-19 (δ^{18}O = 2.20‰ PDB; δ^{13}C = 1.95‰ PDB).

(2000), both of which are based, at least in part, on isotopic measurements in mollusks. Estimates based on inorganic synthesis of aragonite in the laboratory (McCrea, 1950; Zhou and Zheng, 2003) appear to substantially underestimate δ^{18}O values compared with biologically precipitated aragonite, and are not discussed further.

Using the relationship of Boehm et al. (2000) to estimate equilibrium oxygen isotope fractionation in mollusks, it appears that in some cases, differences in the mean δ^{18}O values may be partially explained by seasonal differences in the timing of shell growth (using the model of Grossman and Ku (1986) yields similar answers with slightly poorer agreement). However, it is also evident that growth seasonality alone is an insufficient mechanism for explaining all observed differences. Using shell carbonate isotopic estimates based on measured changes in spring temperature and $\delta^{18}O_{water}$ values, the model of Boehm et al. (2000) suggests that shells of *Tyronia* and *Pyrgulopsis* grew (in POR) in the summer and fall (July—November $\delta^{18}O_{shell\ predicted}$ = −15.57 to −15.74‰) (Fig. 6), in agreement with the observation that most of their shell material is precipitated in the fall (the largest populations were found in the October and January visits). Values for *Melanoides* from POR indicate shell precipitation in the late winter to early summer (February to May: $\delta^{18}O_{shell\ predicted}$ = −15.85 to −15.95‰), also consistent with the observed time of growth of this species.

In contrast, isotopic results from these same species collected from CP do not appear to reflect growth seasonality. Values for *Melanoides* indicate shell growth midwinter (December to January: $\delta^{18}O_{shell\ predicted}$ = −15.50 to −15.56‰) after the late fall die-off, when no *Melanoides* were observed in either of the springs. The very low values observed for *Tyronia* in CP are too low to be predicted for any season by the model ($\delta^{18}O_{shell\ predicted}$ = −15.72 to −15.82‰), and are least consistent with the observed growth of this species during midwinter.

The $\delta^{18}O_{shell}$ values of *Helisoma* and *Physa* shells are also inconsistent with equilibrium precipitation during the growth

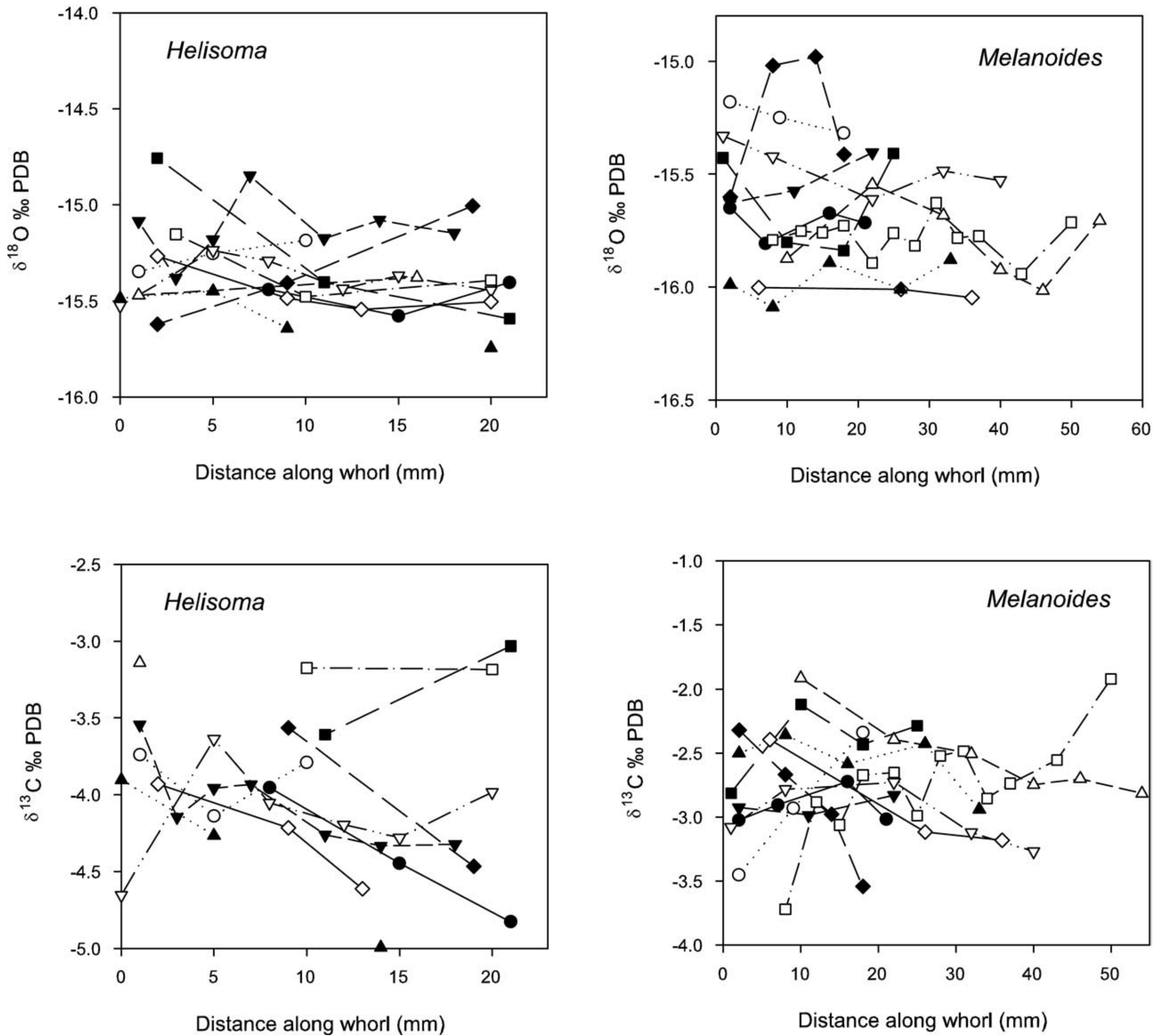

Figure 5. δ^{13}C vs. δ^{18}O values in samples from (a) Crystal Pool and (b) Point of Rocks Spring. Solid lines are regressions through the data for each species. Dashed lines indicate 95% confidence intervals.

season, as was the case for *Tyronia* from CP. Published accounts suggest growth should occur in late spring or summer, consistent with our observations. According to the model, $\delta^{18}O_{shell\ predicted}$ (VPDB) for material precipitated in the summer (June—September) should be around −15.47 to −15.60‰ for CP and −15.76 to −15.83‰ for POR. Measured δ^{18}O values for *Helisoma* from CP were around −15.31‰ and values for *Helisoma* and *Physa* from POR were around −15.36‰. These differences indicate relatively significant (0.2 to 0.5 ‰) departures from equilibrium in these species.

The results from *Melanoides, Tyronia* and *Pyrgulopsis* from POR suggest that some of the isotopic differences between species may be due to growth at different times of the year. The rest of the data indicate that this is not the only controlling factor, and that in at least some situations, it is definitely not the most influential factor. This observation is especially pertinent for the large deviations observed in *Helisoma* and *Physa*, which cannot possibly be caused by the small seasonal changes in spring conditions.

It is clear that within-spring microhabitat differences are also not a major factor in influencing shell chemistry in these springs, and therefore, cannot be the cause of isotopic differences between species. *Tyronia* lives in the nearshore environment, under thick mats of vegetation, where there is the greatest potential for a combination of evaporation and stagnation of isotopically enriched water. Conversely, *Pyrgulopsis* typically lives on rocks at water depths <0.5–1.0 m, and presumably digests mostly algae growing in the spring. However, their δ^{13}C and δ^{18}O values are indistinguishable. This lack of a difference between δ^{18}O values from the two *Hydrobiidae* argues against the possibility of within-spring environmental variations as the cause. Still open to possibility is that some of the differences may be related to biologic factors such as movement in and out of the springs.

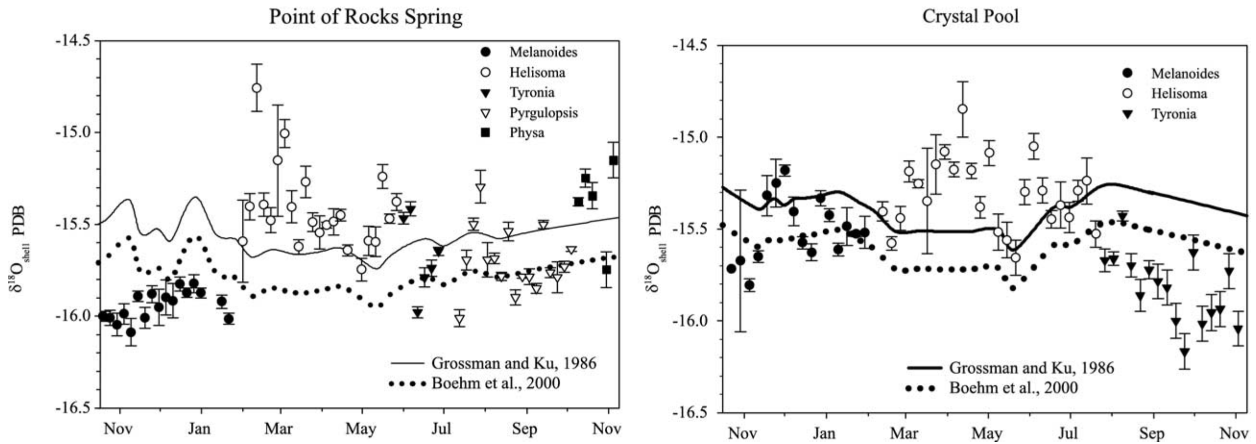

Fig. 6. Predicted $\delta^{18}O$ values of shell material estimated using the equilibrium expressions of Grossman and Ku (1986) (dashed line) and Boehm et al. (2000) (solid line) in (a) POR and (b) CP. $\delta^{18}O$ values of gastropod shells measured during this study are overlain for comparison. *Helisoma* and *Physa* are the pulmonate (lung-breathing snails) whereas *Tyronia, Pyrgulopsis* and *Melanoides* are fully aquatic.

5.2.2. *Biologic factors*

Examination of the data in Table 3 indicates that the largest differences in $\delta^{18}O$ values occur between the lung-breathing and the gill-breathing gastropods. This finding suggests that there may be a biologic control on isotopic composition. Comparison between the measured isotopic results for gastropod shells (Table 3) with computed equilibrium values (Table 1) (assuming as previously that the calibration of Boehm et al. (2000) is most accurate), reveals that the $\delta^{18}O$ values for the gill-breathing gastropods (Prosobranchia: *Melanoides, Hydrobiidae*) lie within the range of predicted values, whereas those for the lung-breathing gastropods (Pulmonata: *Helisoma, Physa*) are significantly higher.

For $\delta^{13}C$, there is unfortunately little data on the carbon isotope composition of DIC in the spring water, so it is difficult to estimate the expected equilibrium $\delta^{13}C$ of shell material. However, using the limited $\delta^{13}C_{DIC}$ values available from Winograd and Pearson (1976) ($\delta^{13}C_{DIC}$ CP: −5.0‰ POR: −4.6 to −4.8‰) and the inorganic aragonite- HCO_3^- fractionation factor of Romanek et al. (1992), we estimate that equilibrium $\delta^{13}C$ values for shells should be in the range of −1.91 to −2.11‰ in POR and −2.31‰ (based on a single $\delta^{13}C$ measurement) in CP (Fig. 7). Comparison with measured values for the gastropods (Table 2) reveals that most of the gastropods have $\delta^{13}C$ values that are more negative than expected. Because the calculations are based on limited $\delta^{13}C_{DIC}$ data that were collected several decades before this study, it is difficult to draw any conclusions regarding the small differences between the isotopic composition of the gill-breathing gastropods and calculated equilibrium values. However, the large (several permil) differences between the pulmonate gastropods and estimated equilibrium values are not likely to be the result of inaccuracies in our estimates of equilibrium $\delta^{13}C$ and indicate that lung-breathing gastropods precipitate their shells with much lighter $\delta^{13}C$ values than expected.

The observed differences between lung- and gill-breathing

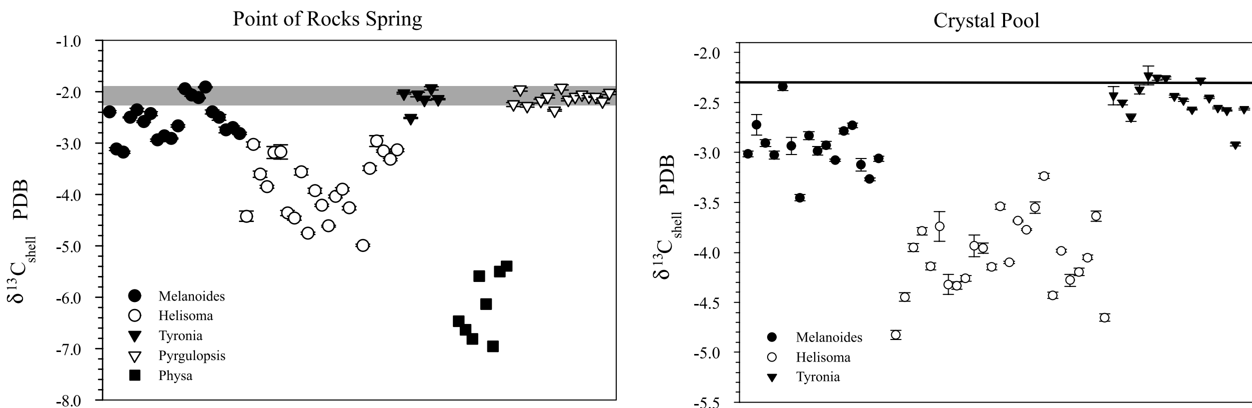

Fig. 7. Predicted $\delta^{13}C$ values of shell material estimated using the equilibrium expression of Romanek (1992) (grey box in a, solid line in b, indicating the different uncertainties in the estimates for the two pools) and the measured $\delta^{13}C$ of gastropod shells measured during this study in (a) POR and (b) CP. *Helisoma* and *Physa* are the pulmonate (lung-breathing snails) whereas *Tyronia, Pyrgulopsis* and *Melanoides* are Prosobranch (gil-breathing snails).

gastropods may suggest the presence of a real vital effect related to the fractionation of isotopes during incorporation of carbon and oxygen in shell material, or it may reflect differences in the life histories of the different species (i.e., movement in and out of the water and utilization of different resources). For *Physa*, life history does appear to be a key factor, as individuals were commonly sampled from the stems of reeds sticking out of the water. In this case, higher average $\delta^{18}O$ values displayed by *Physa* likely reflect the growth of shells in a highly evaporative environment, when the snails emerged from the springs. The lower $\delta^{13}C$ values observed in *Physa* could then be the result of incorporation of atmospheric CO_2 rather than CO_2 in the spring water. This supported by the similarity between the $\delta^{13}C$ of *Physa* and that of land snails collected by previous workers at Ash Meadows (−5.0 to−10.2‰ VPDB) (McConnaughey et al., 1997).

Growth in semiaquatic habitats during at least some parts of its life cycle may also explain the $\delta^{18}O$ values in *Helisoma*, which are intermediate between *Physa* and the gill-breathing, fully aquatic gastropods. *Helisoma* may reflect some combination of residence in the aquatic environment and time spent outside of the springs. However, our observations of *Helisoma* populations are not consistent with this hypothesis. At all times during our study, both *Helisoma* and *Melanoides* occupied very similar positions within the spring pools, on rocks at water depths greater than 25–50 cm. We did not observe individuals of these taxa leaving the spring pool at any time (unlike *Physa*). Furthermore, the variability in $\delta^{18}O$ values within shells and between individuals of *Helisoma* is indistinguishable from that of the fully aquatic gastropods. If the intermediate $\delta^{18}O$ values are a function of the averaging of shell material produced in an aquatic environment and in the atmosphere, one would expect a greater range of isotopic values. Together, these observations suggest that the isotopic values for *Helisoma* are not likely to be a result of migration out of the springs.

Anomalous $\delta^{13}C$ values in *Helisoma* may, however, reflect fundamental differences in the sources of CO_2 used by aquatic and pulmonate organisms. According to the respiratory gas exchange model of McConnaughey et al. (1997), more metabolic carbon will be incorporated into the shells of lung-breathing invertebrates than into the shells of gill-breathing invertebrates because of the higher CO_2:O_2 ratios in water than in air. Assuming that O_2 exchange during gill breathing is accompanied by CO_2 exchange, in gill breathers, a larger proportion of the CO_2 available for shell construction will be derived from the external environment than from respiration. Consequently, the carbon in their shells will be similar to the $\delta^{13}C$ of DIC in the environment. In lung breathers, however, the internal carbon pool is predicted to be less diluted by environmental CO_2, and will more strongly reflect the impact of isotopically depleted respiratory CO_2.

The Ash Meadows system offers a unique opportunity to test this model. According to McConnaughey et al. (1997), the ratio of environmental to respiratory CO_2 incorporated in skeletal material (*E/R*) can be estimated by the following:

$$E/R = (P_c/P_O)\frac{[CO_2]_W}{[O_2]_W}\left(1 - \frac{[O_2]_b}{[O_2]_W}\right)^{-1}$$

where the term P_c/P_o is the ratio between the epithelial permeability of carbon to that of oxygen ($P_c/P_o = 20$), and $[x]_i$, ($x = CO_2, O_2$; $i = W,b$) is the concentration of carbon dioxide and oxygen in water and blood. McConnaughey et al. (1997) suggests that the ratio of blood oxygen to ambient oxygen in invertebrates is around 0.9. Using previously measured values of alkalinity and pH, we estimate the concentration of CO_2 in water to average around 285 micromolar in CP, an extremely high value which we attribute to subsurface sources (U.S.G.S., 2005). Measured dissolved oxygen concentrations in CP were around 87.5 ± 13 micromolar (U.S.G.S., 2005), yielding a water $[CO_2]/[O_2]$ ratio of around 2.5. This leads to an E/R of ca. 580, implying that effectively all of their carbon should be derived from environmental sources (i.e., DIC).

A comparison between the carbon isotopic composition of snail flesh and snail shells (Table 4) supports the hypothesis that respired carbon is a minor component in these shells, but also suggests that the respiration model overestimates the influence of spring oxygen concentrations on the relative proportions of respired and environmental carbon incorporated into shells. Based on the carbon isotope ratios of DIC in spring water at CP (−5.0‰), the average isotopic composition of snail flesh in Table 5, and the average isotopic composition of snail shell material (Table 2), the relative contributions of environmental and respiratory carbon sources can be computed. For *Melanoides*, respiration contributes around 2% of the carbon, leading to an E/R of 39.3. In *Helisoma*, 7% may have been contributed from respiration, and the E/R is 14. These ratios are much lower than predicted by the model, and suggest either that the model or some of its parameterizations are incorrect, or that some other unknown factor is controlling the offset between predicted and measured $\delta^{13}C$. Regardless, the differences in the E/R for *Melanoides* and *Helisoma* are consistent with the expectation that the semiaquatic gastropods may utilize larger amounts of respired carbon due to the duration of time spent out of water, where $[CO_2]/[O_2]$ ratios are much smaller.

The elevated $\delta^{18}O$ values are also difficult to explain. According to both the "kinetic" and the "carbonate" models, vital effects should produce lower $\delta^{18}O$ values in carbonates than those formed at equilibrium. Furthermore, differences in $\delta^{18}O$ values between species do not appear to be constant between the springs. For example, differences in $\delta^{18}O$ values between *Melanoides* and *Helisoma* are larger in CP (~0.5‰) than in POR (0.2‰). Since these observations are not consistent with existing hypotheses, an alternative model for biologic fractionation is needed to explain the difference between lung-breathing and gill-breathing gastropods. More rigorous laboratory experiments examining the impact of physiology on shell chemistry are needed to address this issue.

6. SUMMARY

The results of this study suggest that there can be substantial variations in the isotopic composition of gastropod shell carbonate even in environments with nearly constant temperature and isotopic composition. Gastropod shells collected from geological deposits, where species are likely to have been subject to much more environmental variability, both spatially and temporally (seasonally and annually), should record even larger variations. In addition, bioturbation and postdepositional reworking may result in mixing of sedimentary deposits, so that

shells collected from the same geological unit may differ in age by decades to millennia.

Our results also indicate that not all gastropod species precipitate their shells in equilibrium with host water. Even in our study, springs with uniform environmental conditions, variation in isotopic composition exceeded predicted values. Furthermore, δ^{18}O and δ^{13}C values for gastropod shells exhibited significant internal variations, which are too large to result simply from seasonal differences in the timing of shell precipitation, and show no apparent systematic relationship with shell size. Since these effects are seen in both δ^{13}C and δ^{18}O values, it is possible that these variations reflect vital effects or kinetic effects, though the lack of expected covariation between the two isotopes may mean that the mechanism is different from that suggested by other studies (Adkins et al., 2003). It is clear that the observed isotopic variations limit the usefulness of this technique for reconstructing past environmental changes for situations where the magnitude of these changes is relatively small ($<$1–2‰). Furthermore, the internal variability within shells highlights the problem of interpreting isotopic changes from small subsamples of shell material, as can be measured with devices such as the automated Kiel device. Until the causes of these microscale isotopic variations are better understood, measurement of values from homogenized powders of whole shell material may be more appropriate in studies using isotopes in gastropod carbonate to reconstruct past environmental conditions. Workers attempting to conduct high resolution studies using microsampling techniques should be cautious about the potential for non-environmentally related isotopic variations.

δ^{13}C values of gastropod shells were variable, with some species exhibiting fractionations that agree within uncertainties with predicted equilibrium values, and others showing large (1 to 4 ‰) depletions in ^{13}C. Though the cause of these lower δ^{13}C values is unknown, they are similar in magnitude to those seen in *Unionidae* (Dettman et al., 1999), and may represent the incorporation of metabolic carbon. If so, they indicate that metabolic effects may be more substantial in some aquatic mollusks than previously recognized (Fritz and Poplawski, 1974).

The largest deviations between the isotopic composition of mollusk shell aragonite and predicted equilibrium values appears to occur in the lung-breathing, pulmonate gastropods (*Helisoma duryi, Physa virgata*), whereas near equilibrium values are displayed by the gill-breathing species *Melanoides tuberculata* and the two genera of *Hydrobiidae*. This result suggests that physiology may play an important role in the degree of isotopic equilibrium of shell carbonate. However, we note that these conclusions are based on mean values from the suite of individual measurements. High degrees of internal variability are seen in all the species studied. Therefore, differences in physiology cannot fully account for the anomalous results presented here. Additional work on the internal variability of biologic carbonates, their causes, and the relationships between isotopic values and factors such as ambient temperature, pH, growth rate, seasonality and other causes of vital effects, is needed in the future.

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REFERENCES

Aboul-Ela I. A. and Beddiny E. A. M. (1980) Factors affecting the reproductive capacity and population dynamics of *Helisoma duryi* (Wetherby) 1. Effect of temperature. *J. Egypt. Soc. Parasitol.* **10**,(135–150).

Adkins J. F., Boyle E. A., Curry W. B. and Lutringer A. (2003) Stable isotopes in deep-sea corals and a new mechanism for "vital effects". *Geochim. Cosmochim. Acta* **67 (6),** 1129–1143.

Aharon P. and Chappell J. (1983) Carbon and oxygen isotope probes of reef environment histories. In *Perspectives on Coral Reefs* (ed. D. J. Barnes), pp. 1–15. Australian Institute of Marine Science.

Berry A. J. and Kadri A. H. (1974) Reproduction in the Malaysian freshwater cerithiacean gastropod Melanoides tuberculata. *J. Zoolog. Soc. Lond.* **172,** 369–381.

Boehm F., Joachimski M. M., Dullo W.-C., Eisenhauer A., Lehnert H., Reitner J. and Worheid G. (2000) Oxygen isotope fractionation in marine aragonite of coralline sponges. *Geochim. Cosmochim. Acta* **64,** 1695–1703.

Bottinga Y. (1968) Calculation of fractionation factors for carbon and oxygen isotopic exchange in the system calcite-carbon dioxide-water. *J. Chem. Phys.* **72,** 800–808.

Brand U., Logan A., Hiller N. and Richardson J. (2003) Geochemistry of modern brachiopods: applications and implications for oceanography and paleoceanography. *Chem. Geol.* **198 (3–4),** 305–334.

Brandt R. A. M. (1974) The non-marine aquatic Mollusca of Thailand. *Arch Mollusk.* London. **105,** 1–423.

Brown D. S. (1980) Freshwater snails of Africa and their medical importance. Taylor & Francis. Sunderland, Massachusetts.

Brusca R. C. and Brusca G. J. (1990) Invertebrates. Sinauer Associates.

Chacko T., Mayeda T. K., Clayton R. N. and Goldsmith J. R.(1991) Oxygen and carbon isotope fractionation between CO_2 and calcite. *Geochim. Cosmochim. Acta* **55,** 2867–2882.

Cole J. E., Dunbar R. B., McClanahan T. R. and Muthiga N. A. (2000) Tropical Pacific Forcing of decadal SST variability in the Western Indian Ocean over the past two centuries. *Science* **287,** 617–619.

Curry G. B. and Fallick A. E. (2002) Use of stable oxygen isotope determinations from brachiopod shells in palaeoenvironmental reconstruction. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **182 (1–2),** 133–143.

de Kock K. N. and Joubert P. H. (1991) Life-table experiments with *Helisoma duryi* (Wetherby) and *Biomphalaria pfeifferi* (Krauss) at constant temperatures. *S. Afric. J. Zoo.* **26 (4),** 149–152.

Deniro M. J. and Epstein S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* **42,** 495–506.

Dettman D. L. and Lohmann K. C. (2000) Oxygen isotope evidence for high altitude snow in the Laramide Rocky Mountains of North America during the late Cretaceous and Paleogene. *Geology* **28,** 243–246.

Dettman D. L., Reische A. K. and Lohmann K. C. (1999) Controls on the stable isotope composition of seasonal growth bands in freshwater bivalves (unionidae). *Geochim. Cosmochim. Acta* **63 (7/8),** 1049–1057.

Dudgeon D. (1986) The life cycle, population dynamics and productivity of *Melanoides tuberculata* (Muller 1774) (Gastropoda: Prosobranchia: Thiaridae) in Hong Kong. *J. Zoo. Lond.* **208,** 37–53.

El-Emam M. A. and Madsen H. (1982) The effect of temperature, darkness, starvation and various food types on growth, survival and

reproduction of *Helisoma duryi, Biomphalaria alexandria* and *Bulinus truncatus* (Gastropod: Planorbidae). *Hydrobiol.* **88,** 265–275.

Elliot M., Labeyrie L. and Duplessy J. C. (2002) Changes in North Atlantic deep-water formation associated with the Dansgaard-Oeschger temperature oscillations (60–10 ka). *Quat. Science Rev.* **21 (10),** 1153–1165.

Epstein S., Buchsbaum R. and Lowenstam H. (1953) Carbonate-water isotopic temperature scale. *Geol. Soc. Amer. Bull.* **62,** 417–425.

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

Furla P., Galgani I., Durand I. and Allemand D. (2000) Sources and mechanisms of inorganic carbon transport for coral calcification and photosynthesis. *J. Exp. Bio.* **203 (22),** 3445–3457.

Gasse F. (2002) Diatom-inferred salinity and carbonate oxygen isotopes in Holocene waterbodies of the western Sahara and Sahel (Africa). *Quat. Sci. Rev.* **21 (7),** 737–767.

Gonfiantini R., Stichler W. and Rozanski K. (1995) Standards and intercomparison materials distributed by the International Atomic Energy Agency for stable isotope measurements. In *Reference and intercomparison materials for stable isotopes of light elements* pp. 13–29. I.A.E.A. Techdoc-825.

Goodfriend G. A. (1992) The use of land snails in paleoenvironmental reconstructions. *Quat. Sci. Rev.* **11,** 665–682.

Grossman E. L. (1987) Stable isotopes in modern benthic foraminifera: A study of vital effects. *J. Foramin. Res.* **17,** 48–61.

Grossman E. L. and Ku T. L. (1986) Oxygen and caron isotope fractionation in biogenic aragonite: temperature effects. *Chem. Geol. (Isotope Geosciences Section)* **59,** 59–74.

Guilderson T. P., Fairbanks R. G. and Rubenstone J. L (2001) Tropical Atlantic coral oxygen isotopes: glacial-interglacial sea surface temperatures and climate change. *Mar. Geol.* **172 (1–2),** 75–89.

Herschler R. (1998) A systematic review of the Hydrobiid snails (Gastropoda: RIssooidea) of the Great Basin, Western Uniter States. Part I Genus Pyrgulopsis *The Veliger* **41 (1),** 1–132.

Herschler R. and Sada D. W.(1987) Springsnails (Gastropoda: *Hydrobiidae*) of Ash Meadows, Amargosa Basin, California-Nevada. *Proc. Bio. Soc. Wash.* **100 (4),** 776–843.

Ikeda T. and Tajika E. (2003) Carbon cycling and climate change during the last glacial cycle inferred from the isotope records using an ocean biogeochemical carbon cycle model. *Global Planet. Change* **35** ((**1–2),** 131–141.

Jimenez-Lopez C., Caballero E., Huertas F. J. and Romanek C. S. (2001) Chemical, mineralogical and isotope behavior and phase transformation during the precipitation of calcium carbonate minerals from intermediate ionic solution at 25 degrees C. *Geochim. Cosmochim. Acta* **65 (19),** 3219–3231.

Kalish J. M. (1991) ^{13}C and ^{18}O isotopic disequlibria in fish otoliths: Metabolic and kinetic effects. *Mar. Ecol.-Progr. Ser.* **75,** 191–203.

Kim S. T. and O'Neil J. R. (1997) Equilibrium and nonequilibrium oxygen isotope effects in synthetic carbonates. *Geochim. Cosmochim. Acta* **61,** 3461–3475.

Kunigelis S. C. and Saleuddin A. S. M. (1978) Regulation of shell growth in the pulmonate gastropod *Helisoma duryi*. *Can. J. Zoo.* **56,** 1975–1980.

Kunigelis S. C. and Saleuddin A. S. M. (1983) Shell repair rates and carbonic anhydrase activity during shell repair in *Helisoma duryi* (Mollusca). *Can. J. Zoo.* **61,** 597–602.

Laczniak R. J., DeMeko G. A., Reiner S. R., Smith J. L. and Nylund W. E. (1999) Estimates of ground-water discharge as determined from measurements of evapotranspiration, Ash Meadows area, Nye County, Nevada. In *U. S. G. S. Open File Report.*

Lee D. and Carpenter S. J. (2001) Isotopic disequilibrium in marine calcareous algae. *Chem. Geology* **172 (3–4),** 307–329.

Leveque C. (1971) Equation de Von Bertalanffy et croissance des mollusques benthiques du lac Tchad. *Cahiers d'ORSTROM Sèrie Hydrobiol.* **5,** 263–283.

Livshits G. and Fisgelson L. (1983) Biology and reproduction of the freshwater snail Melanoides tuberculata (Gastropoda: Prosobranchia) in Israel. *Israel J. Ecol.* **32,** 21–35.

Maslin M. A., Thomas E., Shackleton N. J., Hall M. A. and Seidov D. (1997) Glacial northeast Atlantic surface water p(CO_2): Productivity and deep-water formation. *Mar. Geol.* **144 (1–3),** 177–190.

McConnaughey T. (1989a) ^{13}C and ^{18}O isotopic disequilibrium in biological carbonates: I. Patterns. *Geochim. Cosmochim. Acta* **53,** 151–162.

McConnaughey T. (1989b) ^{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 T. A. (2002) Heavy isotope deficiencies in corals: Kinetic and McCrea models. *Geochim. Cosmochim. Acta* **66 (15A),** A498.

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

McConnaughey T. A., Burdett J., Whelan J. F. and Paull C. K. (1997) Carbon isotopes in biological carbonates: Respiration and photosynthesis. *Geochim. Cosmochim. Acta* **61 (3),** 611–622.

McCrea J. M. (1950) On the isotopic chemistry of carbonates and a paleotemperature scale. *J. Chem. Phys.* **18,** 849–857.

McMahon R. F. (1975) Effects of artificially elevated water temperatures on the growth, reproduction and life cycle of a natural population of *Physa virgata* gould. *Ecol.* **56 (5),** 1167–1175.

McMahon R. F. and Payne B. S. (1980) Variation of thermal tolerance limits in populations of Physa virgata Gould (Mollusca: Pulmonata). *Am. Mid. Natural.* **103 (2),** 218–230.

Mladenka G. C. and Minshall G. W. (2001) Variation in the life history and abundance of three populations of Bruneau hot spring snails. *W. N. Amer. Natural.* **61 (2),** 204–212.

Mueller-Lupp T., Erlenkeuser H. and Bauch H. A. (2003) Seasonal and interannual variability of Siberian river discharge in the Laptev Sea inferred from stable isotopes in modern bivalves. *Boreas* **32 (2),** 292–303.

Mulcahy S. A., Killingley J. S., Phleger C. F. and Berger W. H. (1979) Isotopic composition of otoliths from a benthopelagic fish, *Coryphaenoides acrolepis,* Macrouridae: Gadiformes. *Oceanol. Acta* **2,** 423–427.

O'Brien C. and Blinn D. W. (1999) The endemic spring snail *Pyrgulopsis mentezumensis* in a high CO2 environment: Importance of extreme chemical habitats as refugia. *Fresh. Biol.* **42,** 225–234.

O'Neil J. R. (1986) Theoretical and experimental aspects of isotopic fractionation. *Rev. Mineral.* **16,** 1–40.

O'Neil J. R., Clayton R. N. and Mayade T. K. (1969) Oxygen isotope fractionation in divalent metal carbonates. *J. Chem. Phys.* **51,** 5547–5558.

Owen R., Kennedy H. and Richardson C. (2002) Isotopic partitioning between scallop shell calcite and seawater: Effect of shell growth rate. *Geochim. Cosmochim. Acta* **66 (10),** 1727–1737.

Patterson W. P., Smith G. R. and Lohmann K. C. (1993) Continental paleothermometry and seasonality using the isotopic composition of aragonitic otoliths of freshwater fishes. In *Climate Change in Continental Isotopic Records* (ed. P. K. Swart, K. C. Lohmann, J. McKenzie and S. Savin) Vol. 78 pp. 191–202.

Paull C. K., Chanton J. P., Martens C. S., Fullager P. D., Neumann A. C. and Coston J. A. (1991) Seawater circulation through the flank of the Florida platform: Evidence and implications. *Mar. Geol.* **102,** 265–279.

Paull C. K., Chanton J. P., Neumann A. C., Coston J. A., Martens C. S. and Showers W. (1992) Indicators of methane derived carbonates and chemosynthetic organic carbon deposits: Examples from the Florida escarpment. *Palaios* **7,** 361–375.

Paull C. K., Martens C. S., Chanton J. P., Neumann A. C., Coston J., Jull A. J. T. and Toolin L. J. (1989) Old carbon in living organisms and young $CaCO_3$ cements from abyssal brine seeps. *Nature* **342,** 166–168.

Pointier J. P., Delay B., Toffart J. L., Lefèvre M. and Romero-Alvarez R. (1992) Life history traits of three morphs of *Melanoides tuberculata* (Gastropoda: Thiaridae), an invading snail in the French West Indies. *J. Mollusc. Stud.* **58,** 415–423.

Pointier J.-P., Théron A. and Borel G. (1993) Ecology of the introduced snail *Melanoides tuberculata* (Gastropoda: Thiaridae) in relation to Biomphalaria glabrata in the marshy forest zone of Guadeloupe, French West Indies. *J. Mollusc. Stud.* **59 (4),** 421–428.

Radtke R. L., Williams D. F. and Hurley P. C. (1987) The stable isotopic composition of bluefin tuna (*Thunnas thynnus*) otoliths:

Evidence for physiological regulation. *Comp. Biochem. Physiol.* **87A**, 797–801.

Rice J. A. (1995) Mathematical Statistics and Data Analysis. Duxbury Press.

Riggs A. C. (1984) Major carbon-14 deficiency in modern snail shells from southern Nevada springs. *Science* **224,** 58–61.

Romanek C. S., Grossman E. L. and Morse J. W. (1992) Carbon isotope fractionation in synthetic aragonite and calcite: Effects of temperature and precipitation rate. *Geochim. Cosmochim. Acta* **56,** 419–430.

Russell-Hunter W. D. (1978) Ecology of freshwater pulmonates. In *Pulmonates: Systematics, evolution and ecology* (eds. V. Fretter and J. F. Peake) Vol. 2A. Academic Press.

Saleuddin A. S. M., Kunigelis S. C., Khan H. R. and Jones G. M. (1980) Possible control mechanisms in mineralization in Helisoma duryi (Mollusca: Pulmonata). In *The mechanisms of biomineralization in animals and plants* (eds. M. Omori and N. Watabe), pp. 121–129. Proceedings 3rd International Biomineralization Symposium.

Schaffner F. C. and Swart P. K. (1991) Influence of diet and environmental water on the carbon isotopic signatures of seabird eggshell carbonate. *Bull. Mar. Sci.* **48,** 23–38.

Shoeninger M. J. and Deniro M. J. (1982) Carbon isotope ratios of apatite from fossil bones cannot be used to reconstruct diets of animals. *Nature* **297,** 577–578.

Spero H. J., Bijma J., Lea D. W. and Bemis B. E. (1997) Effect of seawater carbonate concentration on foraminiferal carbon and oxygen isotopes. *Nature* **390,** 497–500.

Sullivan C. H. and Krueger H. W. (1981) Carbon isotope analysis of separate chemical phases in modern and fossil bone. *Nature* **292,** 333–335.

Tanaka N., M. C. Monaghan and Rye D. M. (1986) Contribution of metabolic carbon to mollusc and barnacle shell carbonate. *Nature* **320,** 520–523.

Tarutani T., Clayton R. N. and Mayade T. K. (1969) The effect of polymorphism and magnesium substitution on oxygen isotope fractionation between calcium carbonate and water. *Geochim. Cosmochim. Acta* **33,** 987–996.

Thorrold S. R., Campana S. E., Jones C. M. and Swart P. K. (1997) Factors determining $\delta^{13}C$ and $\delta^{18}O$ fractionation in aragonitic otoliths of marine fish. *Geochim. Cosmochim. Acta* **61,** 2909–2919.

Urey H. C. (1947) The thermodynamical properties of isotopic substances. *J. Chem. Soc.* 562–581.

U.S.G.S. (2005) National Water Information System (NWISWeb) data available on the World Wide Web, Vol. 2005.

Veinott G. I. and Cornett R. J. (1998) Carbon isotopic disequilibrium in the shell of the freshwater mussel *Elliptio complanata*. *Appl. Geochem.* **13 (1),** 49–57.

von Grafenstein U., Erlernkeuser H. and Trimborn P. (1999) Oxygen and carbon isotopes in modern fresh-water ostracod valves: assessing vital offsets and autecological effects of interest for palaeoclimate studies. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **148 (1–3),** 133–152.

Von Shirnding Y., Merwe N. J. v. d. and Vogel J. C (1982) Influence of diet and age on carbon isotope ratios of ostrich eggshell. *Archaeom.* **24,** 3–20.

Walker G. E. and Thomas E. (1963) Geology and ground water of Amargosa Desert, Nevada-California., pp. 58. Nevada Department of Conservation and Natural Resources Ground-Water Resources.

White R. M. P., Dennis P. F. and Atkinson T. C. (1999) Experimental calibration and field investigation of the oxygen isotopic fractionation between biogenic aragonite and water. *Rapid Comm. Mass Spectrom.* **13,** 1242–1247.

Wilbur K. M .(1964) Shell formation and Regeneration. In *Physiol. Mollusc.* (ed. K. M. Wilbur and C. M. Yonge), Vol. 1. Academic Press.

Wilbur K. M. and Saleuddin S. M.(1983) Shell Formation. In *Physiology* (ed. K. M. Wilbur and A. S. M. Saleuddin), Vol. 4. Academic Press.

Winograd I. and Friedman I. (1972) Deuterium as a tracer of regional ground-water flow, southern Great Basin, Nevada-California. *Bull. Geol. Soc. Amer.* **83,** 3691–3708.

Winograd I. J. and Pearson F. J. (1976) Major Carbon 14 anomaly in a regional carbonate aquifer: possible evidence for megascale channeling, south central Great Basin. *Water Res. Res.* **12 (6),** 1125–1143.

Winograd I. J. and Thordarson W. (1975) Hydrogeologic and hydrochemical framework, South-central Great Basin, Nevada and California, with special reference to the Nevada Test Site, Nevada. *U.S.G.S. Prof. Paper* **712-C,** 1–126.

Wong V. and Saleuddin A. S. M. (1972) Fine structure of normal and regenerated shell of *Helisoma duryi duryi*. *Can. J. Zoo.* **50,** 1563–1568.

Wurster C. M. and Patterson W. P. (2001) Seasonal variation in stable oxygen and carbon isotope values recovered from modern lacustrine freshwater mollusks: Paleoclimatological implications for sub-weekly temperature records. *J. Paleolimnol.* **26 (2),** 205–218.

Wurster C. M. and Patterson W. P. (2003) Metabolic rate of late Holocene freshwater fish: evidence from delta C-13 values of otoliths. *Paleobiol.* **29 (4),** 492–505.

Zheng Y.-F. (1999) Oxygen isotope fractionation in carbonate and sulfate minerals. *Geochem. J.* **33,** 109–126.

Zhou G.-T. and Zheng Y.-F. (2002) Kinetic mechanism of oxygen isotope disequilibrium in precipitated witherite and aragonite at low temperatures: An experimental study. *Geochim. Cosmochim. Acta* **66 (1),** 63–71.

Zhou G.-T. and Zheng Y.-F. (2003) An experimental study of oxygen isotope fractionation between precipitated aragonite and water at low temperatures. *Geochim. Cosmochim. Acta* **67 (3),** 387–399.