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The influence of diet on the $\delta^{13}C$ of shell carbon in the pulmonate snail *Helix aspersa*

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Abstract

The influence of diet and atmospheric CO_2 on the carbon isotope composition of shell aragonite and shell-bound organic carbon in the pulmonate snail *Helix aspersa* raised in the laboratory was investigated. Three separate groups of snails were raised on romaine lettuce (C3 plant, $\delta^{13}C = -25.8‰$), corn (C4 plant, $\delta^{13}C = -10.5‰$), and sour orange (^{12}C-enriched C3 plant, $\delta^{13}C = -39.1‰$). The isotopic composition of body tissues closely tracked the isotopic composition of the snail diet as demonstrated previously. However, the isotopic composition of the acid insoluble organic matrix extracted from the aragonite shells does not track diet in all groups. In snails that were fed corn the isotopic composition of the organic matrix was more negative than the body by as much as 5‰ whereas the matrix was approximately 1‰ heavier than the body tissues in snails fed a diet of C3 plant material. These results indicate that isotopic composition of the organic matrix carbon cannot be used as an isotopic substrate for paleodietary reconstructions without first determining the source of the carbon and any associated fractionations. The isotopic composition of the shell aragonite is offset from the body tissues by 12.3‰ in each of the culture groups. This offset was not influenced by the consumption of carbonate and is not attributable to the diffusion of atmospheric CO_2 into the hemolymph. The carbon isotopic composition of shell aragonite is best explained in terms of equilibrium fractionations associated with exchange between metabolic CO_2 and HCO_3 in the hemolymph and the fractionation associated with carbonate precipitation. These results differ from previous studies, based primarily on samples collected in the field, that have suggested atmospheric carbon dioxide contributes significantly to the shell $\delta^{13}C$. The culture results indicate that the $\delta^{13}C$ of aragonite is a good recorder of the isotopic composition of the snail body tissue, and therefore a better recorder of diet than is the insoluble shell organic carbon. Because the systematic fractionation of carbon isotopes within the snail is temperature dependent, the $\delta^{13}C$ of the shell could provide an independent technique for estimating paleotemperature changes.

Keywords: Pulmonata; C-13/C-12; diet; shells; paleoclimatology

1. Introduction

The isotopic composition of carbon incorporated into the shells of carbonate secreting organisms is a potential source of paleoenvironmental information. In pulmonate snails the carbon isotopic composition of organic matter encapsulated in the carbonate shell may provide a quantifiable estimate of the types of the vegetation that the organism consumed [1]. This would make it pos-

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sible to reconstruct distribution patterns of C3- and C4-type plants from isotopic analyses of fossil terrestrial snails, providing a valuable tool for paleoclimate research [2,3].

In practice these isotopically based paleoenvironmental reconstructions are not straightforward [3]. Assumptions must be made about a snail's feeding behavior and its physiology in order to reconstruct a snails diet from isotopic results. For example, attempts to use snail $\delta^{13}C$ values to estimate distribution patterns of C4- and C3-type vegetation have assumed that terrestrial snails eat plants indiscriminately and in proportion to the abundances of these plants in the environment [2,4–6]. It has also been assumed that all C3 plants within a community have similar carbon isotopic compositions and that there is an essentially constant $\delta^{13}C$ difference between C3- and C4-type plants. Another assumption that must be made is that there is no isotopic fractionation between a snail and its diet, and in particular between the diet and the snail shell organic matrix. These assumptions are necessary in order to construct a mass balance calculation, which accounts for the contribution of ^{12}C from C3- and from C4-type vegetation. This calculation is formulated as follows:

$$\delta^{13}C \text{ snail} = \delta^{13}C_{C3plant} * (X) + \delta^{13}C_{C4plant}(1-X)$$

where: X = the fraction of C3-type vegetation consumed by the snail = amount of C3 vegetation present in the snails environment.

$\delta^{13}C_{C3plant}$ = isotopic composition of C3 vegetation (typically $-25‰$).

$\delta^{13}C_{C4plant}$ = isotopic composition of C4 vegetation (typically -10 to $-14‰$).

In natural settings the difference in isotopic composition between snails living on pure C3 vegetation versus those that consume a C3+C4 mixed diet differs by as little as 1‰ to 3‰ (e.g. [6]). Given this relatively small isotopic difference it is critical that the assumptions used in the mass balance calculation are confirmed. Even a small 1‰ or 2‰ error in one of the assumptions could significantly effect, or even invalidate, a paleoenvironmental reconstruction.

There are very few data available for laboratory control experiments where snails have been fed diets of fixed isotopic composition. In one experiment DeNiro and Epstein [1] grew *Helix aspersa* on lettuce (C3-type plant, $\delta^{13}C = -26‰$) and $CaCO_3$ or $CaPO_4$. The isotopic compositions of the mantle, foot and the insoluble organic matter extracted from the shells were found to be similar to the lettuce, but were consistently offset by approximately $+1.0‰$. The reason for the offset was not established although the authors suggested that the isotopic composition of uric acid and/or the mucus secreted by the snail might have a different isotopic value that could account for offset. The CO_2 respired by the snail did not differ isotopically from that of the body. From these limited experimental data it is not possible to know whether all snails exhibit similar isotopic relationships to their diet and whether the 1‰ offset observed is constant.

The present study has attempted to address these two questions by culturing three groups of *H. aspersa*, each fed an isotopically different diet. The objective of the study was to test the constancy of the isotopic relationships between snail body tissues and the shell organic matrix that is preserved in fossilized specimen. The second objective was to constrain the isotopic fractionation between the snail's diet and the carbon incorporated into the carbonate shells and to evaluate whether atmospheric $^{12}CO_2$ contributes to the internal pool of CO_2 used to precipitate carbonate.

2. Materials and methods

2.1. Culturing

Juvenile *H. aspersa* were collected for culture from the Palos Verdes Peninsula, Los Angeles County, during the spring. During the spring and early summer seven snails were cultured in a 10-gallon glass aquarium, covered with perforated aluminum foil. At the time of collection each snail shell was marked to indicate the part of the shell formed prior to culturing. These snails were cultured for 4 months on romaine lettuce (C3-type plant) and tap water ad libitum. Four of the seven snails were given small amounts of

reagent-grade $CaCO_3$ as a fine powder sprinkled on the lettuce. The other three snails were fed only lettuce. The calcium used in the construction of shells in this latter group is presumed to have come from the alkaline tap water added to the aquarium daily to maintain a relatively constant humidity. During the 4 months of the culture these snails were active and grew to adult size. A small portion of the lettuce was frozen for isotopic analysis. The aquarium was kept outdoors and allowed to vary in temperature ($^{+}/-\sim 5$°C) with ambient conditions (light/dark cycles during spring summer months).

A second culture group of 11 juvenile snails were cultured in the same 10-gallon aquarium as group one. These snails were fed white corn meal (C4-type plant) and tap water ad libitum for 4 months. Four of the snails were fed powdered $CaCO_3$ and corn meal. Seven of the snails were fed only corn meal. The snails were maintained indoors on a daily light/dark cycle. The temperature within the aquarium was constant 25°C.

A third culture group was grown under conditions similar that of group two. Two snails were fed a mixture of dried sour orange tree leaves and $CaCO_3$ powder ad libitum. Two snails were fed the same leaf diet with finely powdered $CaPO_4$. The sour orange tree, a C3 plant grown in a ^{12}C-enriched atmosphere, has leaf $\delta^{13}C$ values significantly more depleted in ^{12}C than the romaine lettuce. These snails grew to adulthood but were smaller in mass compared to the other two culture groups. This was apparently due to reluctance to consume the sour orange leaves. The shells of snails in this group were not large enough to extract shell matrix carbon. The snails were active throughout the culture period. The snails added sufficient shell aragonite for isotopic analysis of carbonate. At the end of each growing period the snails were frozen. The first group was placed in a freezer (0°C). The snails of the second and third group were first immersed in liquid nitrogen and then placed in the freezer.

2.2. Preparation of isotopic samples

Each snail was sectioned into two halves. The snail body and foot were removed from the shell while frozen. A small portion (~ 5 mg) of the snail foot and body were removed and lyophilized. Care was taken not to include portions of the snail gut that could contain food contamination. The remaining portion was returned to the freezer. The body and foot samples were immersed in 3 N HCl for 20 min, rinsed three times in deionized water and lyophilized. The foot and body samples were ground to a powder.

The powdered samples were loaded into precombusted 9-mm quartz or vycor tubes with precombusted Cu, CuO and Ag foil. The samples were evacuated, sealed and combusted at 850°C for 3 h. Following combustion, the samples were allowed to cool slowly for approximately 12 h. Within 24 h of combustion, CO_2 and N_2 were separated cryogenically and sealed in 6-mm break seal tubes on a high vacuum line.

The acid insoluble organic matrix within the snail shell was extracted in the following manner. The portion of the shell formed prior to culturing was removed. The organic matter adhering to the culture shell wall was removed by placing the shell in a 50-ml bath of 5% sodium hyperchlorite (Clorox) for 48 h while stirring continuously. The shell was rinsed three times with deionized water and lyophilized. The shell carbonate was decarbonated in a 100-ml precombusted Pyrex beaker with 50 ml of 3 N HCl. The insoluble organic fraction was captured on a precombusted quartz fiber filter, rinsed with deionized water, loaded into precombusted quartz tubes and lyophilized. After drying, precombusted Cu, CuO and Ag were added and the tubes were evacuated, sealed and combusted as above. The soluble organic fraction that passed through the quartz fiber filter was discarded. Samples were analyzed on a VG Prism II mass spectrometer for isotopic analysis. Values are reported in ‰ relative to VPDB. Reproducibility of the in-house cellulose standard was ± 0.2‰ for carbon and values fell within 1 S.D. of the long-term mean $\delta^{13}C$ value of -36.2‰. Blanks were run to monitor CO_2 background contamination and were found be too small for analysis on the mass spectrometer. No correction has been made for background contamination.

For isotopic analysis of shell carbonate a small portion of the shell was soaked in 5% sodium

hypochlorite (Clorox) as above, rinsed with deionized water and dried at 50°C. The sample was then crushed to a powder, loaded into a stainless steel boat and placed in an automated carbonate preparation device. The carbonate samples were converted to CO_2 gas by reacting for 10 min in H_3PO_4 at 90°C. CO_2 and water were separated on-line. The CO_2 samples were analyzed isotopically on a VG Prism II triple collector gas ratio mass spectrometer. Carbon isotope values are reported relative to VPDB. Twenty-one carbonate standards (Ultiss marble) were analyzed in conjunction with the carbonate samples during the automated runs of the carbonate device. The mean $\delta^{13}C$ value for these standards was 1.93 ± 0.09‰ (calibrated value is 2.04‰).

3. Results and discussion

3.1. Isotopic relationship between snail body and foot tissue relative to diet

The carbon isotopic composition of each snail (body, foot, shell organic matrix, and carbonate) is listed in Table 1.

Table 1
Carbon isotope results for cultured snails

Snail	Diet $\delta^{13}C$ (‰)	Ca added	Body $\delta^{13}C$ (‰)	Foot $\delta^{13}C$ (‰)	Matrix $\delta^{13}C$ (‰)	Preculture carbonate $\delta^{13}C$ (‰)	Culture carbonate $\delta^{13}C$ (‰)	Diet shell $\Delta\delta^{13}C$ (‰)	Body shell $\Delta\delta^{13}C$ (‰)
Group 1									
#2	−10.0	yes	−11.7	−10.3	−15.8		1.9	−11.9	−13.6
#7	−10.0	yes	−11.9	−10.4	−14.3		1.6	−11.6	−13.5
#8	−10.0	yes	−11.0	−9.9	−13.3		1.9	−11.9	−12.9
#9	−10.0	yes	−9.7	−9.6	−13.9		1.6	−11.6	−11.3
#9 section 1	−10.0	yes			−13.9		1.7	−11.7	−11.3
#9 section 2	−10.0	yes			−16.2		1.8	−11.8	−11.5
#9 section 3	−10.0	yes			−15.9		1.0	−11.0	−10.6
#13	−10.0	no	−14.6			−10.4		−1.8	−14.6
#14	−10.0	no	−13.7			−10.3	1.7	−11.7	−15.4
#15	−10.0	no	−11.2			−2.9	1.7	−11.7	−12.9
#16	−10.0	no	−14.1	−13.9		−9.4		−6.9	−14.1
#17	−10.0	no	−12.1	−11.7		−6.2	2.5	−12.5	−14.6
Average							1.7	−10.5	−13.0
Std							0.4	3.1	1.5
Group 2									
#1	−26	yes	−26.9		−24.5	−12.6	−12.9	−13.1	−14.0
#3	−26	yes	−25.6	−25.5	−24.2				
#3 duplicate	−26	yes	−25.7	−25.4	−24.2		−12.8	−13.2	−12.9
#4	−26	yes	−26.2			−10.9	−12.4	−13.6	−13.8
#5	−26	no	−27.6	−26.2	−23.4		−11.3	−14.7	−16.3
#5 duplicate	−26	no		−26.3	−23.4	−7.7	−12.4	−13.6	−15.2
#6	−26	no	−25.7		−23.3	−8.8	−11.0	−15.0	−14.7
#10	−26	no	−26.3	−24.6		−10.3	−11.5	−14.5	−14.8
Average							−12.0	−14.0	−14.5
Std							0.8	0.8	1.1
Group 3									
#11	−40.0	no					−19.0	−21.0	
#18	−40.0	yes	−34.7			−16.4	−18.2	−21.8	−16.5
#19	−40.0	yes	−30.9	−29.3		−16.3	−18.3	−21.7	−12.6
#20	−40.0	no	−34.5	−33.7		−15.7	−24.3	−15.7	−10.2
#21	−40.0	no	−34.6	−32.6		−15.0	−23.0	−17.0	−11.6
Average							−20.6	−19.4	−12.7
Std							2.9	2.9	2.7

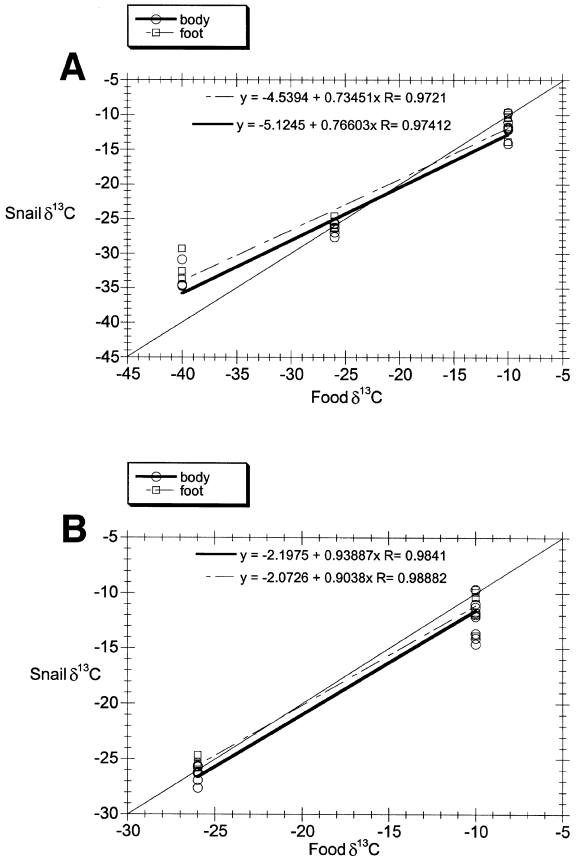

Fig. 1. (A) The carbon isotopic composition of snail body and foot tissues relative to diet. The positive values for orange-fed snails ($\delta^{13}C = -35$ to $-30‰$) is attributed to the fact that these snails did not reach full adult-sized and the values reflect a mixture of preculture and culture carbon. (B) The carbon isotopic composition of the lettuce-fed (C3-type vegetation) and corn-fed (C4-type vegetation) snail versus that of their diet. Here the relationship is close to one as demonstrated previously [1].

The $\delta^{13}C$ of body and foot tissue relative to diet is shown in Fig. 1A. The isotopic composition of the lettuce-fed group varied from $-25.6‰$ to $-27.6‰$ with a mean (including replicates) of $-26.6 \pm 0.8‰$. The foot and body tissues have similar $\delta^{13}C$ values. The $\delta^{13}C$ of the C4-fed group ranged from $-9.7‰$ to $-11.9‰$ with a mean of $-11.2 \pm 0.9‰$. Of the four snails fed orange leaves, three had body $\delta^{13}C$ values of $-34.5 \pm 0.1‰$; one had a value of $-30.7‰$.

The corn-fed group is depleted by 2–3‰ relative to their diet while the lettuce-fed group displays no significant isotopic offset from the diet. The orange-fed group is isotopically heavier than their diet by about 5‰. DeNiro and Epstein [1] also reported a small ($\sim 1‰$) enrichment relative to a diet in snails fed romaine lettuce. There is no difference in $\delta^{13}C$ between the snail's feces and diet. The isotopic relationship between the snail and diet is linear and indicates that as the snails grew they incorporated progressively more carbon from their culture diet into organic tissues.

Three adult snails from Palos Verdes Peninsula were analyzed isotopically for comparison with the cultured snails. The plant and the three adult snails have body and foot $\delta^{13}C$ values of $-25‰$. Comparing these values to those of the cultured snails implies that as much as 22% of the carbon retained in the organic tissues of the orange-fed culture group was derived from the preculture carbon whereas only 8% of the carbon in the corn-fed group would have been preculture carbon.

3.2. Carbon isotopic relationship between diet, body tissue and the shell-bound organic matrix

The $\delta^{13}C$ of the insoluble organic fraction extracted from the cultured portion of the snail shells of the lettuce-fed and corn-fed groups is illustrated in Fig. 2. The $\delta^{13}C$ of the shell-bound organic matrix is enriched in ^{13}C relative to the diet by about 1‰ in the lettuce-fed group. This result is consistent with those of DeNiro and Epstein [1] who found a similar enrichment in snails

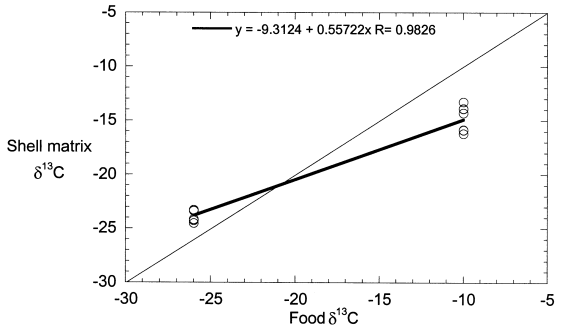

Fig. 2. The carbon isotopic composition of acid insoluble carbon extracted from the aragonite shell relative to diet. Note the offset between diet and the organic matrix, which results in a positive slope of 0.55.

fed romaine lettuce. However, the $\delta^{13}C$ of the organic matrix is depleted in ^{13}C relative to body tissue in the corn-fed group (Fig. 2). This $\delta^{13}C$ offset is not likely to be a result of the incorporation of preculture carbon since only those portions of the shell formed during the culture experiment were analyzed, unless the synthesis of the organic matrix draws upon a pool of carbon formed prior to culturing. It is possible that some of the isotopic difference is due to the loss of carbon in the soluble phase that was not analyzed. However, this seems unlikely since the offset between the matrix and the body tissue is reversed in the two culture groups and the magnitude of the offset is quite different.

The culture results imply that paleoreconstructions of dietary plant communities based on the acid insoluble shell matrix carbon must take into account the fact that there are significant and non-systematic ^{12}C offsets between snail diet and shell organic matrix.

3.3. Carbon isotopic relationships between diet and shell carbonate

The aragonite shells of *H. aspersa* precipitate from a bicarbonate/carbonate pool that derives carbon from metabolic respiration and the break down of urea via urease activity and possibly from atmospheric CO_2 diffusing through the mantle [5]. There may also be a contribution of carbon from ingested carbonates that dissolve in the gut [5].

Goodfriend and Hood [5] presented a model to explain how each carbon source is thought to enter the bicarbonate pool. The model also identifies where isotopic fractionations are thought to occur as carbon is transported in vivo. According to their model, atmospheric $^{12}CO_2$ fractionates as it diffuses through the mantle into the hemolymph. The model does not, however, include a fractionation step for the diffusion of metabolic CO_2 into the internal bicarbonate pool. According to the Goodfriend and Hood [5] model there is no fractionation between $^{13}CO_2$ produced from carbonate dissolved in the gut and the bicarbonate pool. There is a fractionation between the aragonite shell and the bicarbonate pool from which it precipitates. If correct, this model makes it difficult to interpret snail carbonate $\delta^{13}C$ in terms of dietary intake because snails would display a range of values depending on activity levels, dietary intake and pCO_2 at ground level.

In the present study corn-fed snails all have $\delta^{13}C$ values of about 1.6‰ except one snail that is more negative (−3‰). The $\delta^{13}C$ of the lettuce-fed snails is more negative with values ranging between −10.5 and −13.0‰. The snails fed sour orange display the most negative $\delta^{13}C$ values (−18.2 and −24.3‰). There are no systematic isotopic differences between the snails fed $CaCO_3$ in their diet and snails that were not fed $CaCO_3$ (Fig. 3). In the lettuce-fed group, for example, the snails fed $CaCO_3$ are slightly more negative, in contrast to what one would expect if the $CaCO_3$ ($\delta^{13}C = -3.8$‰) were contributing carbon to the shell. Snails that were fed sour orange leaves display values that are isotopically more positive than those that were fed orange leaves and $CaPO_4$. The corn-fed snails show no difference between $CaCO_3$- and non-$CaCO_3$-fed. Based upon these results it does not appear that ingested $CaCO_3$ had any systematic influence on the isotopic composition of the shell carbonate, consistent with previous observations [1,2]. The fact that ingested carbonate does not contribute to the carbon isotopic composition of the shell simplifies dietary reconstructions from $\delta^{13}C$ analysis of snail carbonate. The only other potential sources of carbon are atmospheric CO_2 that dif-

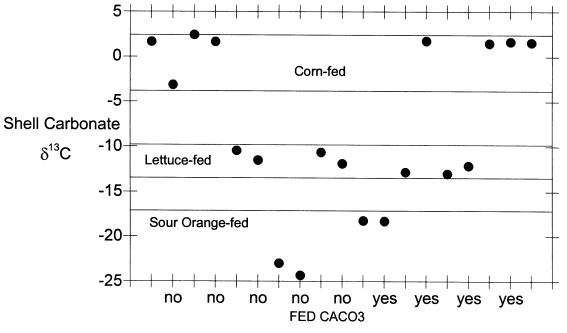

Fig. 3. The carbon isotopic composition of aragonite formed during the culture experiment. The values on the left side of the figure are for those snails that were not fed $CaCO_3$. The values on the right are for snails fed $CaCO_3$ with an isotopic composition of −3.6‰.

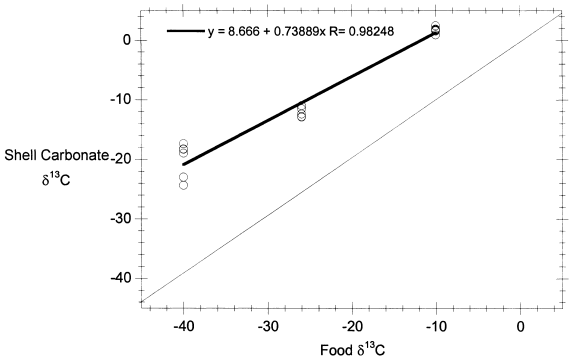

Fig. 4. The carbon isotopic composition of shell aragonite versus diet for each of the culture groups. Note the linear relationship but that the slope is less than one.

fuses into the hemolymph and metabolic CO_2 derived from the digestion of dietary carbon.

The relationship between shell $\delta^{13}C$ and dietary carbon consumed by the snails is shown in Fig. 4. The culture results show a strong correspondence between the snail's isotopic composition and that of the food it consumed (Fig. 4). However, the slope of this relationship is not 1, but is 0.74, similar to the shell organic matrix to diet $\delta^{13}C$ (Fig. 2). If the Goodfriend and Hood [5] model of carbon pathways and fractionations is correct a slope of 0.74 implies there has been a differential incorporation of atmospheric and dietary carbon between the three groups. The model has the form:

$$\delta^{13}C_{aragonite} = \delta^{13}C_d(X) + \delta^{13}C_a(1-X) + 2.7‰$$

where: $\delta^{13}C_d$ = the isotopic composition of metabolic CO_2 = equal to $\delta^{13}C$ of body tissue.

$\delta^{13}C_a$ = the isotopic composition of atmospheric CO_2 ($=-8.5‰$) with a $+9‰$ fractionation = $0‰$.

X = fraction of metabolic CO_2.

$2.7‰$ = isotopic offset between bicarbonate and aragonite precipitated in isotopic equilibrium [8].

Applying this mass balance to the results presented in Fig. 4 requires 30% of the carbon in the orange-fed snails to be from incorporation of atmospheric CO_2. The corn-fed snails would have incorporated approximately 88% atmospheric carbon. The lettuce-fed group would have incorporated approximately 43% atmospheric CO_2. The activity levels between the corn-fed and lettuce-fed snails were similar and there were no significant differences in shell size or mass between the corn-fed and lettuce-fed groups and the environmental conditions within the aquaria during each of the cultures were similar. There is no apparent reason why each of the snail groups should have incorporated such differing amounts of atmospheric CO_2.

Fig. 5 shows the isotopic relationship between aragonite shell and body for each of the snails. The line that describes this relationship is linear and has a slope of one. This relationship does not change significantly if snails fed $CaCO_3$ are excluded. The offset increases slightly but the slope of the line does not change. The linear relationship between the body $\delta^{13}C$ and shell $\delta^{13}C$ with a slope of one implies that metabolically derived CO_2 dominates the bicarbonate pool within the snail and that atmospheric CO_2 does not contribute significantly to the shells of *H. aspersa*. The $12.3‰$ offset between the body and shell carbonate must be explained by ^{12}C fractionations associated with the production of metabolic CO_2 in vivo and by the fractionation of this CO_2 with

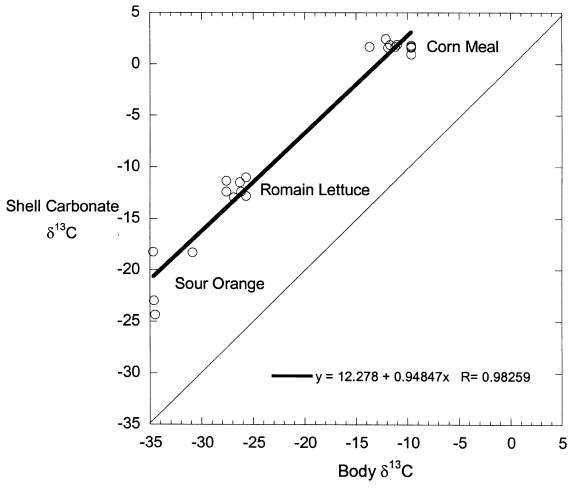

Fig. 5. The carbon isotopic composition of aragonite versus body tissue $\delta^{13}C$ for each of the culture snails. Here the relationship between the shell and body has a slope of one, indicating that the shell $\delta^{13}C$ is derived directly from the body carbon.

respect to the HCO_3 pool from which the carbonate precipitates.

In *H. aspersa* urea is produced in vivo via an arginine–urea pathway [9,10]. The urea formed in this way is not compartmentalized and is broken down into CO_2 and ammonia via reaction with urease. Speeg and Campbell [10] proposed that the formation and volatilization of NH_3 is related to acid–base regulation and that carbonate precipitation is linked to this metabolic process. NH_3 is thought to regulate pH levels in the extrapallial fluid in favor of CO_3^{2-}. Cutenaceous respiration accounts for approximately 40–50% of the CO_2 produced in vivo [10,11]. The remaining CO_2 enters into acid–base reactions raising hemolymph pCO_2. Active snails exposed to experimentally high pCO_2 exhibit large increases in hemolymph [HCO_3], an apparent regulatory response to modulate pCO_2 [11]. The location where CO_2 and HCO_3 exchange is not clear. CO_2 does diffuse from the blood into tissues down a diffusional gradient. This apparently leaves the blood enriched in bicarbonate [10]. When hemolymph pH is elevated to between 8.6 and 8.9 it precipitates carbonate [10]. However, diffusional exchange between the blood and tissue would not likely produce the isotopically heavy HCO_3 pool apparent from the isotopic results shown here. When [^{14}C]HCO_3 is injected into snails between the shell and mantle the [^{14}C]carbon is rapidly incorporated into arginine protein and shell carbonate and respiratory CO_2 [11]. This has led to the inference that amino acids fix carbon directly from HCO_3[10]. In fact, it is apparent from the isotopic results presented here that arginine must be synthesized from a pool of CO_2 isotopically distinct from HCO_3 since body tissues have the same isotopic composition as respired CO_2 whereas the HCO_3 is isotopically heavier by some 10‰. Therefore, any [^{14}C]HCO_3 injected into the shell must first undergo exchange with a CO_2 pool from which arginine is then synthesized. Wherever isotopic exchange between bicarbonate and CO_2aq pools takes place, the constant carbon isotope offset between snail body tissue and shell carbonate leads to an inference that metabolic CO_2 and HCO_3 act as isotopically separate pools and that these reactions are constantly occurring in active snails in order to regulate the acid–base balance.

3.4. A metabolic model for carbonate $\delta^{13}C$

A new model is presented here to explain the systematic δ^{13}C offset between the snail body and shell carbonate. The model differs from that pro-

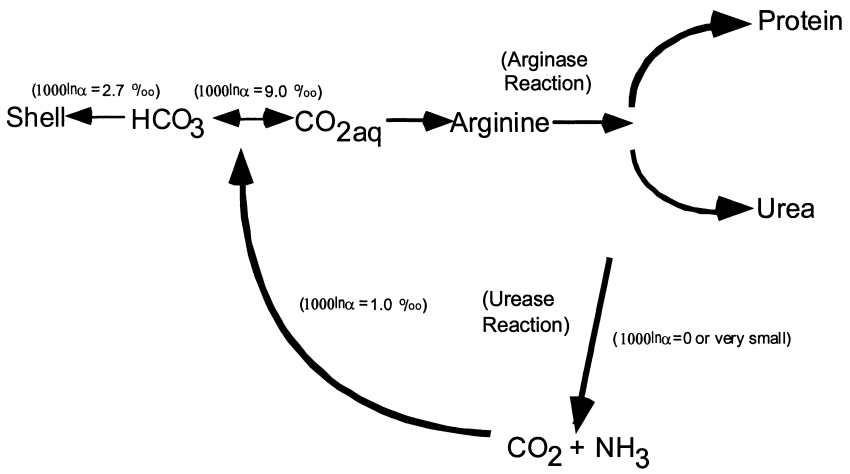

Fig. 6. A model of the fractionation of carbon in vivo that accounts for the 12.3‰ offset between the aragonite and body tissues in *H. aspersa*. The equilibrium fractionation between aragonite and bicarbonate is 2.7‰. The equilibrium fractionation between metabolic CO_2 and bicarbonate via CO_2aq at 25°C is 10‰.

posed by Goodfriend and Hood [5] in that it involves only metabolic CO_2 as a source of carbon and considers the exchange of CO_2 in vivo as one of two isotopic fractionation steps leading to the isotopic offset between body tissue and shell carbonate. The second fractionation step occurs during the precipitation of calcium carbonate. In the model the HCO_3 pool exchanges carbon with metabolic CO_2 via CO_2aq (Fig. 6). This reaction is regulated by hemolymph pH and the production of NH_3. As NH_3 is elaborated into the extrapallial fluid the pH is shifted towards the alkaline region resulting in the precipitation of carbonate. The HCO_3 and CO_2aq are treated as separate pools in the model because each is evidently involved in separate isotopic reactions as discussed above. Arginine fixes CO_2 with no isotopic fractionation. This is based on the observation that body tissue and respired CO_2 have the same isotopic composition [1]. Carbonate, on the other hand, with an isotopic offset characteristic of HCO_3–CO_2aq exchange, appears to precipitate from a HCO_3 pool that is isotopically heavy compared to CO_2.

The acid–base regulation in these snails is intimately tied to the precipitation of calcium carbonate. At temperatures between 20°C and 25°C the model presented here predicts a $\delta^{13}C$ offset between metabolic CO_2 (= snail body tissue $\delta^{13}C$) CO_2aq and HCO_3 of 10‰ (25°C, $1000\ln\alpha = 1‰$, CO_2–CO_2aq, $1000\ln\alpha = 9‰$, [7,12]). The shell aragonite precipitated in isotopic equilibrium from this pool of bicarbonate is fractionationed relative to bicarbonate by 2.7‰ [8]. As a result of these two equilibrium fractionations the carbonate should have a ^{13}C composition that is offset from the body by 12.7‰. This value is very close to the value of 12.3‰ measured on the cultured snails in this study.

4. Applications

4.1. Application to paleovegetation reconstructions

The reconstruction of paleovegetation distributions using the stable isotopic method has relied on the notion that the isotopic composition of the carbon encapsulated in fossilized shells and that of the organism body is the same [6]. In the controlled culture experiments described here this is not the case. There is a significant offset between the acid insoluble carbon extracted from *H. aspersa* shells and the body tissues. These offsets are not the same for snails fed a diet of pure C3 or pure C4 plant material. Because the biosynthetic pathway that leads to the production of the shell organic matrix is not yet known it is not possible to say how these isotopic offsets arise.

In the present study using pure C3 and C4 plant material only the dietary carbon affects the isotopic composition of the shell aragonite in *H. aspersa*. Ingested carbonate and atmospheric CO_2 have no discernible influence on the shell $\delta^{13}C$. This greatly simplifies paleodietary reconstructions since shell carbonates are relatively common in fossil assemblages and can be analyzed easily. The $\delta^{13}C$ offset between the shell aragonite and body tissues of *H. aspersa* in the culture experiment described here is 12.3‰. The offset between the snail body and aragonitic shell can be explained using an equilibrium fractionation model, which takes into account the exchange of metabolic CO_2 and bicarbonate and the fractionation associated with precipitation of aragonite from HCO_3. Using this equilibrium isotope model it should be possible to reconstruct paleodietary carbon and the makeup of paleovegetation from fossils snail without having to account for other sources of carbon.

Not all pulmonate snails consume only live plant material. *H. aspersa* appears to prefer live plant material for its primary diet. However, other pulmonate snails tend to either subsist on both live and dead or primarily on dead and decaying plant material [13]. The decay of plant material could affect the isotopic composition of the organic carbon ingested by the snail, and therefore the $\delta^{13}C$ of its aragonitic shell. However, this variability may be relatively small in comparison to the change in ^{13}C fractionation associated temperature changes the snails experience throughout the year. For example, in a previous study Margaritz et al. [13] recorded a range of $\delta^{13}C$ values for *Xeropicta vestalis*, a Mediterranean helicellid snail from Israel that feeds mainly on dead vegetable

material and saprophytic fungi. In their study the average $\delta^{13}C$ value of adult snail shells varied seasonally from −7.0‰ in winter to −9.5‰ in summer. The authors suggested that the variability in $\delta^{13}C$ reflected differences in the amount of soil $^{12}CO_2$ incorporated into the shell. The range of seasonal temperature change in this part of Israel is approximately 12°C. Winter temperatures average 12°C and summer temperatures are around 24°C [13]. According to the model presented here the 12° change in temperature would account entirely for the 1.5‰ change in $\delta^{13}C$ recorded by snails in Israel. Assuming a constant $\delta^{13}C$ of plant material consumed by the snails of −21.5‰, the $\delta^{13}C$ of shell aragonite would vary from −7.8‰ in winter to −9.4‰ in summer, essentially indistinguishable from the measured values in their study [13]. There is good reason to consider therefore that even snails that consume dead plant material display the same carbon isotope fractionations as those that consume live plant material.

4.2. A paleothermometry

One important implication of the model presented here is the potential for using the $\delta^{13}C$ of fossil snails to reconstruct temperature changes as well as reconstructing changes in vegetation. The carbon isotope fractionation model predicts a systematic change in $\delta^{13}C$ of shell aragonite in response to changes in ambient temperature as illustrated in Fig. 7. The slope of the fractionation change with respect to temperature is −0.13/°C. This can be written as an empirical relationship of the following form:

$$\Delta\delta^{13}C = -0.13/^{\circ}C * \Delta T$$

If the snails diet remains isotopically constant it should be possible to reconstruct changes in average air temperature through analysis of $\delta^{13}C$ changes within a snail community. This approach to paleothermometry is potentially better than using shell $\delta^{18}O$ because of the difficulty in constraining the $\delta^{18}O$ of source water and the effects of evaporation and condensation [3,13,14]. The analytical precision of the $\delta^{13}C$ measurement is typically less than 0.1‰ so there is the possibility of reconstructing changes in temperature with a resolution of 1°C. Clearly in the case of the Israel example it is possible to resolve the entire range of seasonal air temperatures. Preservation of the aragonite is a critical factor for such estimates and constraints must be placed on the range of $\delta^{13}C$ variability within the plant community that was available to the snail community.

There are few time series records of snail $\delta^{13}C$ from which to evaluate the potential of a $\delta^{13}C$ paleothermometer. In an earlier study Yapp [14] measured the $\delta^{13}C$ of *Vallonia*, a terrestrial snail, from Ivie Creek Utah. The samples analyzed ranged in age from about 3500 BP to 7480 BP based on carbon-14 age estimates. At this site, $\delta^{13}C$ values of *Vallonia* increased gradually from about −7‰ at 7800 BP to about −6‰ at 3400 BP. This increase in $\delta^{13}C$ was accompanied by a gradual decrease in $\delta^{18}O$ of about 1.5‰. Based upon the change in $\delta^{18}O$ the authors suggested the period around 7800–7000 BP was warmer and drier in central Utah than the period 4400–3500 BP. The gradual change in both $\delta^{13}C$ and $\delta^{18}O$ suggests that climate change was gradual and progressive. Applying the model presented here to the *Vallonia* data would suggest that the period between 7800 BP and 7000 BP was approximately 7°C warmer than the period between 4400 and

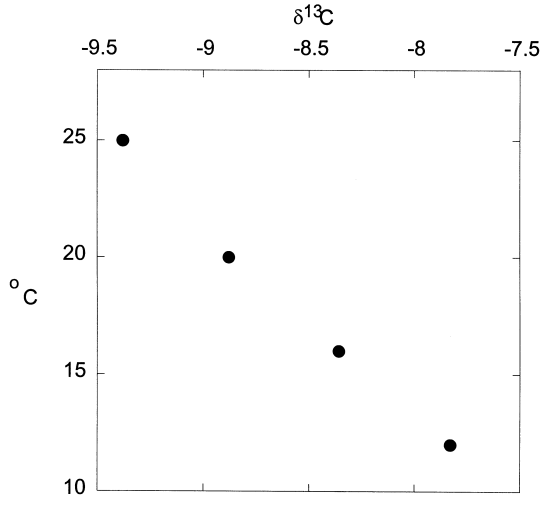

Fig. 7. Effect of seasonal changes in temperature on the carbon isotopic composition of shell aragonite.

3500 BP. Without constraints on the isotopic composition of the snail diet and habitat it is difficult to assess how accurate such an estimate is. However, the change in temperature predicted by this model is consistent with the direction and magnitude of $\delta^{18}O$.

5. Conclusions

The isotopic composition of the acid insoluble organic matrix extracted from the aragonite shells does not track diet when the diet varies. The isotopic composition of the organic matrix carbon cannot be used as an isotopic substrate for paleodietary reconstructions without first determining the source of the carbon and any associated fractionations.

The isotopic composition of the shell aragonite is offset from the body tissues by 12.3‰. This offset is not influenced by the consumption of carbonate and is not attributable to the diffusion of atmospheric CO_2 into the hemolymph. The carbon isotopic composition of shell aragonite is best explained in terms of equilibrium fractionations associated with exchange between metabolic CO_2 and HCO_3 in the hemolymph and the fractionation associated with carbonate precipitation. The $\delta^{13}C$ of aragonite is a good recorder of the isotopic composition of the snail body tissue, and therefore a better recorder of diet than is the insoluble shell organic carbon. Because the systematic fractionation of carbon isotopes within the snail is temperature dependent the $\delta^{13}C$ of the shell could provide an independent technique for estimating paleotemperature changes.

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