Carbon isotope signatures from land snail shells: Implications for palaeovegetation reconstruction in the eastern Mediterranean

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Abstract

In this study we compare carbon isotope values in modern Helix melanostoma shell carbonate ($\delta^{13}C_{shell}$) from the Gebel al-Akhdar region of Libya with carbon isotope values in *H. melanostoma* body tissue ($\delta^{13}C_{body}$), local vegetation ($\delta^{13}C_{plant}$) and soil ($\delta^{13}C_{soil}$). All vegetation in the study area followed the C3 photosynthetic pathway. However, the $d^{13}C_{plant}$ values of different species formed two distinct isotopic groups. This can be best explained by different water use efficiencies with arid adapted species having significantly more positive $\delta^{13}C_{plant}$ values than less water efficient species. The ranges and means of $\delta^{13}C_{body}$ and $\delta^{13}C_{plant}$ were statistically indistinguishable from one another suggesting that $\delta^{13}C_{body}$ was primarily a function of local vegetation composition. *H. melanostoma* $\delta^{13}C_{shell}$ reflected the $\delta^{13}C_{plant}$ of local vegetation with a positive offset between body/diet and shell of 14.5 ± 1.4‰. Therefore, in the Gebel al-Akhdar where only C3 plants are present, higher mean $\delta^{13}C_{shell}$ values likely reflect greater abundances of water-efficient C3 plants in the snails diet and therefore in the landscape, whilst lower mean $\delta^{13}C_{shell}$ values likely reflect the consumption of less water-efficient C3 plants. The distribution of these plants is in turn affected by environmental factors such as rainfall. These findings can be applied to archaeological and geological shell deposits to reconstruct late Pleistocene to Holocene vegetation change in the southeast Mediterranean

1. Introduction

Terrestrial snail shells are some of the most commonly preserved biological remains in archaeological deposits (Evans, 1972; Prendergast and Stevens, 2014) and geological deposits such as loess sequences (Rousseau, 1990), paleosols (Marcolini et al., 2003), and tufa (Preece and Day, 1994). They can provide a record of continental climatic conditions both through their biology and

their shell chemistry (Goodfriend, 1999). The application of stable isotopes from land snail carbonate ($\delta^{18}O_{shell}$, δ^{13}Cshell) for palaeoclimate reconstruction has steadily increased over the last decade (e.g. Balakrishnan et al., 2005a; Colonese et al., 2007, 2011; Yanes et al., 2011, 2013; Stevens et al., 2012). However, the interpretation of δ^{13}Cshell as a palaeovegetation proxy can be complicated by factors such as water stress in plants, the ingestion of foreign carbonates, and uptake of atmospheric CO_2. These effects differ from species to species. It is therefore important for any species of interest in any new region of study, to test the empirical relationships between terrestrial shell stable isotopes and environmental parameters with a modern dataset before attempting to use $\delta^{13}C_{shell}$ for palaeoenvironmental reconstruction. This study presents a modern carbon isotope calibration on a previously unstudied species, *Helix melanostoma*. This species is one of the most common land snails in the Mediterranean and northern Africa and one of the most abundant species in Mediterranean archaeological sites from the Middle Palaeolithic to the Roman period (Lubell, 2004; Barker et al., 2010; Lubell and Barton, 2011). Therefore, if this species can be validated as a useful palaeoenvironmental proxy, it can provide valuable information on late Pleistocene to Holocene past environments, in a region with comparatively little palaeoenvironmental data.

1.1. Carbon isotopes from land snails for palaeoenvironmental reconstruction

Terrestrial mollusc shell carbon is derived from the bicarbonate in snail body fluids. The CO_2 in these body fluids has three major carbon sources: metabolic CO_2, atmospheric CO_2, and CO_2 generated via the reaction of acid with ingested carbonates including limestone rock and recycled snail shell in the snail's stomach (Goodfriend and Ellis, 2002). Therefore, the carbon isotope ratios in land snail shells ($\delta^{13}C_{shell}$) are affected by three main factors: diet; atmospheric carbon; and ingested carbonates (Goodfriend and Ellis, 2002).

Previous studies have found that the primary variable affecting δ^{13}C in terrestrial snail shells is diet. Therefore, in herbivorous species, the carbon isotope ratios may provide a proxy for palaeovegetation patterns in terms of the distribution of C3 and C4 plants and any changes due to water stress (Yapp, 1979; Francey, 1983; Goodfriend and Ellis, 2002; Stott, 2002; Metref et al., 2003; Balakrishnan et al., 2005a, 2005b; Baldini et al., 2007).

There are three metabolic pathways in plants: C3 (Calvin-Benson), C4 (Hatch-Slack), and CAM (Crassulacean Acid Metabolism). These pathways isotopically fractionate atmospheric CO_2 differently due to the presence of different carboxylating enzymes (Farquhar et al., 1989). Such differences can be discriminated isotopically. The majority of terrestrial plants including temperate grasses, all trees and shrubs use the C3 metabolic pathway and have a δ^{13}C range between -33‰ and -21‰ (Cerling and Quade, 1993). The δ^{13}C in C3 plants ($d^{13}C_{plant}$) is a result of the δ^{13}C of atmospheric CO_2 (δ^{13}Catmosphere) and the fractionation processes that occur as CO_2 is incorporated into the plant. Atmospheric CO_2 diffuses into the gas space of the leaf through stomatal openings. CO_2 is then combined with the enzyme ribulose bisphosphate in a reaction called carboxylation (Farquhar et al., 1989).

The fractionation of δ^{13}C in C3 plants can be affected by processes that alter the rate of photosynthesis or limit the CO_2 that passes through the stomatal pores. These factors include water availability, the canopy effect, air temperature, light availability, salinity, and the concentration of

atmospheric CO_2. The most important factor to consider is water availability. When more soil water is available, the plant's stomata open wider, allowing more CO_2 into the plant, which causes ^{13}C-depletion. Conversely, when less water is available, the plant's stomata close, causing ^{13}C-enrichment (Farquhar et al., 1989; Condon et al., 1992). Water availability is a function of rainfall amount, relative humidity and aspects of the physiology of the plant. Plants that are morewater-efficient tend to modulate their stomatal conductance, thus have more positive $\delta^{13}C$plant ratios (Farquhar and Sharkey, 1982; O'Leary, 1988). Within the range of C3 variation, there are marked inter and intra-specific $\delta^{13}C$ ratios which are likely due to physiological factors based on the above mentioned mechanisms as well as potentially genetic variation (Handley et al., 1994).

Modern field studies in various regions of the world have found significant correlations between the carbon isotopic composition of local vegetation ($\delta^{13}C_{plant}$) and modern terrestrial snail $\delta^{13}C_{shell}$ (e.g. Francey, 1983; Goodfriend and Ellis, 2002; Baldini et al., 2007; Yanes et al., 2008; Colonese et al., 2014). Snails in regions of predominantly C3 vegetation have significantly more negative $\delta^{13}C_{shell}$ ratios than snails found in regions with predominantly C4 vegetation, whilst snails that eat a mixed diet will have intermediate $\delta^{13}C_{shell}$ values. For example, Balakrishnan et al. (2005b) measured $\delta^{13}C_{shell}$ ratios from -10.1 to -8.8‰ in areas of C3 vegetation, and $\delta^{13}C_{shell}$ ratios from -4.3 to þ1.9‰ in areas of C4 vegetation. Similar field correlations were achieved between the acid insoluble organic matrix of terrestrial shell carbonates and local vegetation, although this proxy is less commonly used than terrestrial snail carbonate (Goodfriend, 1988, 1990).

Laboratory controlled feeding experiments on the land snail *Helix aspersa* showed that diet-derived metabolic CO_2 was the primary influence on $\delta^{13}C_{shell}$ (Stott, 2002; Metref et al., 2003). Snails fed on an entirely C3 diet ($d^{13}C_{diet}$ = -27‰) had significantly higher $\delta^{13}C_{shell}$ ratios than snails fed on entirely C4 diets ($d^{13}C_{diet}$ = -11.7‰). Animals fed on mixed diets had intermediate values, but they were closer to the C3 range suggesting a preference for C3 diet in this species. Likewise, the offsets between $\delta^{13}C_{diet}$ and $\delta^{13}C_{shell}$ was greater for the C3 diet (D $\delta^{13}C$ = 13.75 ± 0.52‰) than for the C4 diet (D $\delta^{13}C$ = 4.89 ± 0.87‰), suggesting that the animals may metabolise these plants in different ways (Stott, 2002).

The correlation between $\delta^{13}C_{plant}$ and $\delta^{13}C_{shell}$ has implications for palaeoclimate reconstruction as the distribution of vegetation types is related to other environmental variables such as rainfall. For example, Goodfriend (1988, 1990) used $\delta^{13}C$ from the organic component of land snail shells to map the shifting distribution of C3 and C4 vegetation during the Holocene in the Negev Desert and by inference the shift in the desert boundary due to changing rainfall patterns. The effects of aridity may also be seen within entirely C3 plant communities as water stress causes a ^{13}C-enrichment in $\delta^{13}C_{plant}$ ratios. Therefore, areas with high rainfall, tend to have more negative $\delta^{13}C_{plant}$ ratios (Goodfriend and Magaritz, 1987; Goodfriend and Ellis, 2000, 2002). Such changes will be recorded in land snail $\delta^{13}C_{shell}$ ratios.

Some studies have suggested that atmospheric CO_2 from gas exchange across the snail's body surface is an important source of $\delta^{13}C_{shell}$(Magaritz and Heller, 1980, 1983a, 1983b). The carbon isotopic composition of the local atmosphere can vary microgeographically due to the output of plant respiration and soil CO_2, so more heavily vegetated regions have more ^{13}C-depleted $\delta^{13}C_{atmosphere}$ values at the ground surface, therefore more ^{13}C-depleted shell ratios (Magaritz et al., 1981; Magaritz and Heller, 1983b). However, it is likely that the effect of exchange with atmospheric

CO_2 on $\delta^{13}C_{shell}$ is more important for smaller snail species that have more of their surface area in contact with the atmosphere (Goodfriend and Hood, 2006). Regardless of the direct influence of atmospheric CO^2 on land snail $\delta^{13}C_{shell}$, plants derive their CO_2 from the atmosphere so changing concentrations of CO_2 in the atmosphere over glacial/ interglacial timescales (e.g. Arens et al., 2000), and as a result of the input of depleted CO_2 into the atmosphere since the industrial revolution (Suess effect, e.g. Stuiver et al., 1984; Friedli et al., 1986) should be accounted for when reconstructing palaeoenvironment from land snail carbonates.

Various studies have shown that $\delta^{13}C_{shell}$ ratios of land snails can be significantly affected by the ingestion of carbonates as a result of grazing on limestone rocks and carbonate-rich soils since these are usually much more positive in $d^{13}C$ than plants (Goodfriend and Stipp, 1983; Goodfriend, 1999, 2006; Goodfriend and Ellis, 2002; Yates et al., 2002; Li et al., 2007; Romaniello et al., 2008; Yanes et al., 2008). This leads to $\delta^{13}C_{shell}$ ratios that are more positive than expected from diet alone. This effect seems to be highly species- specific and varies from location to location (Goodfriend and Ellis, 2000). Some species such as *H. aspersa* showed no detectable influence of foreign carbonate ingestion (e.g. Stott, 2002; Chiba and Davison, 2009), whilst others have shown that carbonate ingestion can account for up to 40% of the $\delta^{13}C_{shell}$ ratios (Goodfriend and Ellis, 2000; Yanes et al., 2008). If a significant proportion of $\delta^{13}C_{shell}$ is derived from carbonate ingestion, Goodfriend (1988, 1990) suggests analysing the acid insoluble organic matter within the shells for palaeoenvironmental reconstruction.

1.2. *H. melanostoma*

H. melanostoma (Draparnaud 1801) is a herbivorous air breathing land snail (terrestrial pulmonate gastropod) in the family Helicidae. This species has been reported across the Mediterranean in northern Algeria, Tunisia, Greece, Albania, Italy, and on the south coast of France (Baker, 1938; Morel, 1973; Lubell et al., 1975; Kerney et al., 1983). This study confirms its existence in Libya. Previous surveys of *H. melanostoma* in Algeria and Tunisia have shown that it inhabits humid shady areas in bush and parkland habitats, often favouring calcareous substrates (Baker, 1938; Morel, 1973; Lubell et al., 1975). During dry periods, the snail digs into the soil and aestivates (Baker, 1938; Lubell et al., 1975). In Tunisia, active *H. melanostoma* were observed from February until June, particularly following rainfall events. The snails probably aestivate from November to January as they were not seen in the landscape during those months (Morel, 1973). Lubell et al. (1975) noted that in the Algerian winter, the snails formed a thick epiphragm suggesting longer periods of inactivity whereas in summer they formed several thin epiphragms suggesting shorter periods of aestivation.

1.3. Study area

The Mediterranean is located in a transitional zone where tropical and mid-latitude systems both affect climate variability. Therefore, Mediterranean moisture sources are varied. The region is characterised by hot dry summers and cool wetter winters. During summer, a high pressure belt steers storm tracks away from the region. During winter, the sub-tropical high pressure belt moves southwards, bringing cold air with it. The mixing of cold air from the high pressure belt with relatively warmer sea water causes increased evaporation and cyclonogenesis (Gat and Carmi, 1970). Rainfall in the eastern Mediterranean primarily occurs in winter and is of cyclonic origin (Wigley and Farmer, 1982; Kostopoulou and Jones, 2007).

The Gebel al-Akhdar is a limestone massif in the southeastern Mediterranean that rises to a height of >850 m above sea level over 10 km (Fig. 1). It forms a condensed climatic gradient with sharp differences in temperature and rainfall amount over a short distance. At sea level, rainfall is around 250 mm/yr rising to >700 mm/yr on the upper escarpments. Rainfall falls sharply on the southern slopes before dropping to arid levels in the predesert (Libyan National Meteorological Center, 2012). McBurney and Hey (1955) note that the Gebel al-Akhdar is "the only area of high ground along 2500 km of flat coastline between Homs in Tripolitania and Mount Carmel in Palestine". The high elevation of the Gebel al-Akhdar, which acts as a trap for rainfall from the westerlies, ensures that climate and vegetation patterns are distinct from the surrounding regions. Today, the area provides a refuge of fertile Mediterranean vegetation bounded by arid coastal corridors and the Sahara Desert. The region has the richest species diversity of any region in Libya (Hegazy et al., 2011). The vegetation assemblage is predominantly maquis scrubland dominated by *Juniperus phoenecia*, *Quercus coccifera*, *Pistacia lentiscus*, and *Ceratonia siliqua* with some areas of steppic vegetation including *Sarcopterium spinosum* and *Artemisia* (Al-Sodany et al., 2003; El-Darier and El-Mogaspi, 2009; Simpson and Hunt, 2009). Like the Magreb in northwestern Africa, it may have served as a refugium for vegetation, animals and human populations at times of climatic extremes during the late Quaternary (McBurney, 1967; Barker et al., 2010).

The Gebel al-Akhdar contains many important archaeological sites spanning from the last interglacial (c. 130,000 years ago) to present. Many of these sites, particularly the multi-period cave site of Haua Fteah, contain abundant land snail remains (McBurney, 1967; Barker et al., 2007, 2008, 2009; 2010, 2012). These sites therefore offer enormous potential for palaeoenvironmental reconstruction from land snail stable isotopes.

2. Methodology

Live, aestivating and recently dead (with remnant organic body matter in the shell and excellent shell colour preservation) *H. melanostoma* snails were collected from their natural habitats in April 2010 and May 2012 from a north-south transect across the Gebel al-Akhdar (Fig. 1). Active terrestrial gastropods can be difficult to find, particularly during spring when the climate is drier. Collection was primarily achieved by digging under vegetation to find buried aestivating snails. This was supplemented by collection during periods immediately following rainfall events when active snails emerged in the landscape. At each collection site, vegetation assemblages, geological substrate and altitude were noted. Samples of the most common plant species associated with *H. melanostoma* in the Gebel al-Akhdar and samples of the sediments surrounding the aestivating snails were collected during the land snail survey in 2010.

Modern aestivating *H. melanostoma* were transported back to the laboratory in Cambridge in the soil in which they were found. Adhering particles were cleaned from the shells using a brush and distilled water. The epiphragms were removed from the shell aperture and retained for future analysis. Snail bodies were removed from their shells with a dissecting needle and were placed in a sealed Exetainer® (Labco, High Wycombe, UK). Snail body fluid was removed by cryogenic vacuum distillation. Stable isotope analyses of these body fluids are reported in another paper on the oxygen isotope composition of *H. melanostoma* (Prendergast et al., under review). The remaining snail bodies were transferred to micro-centrifuge tubes and oven dried for 12 h at 50 ^{o}C. Snail bodies were homogenised using a ball mill. Approximately 100 mg was subsampled from each individual

and transferred into a tin capsule. Vegetation and soil samples were dehydrated and then crushed using an agate mortar and pestle. The samples were pretreated with 4% hydrochloric acid to remove contaminants. Soil samples were heated on a hotplate at 60 ^{o}C for 20 min. After this all samples were washed repeatedly with deionized water until neutral, then dried and transferred into a tin capsule.

After cleaning with distilled water, whole shells were pretreated in a bath of 5% aqueous reagent-grade sodium hypochlorite at room temperature for 48 h to remove organic matter. After pretreatment, shells were rinsed in an ultrasonic bath for five minutes in distilled water three times before being oven-dried at 50 ^{o}C for 12 h. Pretreated whole shells were crushed into a fine powder (<90 µm) using an agate mortar and pestle. A sub-sample of approximately 100 mg was used for stable isotope analysis.

Isotopic analyses of all samples were carried out using continuous flow isotope ratio mass spectrometry. Powdered shell samples were transferred to Exetainer vials and sealed with silicone rubber septa using a screw cap, then flushed with CP grade helium, acidified with H_3PO_4, left to react for 1 h at 70 ^{o}C and then analysed using a Thermo Gasbench preparation system attached to a Thermo MAT 253 mass spectrometer in continuous flow mode. Each snail body sample was analysed in duplicate using a Costech elemental analyser coupled in continuous-flow mode to a Thermo MAT 253 mass spectrometer. Soil and vegetation were measured in duplicate using a Costech elemental analyser coupled in continuous-flow mode to a Thermo Delta V Advantage. Carbon isotopic ratios are given in parts per mil (‰) relative to VPDB. Repeated measurements on international and in-house standards showed that the analytical error was <0.1‰ for shells, <0.2‰ for snail bodies, and <0.2‰ for plants and soil.

Statistical analyses were undertaken using SPSS version 19. Statistical significance was set at 0.05. After testing for normal distribution, parametric data were investigated using Student's t- tests for two groups and independent one-way ANOVA with Tukey post-hoc tests for three or more groups. Simple and multiple linear regression were performed to investigate the relationships be- tween the variables.

3. Results

3.1. Land snail distribution in the modern landscape

In the Gebel al-Akhdar, *H. melanostoma* were found in loose, carbonate-rich soils on the well watered northern slopes of the Gebel al-Akhdar (Table 1). The maximum basal diameter (MBD) of adult specimens ranged from 12 to 35 mm (Appendix 1). Aestivating specimens of *H. melanostoma* were frequently found under *C. siliqua* (carob), *P. lentiscus* (pistachio) and *Rhus tripartita* (sumac) bushes at elevations below 200 m and under *C. siliqua* (carob), *P. lentiscus* (pistachio), *Juniperus phonenicea* (juniper) and *Ziziphus* sp. (buckthorn) bushes above 500 m. The snails had a relatively broad ecological range but were particularly abundant around *C. siliqua* trees near the Haua Fteah and in shaded, well watered slopes above 400 m. No live snails were recovered between 200 and 400 m where slopes were steeper and soils were shallower and better drained. The animals usually occurred in clusters of two to six snails buried 10-20 cm deep in loose soil underlying the above mentioned shrubs. *H. melanostoma* routinely occupied areas of soil towards the centre or the

eastern edges of shrubs. These areas were noticeably cooler than the surrounding soils as they were sheltered from the harsh afternoon sun.

All aestivating *H. melanostoma* had well formed calcareous epiphragms several mm thick (Fig. 2) that completely sealed the shell aperture suggesting that in spring when they were collected, they were in long-term aestivation. Several juvenile *H. melanostoma* were found on the upper and lower slopes of the Gebel al-Akhdar. The juvenile shells (<10 mm maximum basal diameter) had thin, delicate shells, and thin epiphragms that could be easily crushed between two fingers.

3.2. Carbon isotopes in vegetation and sediments

The $\delta^{13}C_{plant}$ ranged from -30‰ to -22.6‰ with a mean of -25.7‰ (Table 2). All $\delta^{13}C_{plant}$ values were within the range expected for the C3 photosynthetic pathway but they plotted in two distinct clusters based on species (Fig. 3): oak and carob (n = 4, mean = -28.7 ± 1.4‰), and juniper (n = 6, mean = -23.7 ± 1.4‰). An independent samples t-test revealed that there was a statistically significant difference between the $\delta^{13}C_{plant}$ ratios of the two groups ($t(8) = 7.578$, $p < 0.001$).

The $d^{13}C$ signatures of soil samples collected adjacent to aestivating *H. melanostoma* ($\delta^{13}C_{soil}$) had a narrow range between -24.9‰ and -25.0‰ (n = 4, mean = -24.9‰) (Table 3). An independent samples t-test showed that $d^{13}C_{soil}$ was not significantly different from mean $\delta^{13}C_{plant}$ ($t(12) = -5.35$, $p > 0.05$).

3.3. Carbon isotope composition of body tissue

Carbon isotope values of *H. melanostoma* body tissue ($\delta^{13}C_{body}$) ranged from -29.5‰ to -20.7‰ (Table 4). The mean of juvenile $\delta^{13}C_{body}$ (n = 4) was slightly more negative than adult $\delta^{13}C_{body}$ (n = 25) (-26.1‰ compared to -25.0‰, Appendix 1) but these differences were not significant ($t(31) = 1.3$, $p = 0.2$) so adults and juveniles were treated together in the following analysis and discussion. A one-way ANOVA with a Tukey post-hoc test revealed that there were no statistically significant differences between $\delta^{13}C_{body}$, $\delta^{13}C_{soil}$, and $\delta^{13}C_{plant}$ ($F(2, 38) = 0.262$, $p > 0.05$).

3.4. Carbon isotope composition of *H. melanostoma* shell and comparison with body tissue

The $\delta^{13}C$ of modern *H. melanostoma* shell ($\delta^{13}C_{shell}$) from the Gebel al-Akhdar ranged from -12.3‰ to -7.8‰ (Table 4, Appendix 1). There was no significant difference between adult and juvenile $\delta^{13}C_{shell}$ (means = -10.4‰, and -11.2‰, $t(57)$ ¼ 1.595, $p > 0.05$) therefore adults and juveniles were treated together in the following analysis and discussion. These values were in the lower range of $\delta^{13}C_{shell}$ reported from previous studies worldwide (-13.5‰ to þ1.7‰, Balakrishnan et al., 2005a, 2005b; Baldini et al., 2007). Shell carbon isotope composition was always more positive than body. The offset between $\delta^{13}C_{body}$ and $\delta^{13}C_{shell}$ ranged between 12.1 and 17.2‰, with a mean offset of 14.5 ± 1.4‰.

4. Discussion

4.1. Vegetation in the Gebel al-Akhdar

The $\delta^{13}C_{plant}$ results confirmed that all measured vegetation in the study area use the C3 photosynthetic pathway. However, the significant $\delta^{13}C_{plant}$ differences between species suggest that it may be possible to distinguish some genera based on their $\delta^{13}C_{plant}$ signatures. Differences in the

$\delta^{13}C$ ratios of different species are probably due to water use efficiency. Plants exposed to water stress from phenomena such as sun or dry soils protect themselves from evapotranspiration by closing their stomatal pores, which prevents the diffusion of CO_2 to the leaf. Species that are more water-efficient tend to modulate their stomatal conductance thus have more positive $\delta^{13}C_{plant}$ ratios and can thus live in slightly drier environments (O'Leary, 1988; Farquhar et al., 1989). Juniper consistently had the most positive $\delta^{13}C_{plant}$ values of the plants measured in this study. This genus prefers dry, sunny environments in open forest and it tends to grow in sunnier locations in the study area. Similar results of more positive $\delta^{13}C_{plant}$ ratios have been found in other studies of juniper stable isotopes (e.g. Schubert and Jahren, 2012). Therefore, it is likely that juniper had the most positive $\delta^{13}C_{plant}$ ratios as it was the most water-efficient of the three analysed species whereas oak and carob have more negative $\delta^{13}C_{plant}$ ratios as they are less water-efficient. If climates became drier, they would favour a greater abundance of juniper over oak and carob, which tend to need wetter, more shaded environments for growth.

4.2. From vegetation to snail bodies

The range and mean of *H. melanostoma* $\delta^{13}C_{body}$ values (-29.5‰ to -20.7‰, mean = 25.3‰) are remarkably similar to Gebel al-Akhdar $\delta^{13}C_{plant}$ values (-30.0‰ to -22.6‰, mean = -25.7‰). This suggests that $\delta^{13}C_{body}$ is a reflection of snail diet, therefore of the local vegetation. This observation is in line with other studies of land snails, which have shown that $\delta^{13}C_{body}$ reflects diet with a possible offset of up to þ1‰ (DeNiro and Epstein, 1978; Stott, 2002; Metref et al., 2003). The similar values and ranges of $\delta^{13}C_{body}$ and $\delta^{13}C_{plant}$ show that *H. melanostoma* consume a variety of the C3 vegetation in the Gebel al-Akhdar. More positive $\delta^{13}C_{body}$ values (e.g. 22‰) may reflect ingestion of a greater proportion of water-efficient species such as juniper, whilst more negative values (e.g. -29‰) may reflect ingestion of more shade-adapted species such as oak and carob whilst intermediate values (e.g. -25‰) may reflect a mixed diet. The majority of δ $d^{13}C_{body}$ values were between -24 and -25‰ (Fig. 4), indicating that most snails probably consumed a mixed diet of the available vegetation within their home range.

4.3. From snail bodies to shell

Several studies have shown that for many herbivorous species, snail diet has a significant control on $\delta^{13}C_{shell}$ (Stott, 2002; Metref et al., 2003; Yanes et al., 2008; Chiba and Davison, 2009). In this study, individual snail $\delta^{13}C_{body}$ was significantly correlated with $\delta^{13}C_{shell}$ (Fig. 5A, $R = 0.28$, $p < 0.05$). When averaged by collection site, the correlation became stronger (Fig. 5B, $R2 = 0.48$, $p < 0.05$). However, this correlation was only moderate, so the possibility that other factors may also influence $\delta^{13}C_{shell}$ in *H. melanostoma* was explored.

One factor that may influence land snail $\delta^{13}C_{shell}$ is the incorporation of $\delta^{13}C$ from carbonate-rich rocks and sediments. Limestone and carbonate-rich sediments generally have more positive $\delta^{13}C$ values than plants. If carbonate ingestion affected shell isotope ratios, it would cause an ^{13}C-enrichment in snail shell that could be estimated using a simple isotope mass balance equation (e.g. Yanes et al., 2008). Stott (2002) found a strong significant linear relationship between δ $^{13}C_{shell}$ and $\delta^{13}C_{body}$ in laboratory-reared *H. aspersa* ($\delta^{13}C_{shell} = 0.9485(\delta^{13}C_{body})$ þ 12.278, $R2 = 0.98$). The diets of some of these snails included carbonate powder, yet their $\delta 13C_{shell}$ values were statistically indistinguishable from those fed on pure C3 and pure C4 plants. This was interpreted as evidence

that $\delta^{13}C_{plant}$ was the primary determinant of $\delta^{13}C_{shell}$ without any influence from foreign carbonate ingestion.

To test whether $\delta^{13}C_{shell}$ in this study was influenced by foreign carbonate ingestion, we applied Stott's equation to predict *H. melanostoma* $d^{13}C_{body}$ from measured $\delta^{13}C_{shell}$ (Table 4). Predicted mean $\delta^{13}C_{body}$ was lower in $\delta^{13}C$ by 0.9‰ compared to measured mean $\delta^{13}C_{body}$. This difference, whilst small, was significant ($t(52) = 2.716$, $p < 0.05$). The difference between calculated $\delta^{13}C_{body}$ and measured $\delta^{13}C_{body}$ was not constant. Around 43% of measured shells were enriched in $\delta^{13}C$ by >1‰, ~7% were depleted in $\delta^{13}C$ by >1‰, whilst ~50% were within 1‰ of predicted values. All samples were collected from areas with abundant Tertiary limestone outcrops. The fact that not all shells exhibit ^{13}C-enrichment suggests that like *H. aspersa*, *H. melanostoma* $d^{13}C_{shell}$ is not affected by foreign carbonate ingestion. Furthermore, the differences between measured $\delta^{13}C_{plant}$, $\delta^{13}C_{soil}$, and $\delta^{13}C_{body}$ were statistically insignificant which suggests that $\delta^{13}C_{body}$ is a reflection of diet with little to no offset.

Stott's (2002) equation was determined by analysing $\delta^{13}C$ from shells that had grown over a four month period. In this study $\delta^{13}C_{shell}$ was analysed from whole shells that had grown over several years. Snail body tissue has a high turnover rate so measurements of $\delta^{13}C_{body}$ reflect snail diet from the most recent days to weeks of activity (Goodfriend and Hood, 1983). There was a disparity in the time-spans reflected in $\delta^{13}C_{shell}$ and $\delta^{13}C_{body}$ in this study, which may account for the imperfect correlations between measured $\delta^{13}C_{shell}$ and $\delta^{13}C_{body}$ and the inconsistent offsets between measured and calculated $\delta^{13}C_{body}$. For example, if the animals ate a mixed diet of higher ^{13}C and lower ^{13}C C3 plants throughout their lifetime, yet ate only higher ^{13}C C3 plants during their last activity period, their $\delta^{13}C_{body}$ would be more enriched than expected from their $\delta^{13}C_{shell}$ values.

The $\delta^{13}C_{shell}$ values were consistently ^{13}C-enriched relative to $\delta^{13}C_{body}$ with a fairly constant offset of 14.5 ± 1.4‰ (Table 4, Fig. 6). This offset was similar to the offset between soft tissue and carbonate found for other land snail species (DeNiro and Epstein, 1978; Stott, 2002; Metref et al., 2003) which is another indication that $\delta^{13}C_{shell}$ is primarily a function of $\delta^{13}C_{body}$.

4.4. Carbon isotope composition of *H. melanostoma* shell correlation with climate

Weak but significant correlations were found between individual $d^{13}C$body and elevation, and instrumental climate data (mean annual temperature, mean annual relative humidity and mean annual precipitation) from the Libyan National Meteorological agency (Appendix 2: $R2 = 0.008$, $p < 0.05$). These correlations were not significant when $\delta^{13}C_{body}$ and $\delta^{13}C_{shell}$ were averaged by collection site. This suggests that at the local scale, a climatic control on $\delta^{13}C_{shell}$ was not evident in the Gebel al-Akhdar. A one-way ANOVA showed that there were significant differences in $\delta^{13}C_{shell}$ between some collection sites ($F(10, 42) = 4.464$, $p < 0.01$) but a Tukey post-hoc test revealed that for the majority of sites mean $\delta^{13}C_{shell}$ ratios were not significantly different ($p > 0.05$). This suggests that the vegetation source of carbon was similar across the sites and did not differ in any regular way with elevation or climatic parameters. This finding is not unexpected as the transect was fairly small (~10 km). Other studies have found stronger corrections between $\delta^{13}C_{shell}$ and environmental predictors but they surveyed a much broader geographic region (e.g. Goodfriend and Ellis, 2002; Yanes et al., 2008).

5. Conclusion

The evidence presented in this study shows that *H. melanostoma* carbon isotopes reflect the $\delta^{13}C_{plant}$ of local vegetation with a mean positive offset between diet/body and shell of 14.5 ± 1.4‰. In the Gebel al-Akhdar, higher mean $\delta^{13}C_{shell}$ values likely reflect the consumption, and therefore greater availability of water-efficient C3 plants whilst lower mean $\delta^{13}C_{shell}$ values likely reflect the consumption of less water-efficient C3 plants. The distribution of these plants is in turn affected by environmental factors such as rainfall. At the scale of this study, $\delta^{13}C_{shell}$ was not strongly controlled by temperature or rainfall amount, however, correlations may be found with additional data at a more regional scale. The findings from this study can be applied to *H. melanostoma* shells from archaeological and geological sites and hold promise for reconstructing past vegetation parameters in the eastern Mediterranean. If the records are derived from archaeological land snail shells, the palaeoenvironmental reconstructions can be used to study late Pleistocene to Holocene human-environment interactions.

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Figures

Fig. 1. A: Location of the Gebel al-Akhdar (rectangle) in the eastern Mediterranean. B: The Gebel al-Akhdar showing the closest major cities. Dark grey shading corresponds to higher elevation. Numbers are land snail collection sites outlined in Table 1.

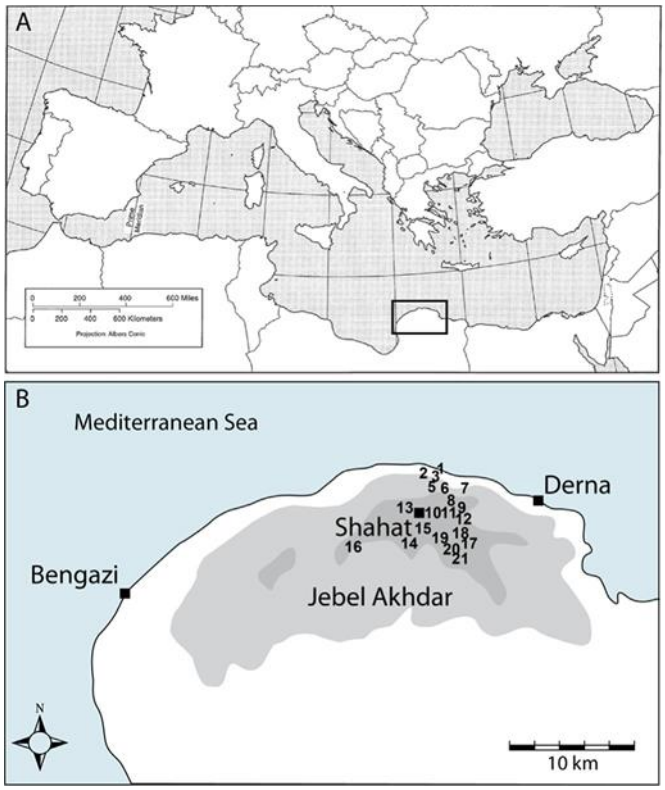


Fig. 2. Land snails in the Gebel al-Akhdar: A: Aestivating *H. melanostoma*, note the thick opaque epiphragm, B: Active *H. melanostoma*.

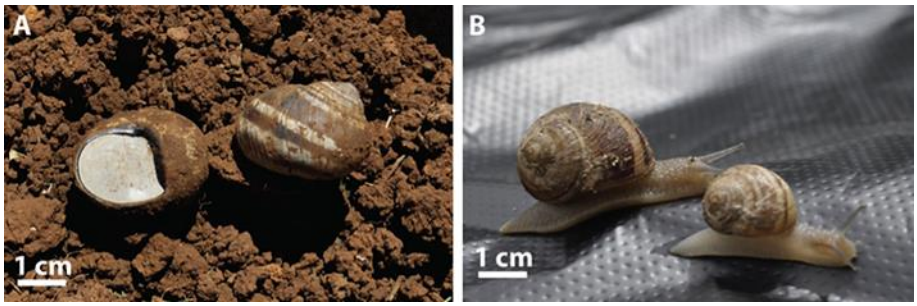


Fig. 3. Stable isotopes from vegetation in the Gebel al-Akhdar (oak and carob n = 4, juniper n = 6). The SD is 0.1‰.

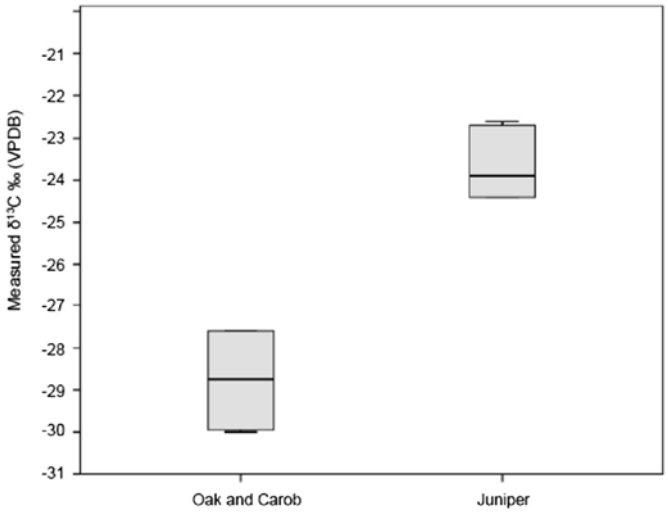


Fig. 4. Frequency distribution of snail $\delta^{13}C_{body}$ values in the Gebel al-Akhdar.

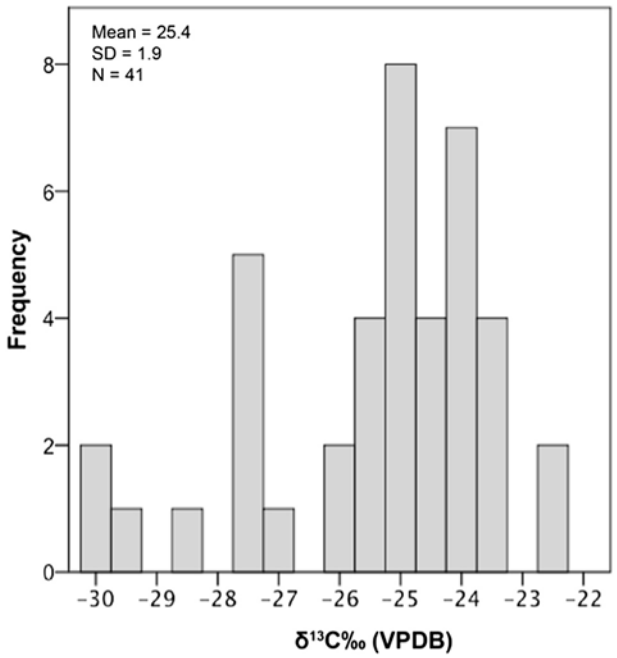


Fig. 5. Correlations between $\delta^{13}C_{body}$ and $\delta^{13}C_{shell}$. A: Correlation between *H. melanostoma* $\delta^{13}C_{body}$ and $\delta^{13}C_{shell}$ from this study in comparison to the empirical correlation found by Stott (2002) for laboratory-raised *H. aspersa*. B: Correlation between site mean $\delta^{13}C_{shell}$ and $\delta^{13}C_{body}$. Error bars are one SD.

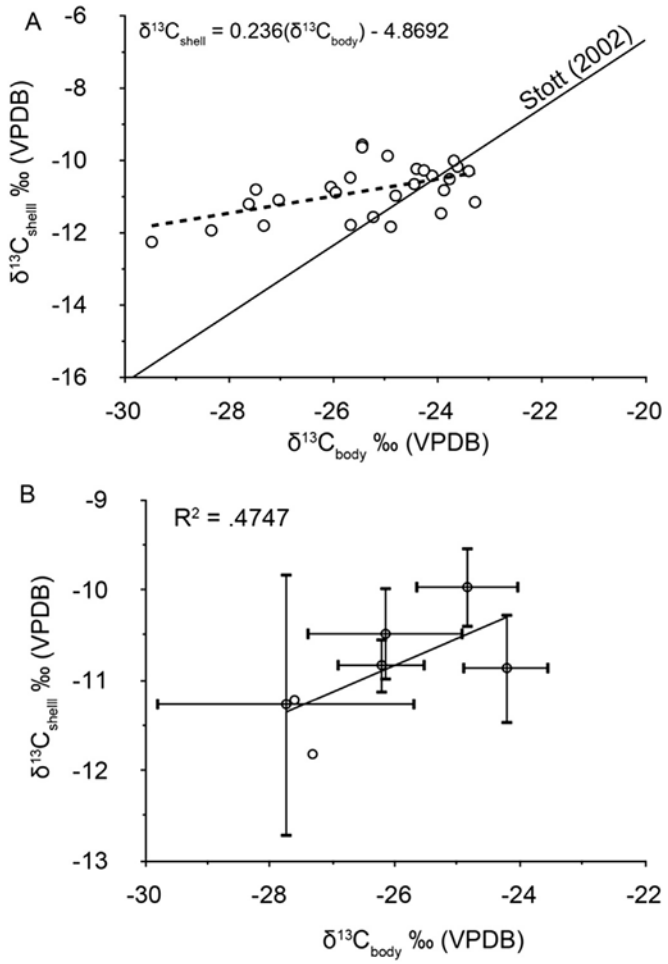
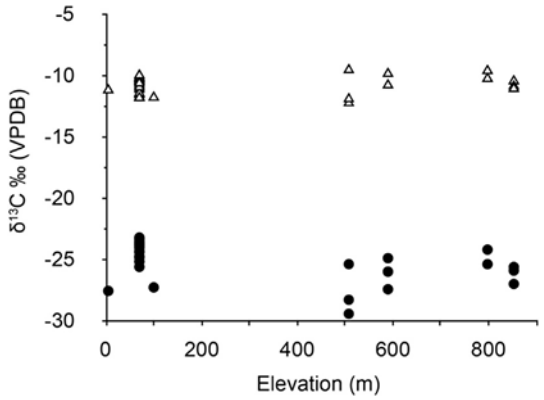


Fig. 6. *H. melanostoma* $\delta^{13}C_{shell}$ (triangles) and $\delta^{13}C_{body}$ (circles) plotted against elevation.



Tables

Table 1: Summary of the land snail collection survey in the Gebel al-Akhdar

Site	Site code	Location	Elevation	Substrate	Vegetation
1	AP12-1	32° 54.176,	5	LO	LS

		21° 58.524			
2	MHWHG	32° 53.951, 22° 2.646	50	CS	PL, JO, CS
3	ECOHF	32° 54.017, 22° 3.011	70	CS, LL	PL, JO, CS
4	T01-2	32° 54.015, 22° 3.0035	74	LO	PL, JO, CS
5	T01-10	32° 53.981, 22° 2.034	80	LO	PL, JO
6	T01-6	32° 53.978, 22° 3.074	89	LO	PL, JO
7	ML12-1	32° 52.748, 22° 10.617	100	CS	PL, JO
8	ML12-2	32° 51.676, 22° 10.648	348	CS	PL, JO
9	AS1-2	32° 52.285, 22° 5.091	390	CS	PL, JO
10	AS1-A	32° 51.947, 22° 5.085	435	CS	PL, JO
11	ML12-6	32° 50.779, 22° 09.619	508	CS	PL, JO
12	ML12-5	32° 50.915, 22° 09.589	514	CS	PL
13	RH12-1a	32° 49.579, 21° 51.730	590	CS	CS
20	ML12-9	32° 40.038, 21° 51.789	798	CS	SS, LS
21	ML12-10	32° 39.647, 21° 51.308	853	CS	SS, LS

Vegetation key: PL = *Pistacia lentiscus*, CS = *Ceratonia siliqua*, JO = *Juniperus oxycedrus*, SS = *Sarcopterium spinosum*, AS = Asteraceae sp., LS = Lamiaceae sp.

Substrate key: LO = limestone outcrop, CS = carbonate soils, LL = leaf litter.

a Collected whilst active. All other snails collected whilst aestivated.

Table 2: Carbon stable isotope composition of vegetation in the Gebel al-Akhdar.

Genus	**Sample number**	**Sample type**	**$\delta^{13}C$ ‰ VPDB**
Ceratonia	C1	Bulk vegetation	-29.9
Ceratonia	C2	Bulk vegetation	-30.0
Quercus	Q1	Bulk vegetation	-27.6
Quercus	Q2	Bulk vegetation	-27.6
Juniperus	J1	Bulk vegetation	-22.6
Juniperus	J2	Bulk vegetation	-22.7
Juniperus	J3	Bulk vegetation	-24.4
Juniperus	J4	Bulk vegetation	-24.4
Juniperus	J5	Bulk vegetation	-23.9
Juniperus	J6	Bulk vegetation	-23.9
Mean			-25.7

Table 3: Isotopic compositions of sediment surrounding aestivated *H. melanostoma* samples.

Sample	**Sample code**	**$\delta^{13}C$ ‰ VPDB**
Soil	CP10 HF EC002 02-01-S1	-24.9
Soil	CP10 HF EC002 02-01-S1	-25.0
Soil	CP10 HF EC006 06-01-S1	-24.9
Soil	CP10 HF EC006 06-01-S1	-24.9
Mean		-24.9

Table 4: Carbon isotopes in *H. melanostoma* shell and body tissue, offsets between $\delta^{13}C_{shell}$ and $\delta^{13}C_{body}$, and comparison between measured and predicted $\delta^{13}C_{shell}$ and $\delta^{13}C_{body}$ calculated using the Stott (2002) equation. Predicted values are shown in bold typeface.

Elevation (m)	**Sample code**	**Measured $\delta^{13}C_{body}$**	**Measured $\delta^{13}C_{shell}$**	**$\delta^{13}C_{shell}$ - $\delta^{13}C_{body}$**	**Predicted $\delta^{13}C_{body}$**	**Predicted $\delta^{13}C_{shell}$**
5	APH121A	-27.6	-11.2	16.4	**-24.8**	**-13.9**
70	ECO0201	-23.4	-10.3	13.1	**-23.8**	**-9.9**
70	ECO0202	-24.1	-10.4	13.6	**-24.0**	**-10.6**
70	ECO0203	-24.4	-10.3	14.1	**-23.8**	**-10.8**
70	ECO0204	-23.9	-10.8	13.0	**-24.4**	**-10.4**
70	ECO0205	-23.6	-10.2	13.4	**-23.7**	**-10.1**
70	ECO0301	-23.8	-10.5	13.2	**-24.0**	**-10.3**
70	ECO0302	-23.7	-10.0	13.6	**-23.5**	**-10.2**
70	ECO0602	-25.2	-11.6	13.6	**-25.2**	**-11.6**
70	ECO0603	-25.6	-11.8	13.8	**-25.4**	**-12.0**
70	ECO0604	-23.3	-11.2	12.1	**-24.7**	**-9.8**
70	ECO0605	-23.9	-11.5	12.4	**-25.0**	**-10.4**
70	ECO0606	-24.9	-11.9	13.0	**-25.4**	**-11.3**
70	ECO0607	-24.8	-11.0	13.8	**-24.5**	**-11.2**
70	ECO0608	-24.4	-10.7	13.8	**-24.2**	**-10.9**
100	MLH121A	-27.3	-11.8	15.5	**-25.4**	**-13.6**
508	MLH12-6C	-29.5	-12.3	17.2	**-25.9**	**-15.7**
508	MLH12-6D	-28.3	-11.9	16.4	**-25.5**	**-14.6**
508	MLH12-6E	-25.4	-9.6	15.8	**-23.0**	**-11.8**
590	RHH12-2A	-24.9	-9.9	15.0	**-23.4**	**-11.4**
590	RHH12-2C	-27.5	-10.8	16.6	**-24.4**	**-13.8**
590	RHH12-2D	-26.0	-10.7	15.3	**-24.3**	**-12.4**
798	MLH12-9A	-24.2	-10.3	14.0	**-23.8**	**-10.7**
798	MLH12-9B	-25.4	-9.6	15.8	**-23.1**	**-11.8**
853	MLH1210A	-25.7	-10.5	15.2	**-24.0**	**-12.1**
853	MLH1210B	-25.9	-10.9	15.0	**-24.4**	**-12.3**
853	MLH1210D	-27.0	-11.1	15.9	**-24.7**	**-13.4**
Mean		-25.3	-10.8	-14.5	-24.4	-11.7
SD		1.6	0.7	1.4	0.8	1.5

Appendices

Appendix 1: Stable isotope results from *H. melanostoma* bodies and shells. Highlighted cells are juvenile samples (<10 mm maximum basal diameter).

Elevation (m)	Sample code	Body $\delta^{13}C$ ‰ VPDB	Shell $\delta^{13}C$ ‰ VPDB
5	APH121A	–27.6	–11.2
5	APH12-1B		-10.8
5	APH12-1C		-11.0
5	APH12-1E		-11.7
5	APH12-1F		-11.2
50	MHWHG-A		-11.1
50	MHWHG-B		-9.9
50	MHWHG-C		-10.5
70	ECO0201	-23.4	-10.3
70	ECO0202	-24.1	-10.4
70	ECO0203	-24.4	-10.3
70	ECO0204	-23.9	-10.8
70	ECO0205	-23.6	-10.2
70	ECO0301	-23.8	-10.5
70	ECO0302	-23.7	-10.0
70	ECO0401		-10.5
70	ECO0602	-25.2	-11.6
70	ECO0603	-25.7	-11.8
70	ECO0604	-23.3	-11.2
70	ECO0605	-23.9	-11.5
70	ECO0606	-24.9	-11.9
70	ECO0607	-24.8	-11.0
70	ECO0608	-24.4	-10.7
74	TO12A.A		-10.4
74	TO12A.B		-10.3
74	TO12B		-10.6
74	TO12C		-11.1
74	MHT012-A		-10.8
80	TO110A.A		-9.7
80	TO110B		-9.5
80	TO110C		-10.4
89	MHT016-A		-10.9
89	MHT016-B		-10.8
100	MLH121A	-27.3	-11.8
100	MLH121B		-11.7
390	MHAS1-2B		-10.5
390	MHAS1-2C		-9.7
390	MHAS1-2D		-7.8
435	AS1A.A		-9.1

435	AS1A.B		-9.2
435	AS1B		-8.6
435	AS1C		-10.0
435	AS1D		-8.8
435	AS1E.A		-9.9
508	MLH12-6C	-29.5	-12.3
508	MLH12-6D	-28.3	-12.0
508	MLH12-6A		-9.9
508	MLH12-6B		
508	MLH12-6E	-25.4	-9.6
514	MLH12-5A	-24.6	-9.6
590	RHH12-2A	-24.9	-9.9
590	RHH12-2C	-27.5	-10.8
590	RHH12-2B		
590	RHH12-2D	-26.0	-10.8
798	MLH12-9C		-10.2
798	MLH12-9A	-24.2	-10.3
798	MLH12-9B	-25.4	-9.7
853	MLH1210A	-25.7	-10.5
853	MLH1210B	-25.9	-10.9
853	MLH1210C	-25.7	
853	MLH1210D	-27.0	-11.1

Appendix 2: Results of simple linear regression on individual and site mean $\delta^{13}C_{shell}$. The coefficient of determination is shown in bold typeface.

Predictors	F	t	Constant	B	p	SE mod	R	R2
Individual shells								
Elevation	4.971	2.230	−68.999	−10.74	<0.05	0.85	0.29	**0.08**
Mean relative humidity	69.762	8.352	−28.452	0.409	<0.05	0.85	0.29	**0.08**
Mean annual temperature	4.977	−2.231	−6.231	−0.147	<0.05	0.85	0.29	**0.08**
Mean annual precipitation	4.972	2.230	−11.189	0.002	<0.05	0.85	0.29	**0.08**
Site average								
Mean relative humidity	1.369	−1.170	3.416	−0.197	ns	0.71	0.32	**0.10**
Mean annual temperature	1.369	−1.170	−8.130	−0.125	ns	0.71	0.32	**0.10**
Mean annual precipitation	1.360	1.170	−11.074	0.002	ns	0.71	0.32	**0.10**

Dependent variable = $\delta^{13}C_{shell}$.

B = unstandardised beta coefficient, SE B = standard error. SE mod = Standard error of the model.

t = t-test statistic, p = significance. ns = not significant.