Paleoenvironmental implications of carbon stable isotope composition of land snail tissues

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ABSTRACT

Land snail shell δ¹³C value is often used as a paleovegetation proxy assuming that snails ingest all plants in relation
to their abundance, and that plants are the only source of carbon. However, carbonate ingestion and variable met-
abolic rates complicate these relationships. We evaluate if live-collected snails from Lanzarote (Canary Islands)
reflect the abundance of C₃ and CAM plants. Snails were collected on either CAM or C₃ plants for isotope analysis
of shell and body, and shell size. Respectively shell and body δ¹³C values of snails collected on CAM plants averaged
−8.5 ± 1.7‰, and −22.8 ± 1.6‰, whereas specimens from C₃ plants averaged −10.1 ± 0.7‰ and −24.9 ±
1.1‰. A flux balance model suggests snails experienced comparable metabolic rates. A two-source mass balance
equation implies that snails consumed ~10% of CAM, which agrees with their abundance in the landscape. Snails
collected on CAM plant were smaller than those on C₃ plants. Conclusively: 1) snails consume CAM plants when
they are available; 2) migration of snails among C₃ and CAM plants is a common phenomenon; and 3) C₃ plants
may be a more energetic food for growth than CAM plants. This study shows that shell δ¹³C values offer approximate
estimates of plants in C₃-CAM mixed environments.

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Introduction

Plants differ in their carbon isotopic composition (δ¹³C) as a conse-
quence of variable carbon isotope fractionation pathways during photo-
synthesis (e.g., O’Leary, 1981; Farquhar et al., 1989). C₃ plants have
significantly higher δ¹³C values, from −15% to −11‰, than C₄ plants,
which range between −35% and −20‰. CAM plants, which follow a
crasulacean acid metabolism, use C₃ or C₄ pathways depending on the
times of the day and exhibit δ¹³C values between C₃ and C₄ plants
(see review in Dawson et al., 2002). C₃ plants, which include most
trees, shrubs and grasses that grow in cold seasons, are favored some-
what cooler and wetter conditions, and relatively higher atmospheric
CO₂ concentrations (e.g., Quade and Cerling, 1995). In contrast, C₄
plants, which include some shrubs and grasses that grow in warm
seasons, are widespread in low latitude, hot and/or dry settings (e.g.,
Quade and Cerling, 1995), and their carbon demand is reached more
efficiently under lower concentration of atmospheric CO₂ than
C₃ plants (e.g., Koch et al., 2004). CAM plants are common in desert
and semi-desert areas and survive under dry conditions by advanced
water storage strategies (e.g., Dawson et al., 2002). Because plants
with differing photosynthetic pathways are associated with different
climates, variations in the proportion of plant type in a landscape may
offer insight into environmental conditions, or even changes
in land use over time (e.g., Dawson et al., 2002). Consequently, surficial
materials that preserve remnants of the carbon isotopic composition
of indigenous vegetation, such as pollen (Jahren, 2004; Nelson et al.,
2006), pedogenic carbonates, soil organic matter (e.g., Quade and
Cerling, 1995), and the teeth and bones of animals (e.g., Koch et al.,
2004; Clementz, 2012), are valuable proxies for paleoenvironmental
studies.

Aragonitic shell material and the shell organic matrix of land snails
exhibit high preservation potential and have the ability to track the iso-
topic signature of fossil plant material (Goodfriend, 1990; Goodfriend
and Ellis, 2000, 2002). The great majority of land snails are primary con-
sumers, mostly feeding on living and decayed vascular plants (Speiser,
2001; ZongXiu et al., 2007). The δ¹³C values of snail tissues, thus, reflect
the signature of the diet (DeNiro and Epstein, 1978). Previous laboratory
studies demonstrate that the δ¹³C values of the snail body tissue and the
aragonitic shell mainly reflect the δ¹³C values of the consumed C₃ or C₄
plant (Stott, 2002; Metret et al., 2003; ZongXiu et al., 2007). Accordingly,
the δ¹³C values of the shell may be used to reconstruct past variations
in the relative abundance of C₃ and C₄ plants in paleolandscapes
(Goodfriend and Ellis, 2000, 2002; Balakrishnan et al., 2005; Baldini
et al., 2007; Yanes et al., 2008, 2009). However, accurate estimations
of the proportion of C₃/C₄ plants derived from the isotopic signature
of snail shells are complicated by other factors, such as the ingestion

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of limestone and potential variations in metabolic rates among snails (Goodfriend and Hood, 1983; Goodfriend, 1987; Goodfriend et al., 1999; Balakrishnan and Yapp, 2004). Thus, some published studies recommend caution when interpreting the δ\(^{13}\)C values of snail shells to reconstruct the relative percentage of C\(_3\) vs. C\(_4\) plants in the environment (Balakrishnan and Yapp, 2004; Yanes et al., 2008). While many studies have evaluated the relationship between the proportion of C\(_3\)/C\(_4\) plants in the diet and the δ\(^{13}\)C value of land snail shells in the laboratory (Stott, 2002; Metref et al., 2003) and in the field (e.g., Balakrishnan et al., 2005; Baldini et al., 2007), studies that survey if CAM plants are consumed by land snails and isotopically recorded in their tissues are rare (but see Goodfriend and Ellis, 2000, 2002).

Lanzarote Island of the Canary Archipelago is a semiarid (<150 mm annual rainfall) subtropical island (29°N) with abundant endemic CAM plants. Herbivorous endemic snails like the helicid Theba geminata (Mousson, 1857), appear to feed on succulent plants from the families Euphorbiaceae, Crassulaceae and Cactaceae. This offers an excellent opportunity to evaluate the potential effect of dietary carbon derived from CAM plants on the δ\(^{13}\)C values of snail tissues in a natural setting. In the present study, specimens of the helicid T. geminata were live-collected directly from either C\(_3\) or CAM plants and studied isotopically and morphometrically to: (1) elucidate if snails track the δ\(^{13}\)C values of the plants on which they were collected (i.e., do snails feed on a single plant type throughout their life or do they migrate among plant species), (2) explore if snail growth is affected by the type of diet (i.e., do snails that feed mostly on C\(_3\) plants exhibit equivalent adult body size as those that feed on CAM plants), and (3) evaluate the potential of snail shell δ\(^{13}\)C values as a paleovegetation proxy in oceanic semiarid settings (i.e., do shell δ\(^{13}\)C values of fossil land snails have the potential to mirror the isotopic signature of the succulent vegetation). Data are evaluated using a two-source input mass balance equation and a published snail flux balance mixing model, and the results are compared to published records from the region.

Material and methods

Geographical and environmental setting

The volcanic island of Lanzarote (29°N, 13°W) is the easternmost oceanic island of the Canary Archipelago (Fig. 1A), located ~125 km from the northwest African coast. Lanzarote is the fourth largest island, with an area of ~807 km\(^2\); it is the lowest lying island, with a maximum elevation of ~607 m above sea level (asl). The island is ~15.5 million years old (Ma), and it is the second oldest of the seven main islands of the Canary Archipelago (Fernández-Palacios and Whittaker, 2008).

Lanzarote is a semiarid island. For the recording period 1972–2000, the meteorological station of Arrecife Airport (14 m asl), reported (http://www.aemet.es): (1) air temperature varied from 17°C in January to 24.7°C in August; (2) precipitation ranged from 0 mm during several summer months to 27 mm in December; and (3) mean relative humidity ranged from 69% for the March–June period to 73% for the September–December time interval. Annual mean temperature, precipitation and relative humidity in Lanzarote are ~20°C, 109 mm, and 71%, respectively. Relative humidity values measured with a hygrometer directly on the soil–air interface next to a living snail assemblage, at 8:00 AM in March of 2012, was ~90–91%. The weighted (mean annual) δ\(^{18}\)O value of the rainfall in the study area is ~−3.5‰ vs. SMOW (Yanes et al., 2011).

The vegetation on Lanzarote is principally xerophytic (adapted to dry conditions), dominated by sub-desert coastal scrub below 300–500 m (asl), with abundant succulent-type plants. Succulent plants include native species such as those from the Euphorbiaceae and Crassulaceae families, as well as introduced species from the family Cactaceae. The

Figure 1. Geographical setting of sampling localities and photographs of snails and plants in the field. (A) Map of Lanzarote Island and sampling sites: Gayo (latitude: 29°10′10″, longitude: 13°30′40″, altitude: 450 m asl) and Loma de San Andrés (latitude: 29°02′12″ N, longitude: 13°36′48″, altitude: 280 m asl). (B) Snails resting on the C\(_3\) plant Euphorbia balsamifera. (C) Snails resting on the CAM plant Aeonium lancerottense. (D) Snails resting on the CAM plant Opuntia dilenii (photographs taken by Y. Yanes, 2010–2012).
genera Euphorbia (Euphorbiaceae) and Aeonium (Crassulaceae) have experienced considerable radiation and speciation in the Canary Islands and consequently, they both are relatively rich in the archipelago (Fernández-Palacios and Whittaker, 2008). Besides succulent plants, many other vascular plants (mostly C3 plants) are present in Lanzarote. Overall, C3 plants dominate the landscape, but C4 and CAM plants are also present and can be locally important in the eastern Canary Islands (Yanes et al., 2008).

Sampling approach

Living land snails of the helicid T. geminata (n = 58) were collected at two localities in the northwest and center of Lanzarote Island: Gayo (LGA) and Loma de San Andrés (LLA), respectively (Fig. 1A). These localities were chosen because the bedrock of the areas contained little limestone compared to other locales in the island. Field observations indicated that the Gayo locality (29°10′10″ N), at ~450 m asl, supported a denser vegetative cover than Loma de San Andrés (29°2′12″ N), at ~280 m asl near the center of the island. In addition, Gayo exhibited negligible anthropogenic impact while Loma de San Andrés was closer to urbanized areas. Adult specimens were collected resting on three different plants at two localities in the northwest and center of Lanzarote Island: Gayo (Fig. 1C) and the introduced Opuntia dilleni (Fig. 1D). In Gayo, specimens were collected from Aeonium (n = 14) and Euphorbia (n = 15) whereas individuals from Loma de San Andrés locality were collected from Aeonium (n = 14) and Euphorbia (n = 15). C3 plants were not found at those localities during fieldwork. Snail specimens were stored in 80% ethanol for 20 days and samples of eight fresh leaves on which snails were feeding (Figs. 1B–D) were oven-dried at 50°C for 48 h shortly after collection and prior to laboratory analysis. Biological tissues preserved in ethanol may vary slightly in δ13C value as a result of lipid extraction (e.g., Sarakinos et al., 2002). However, land snail body tissue is poor in fat content (~2–4% by weight) and therefore, the preservation method employed here should have not affected the δ13C values of the original body tissue to a measureable extent. Moreover, potential effects of ethanol should have affected equally analyzed samples because they all were preserved using the same procedure.

Laboratory analyses

Samples were prepared and analyzed in the stable isotope laboratory of the Department of Earth and Environmental Sciences, University of Kentucky. Leaf samples were washed with distilled water and oven-dried at 40°C for 48 h. Samples were then ground using an electric blender. The snail body was separated manually from the shell, rinsed with deionized water, oven-dried at 40°C overnight and homogenized with an electric blender. The snail body was finely ground using an agate mortar and pestle. About 1.5 mg of each organic sample was rinsed with deionized water, oven-dried at 40°C overnight and homogenized using an electric blender. About 1.5 mg of each organic sample was weighed in a pre-cleaned tin capsule, crimped and combusted in a Costech Elemental Analyzer (ESC 4010). The CO2 produced after combustion was analyzed using a Finnigan DeltaPlus XP isotope ratio mass spectrometer. Analytical uncertainty was ±0.1‰ based on the repeated measurements of in-house and international standards dispersed periodically throughout each run sequence (n = 10).

Shells were cleaned in DI water by ultrasonication, and oven-dried at 40°C overnight. Entire shells were finely ground using an agate mortar and pestle. Shell powder was treated with 3% NaOCl (reagent grade) overnight at room temperature (~22°C) to remove external organic contaminants and shell organic matrix. About 150 μg of carbonate was placed in a 6 mL Exetainer™ vial that was subsequently flushed with helium to replace the headspace. The carbonate was then converted to CO2 gas by adding 0.1 ml of 104% H2PO4 at 25°C. The resulting CO2 was analyzed after 24 h using a GasBench II peripheral device connected to the isotope ratio mass spectrometer. Analytical uncertainty was ±0.1‰ based on the repeated measurement of in-house and international standards throughout each sequence (n = 20).

All stable isotope results are reported in δ notation relative to the international standard Pee Dee Belemnite (PDB). The δ value is defined as:

$$\delta^{13}C = \frac{R_{sample}}{R_{standard}} - 1 \times 1000 \text{‰}$$

Statistical analyses

Statistical analyses were carried out using PAST 2.17 software (Hammer et al., 2001) considering statistical significance at α = 0.05. Isotopic and morphometric data were normally distributed (Shapiro-Wilk test, p < 0.05) and generally displayed equality of variances (F test, p > 0.05). Pearson correlation coefficients were used to estimate the relationship between two variables. Ordinary least-square regression was employed to estimate the slope and intercept of the linear relationships between variables.

Morphometric analyses

Six measurements were made on each of 58 shells following methodologies from Kerney and Cameron (1979) and Huntley et al. (2008) prior to geochemical analyses: (1) shell length, (2) shell width, (3) height of the two last spires, (4) height of the last spire, (5) aperture height, and (6) aperture width. Measurements were made using an electronic caliper to the nearest 0.02 mm. Morphometric data was subjected to a principal component analysis (PCA) on a variance-covariance matrix. Because the six bidimensional measures correlated with PC1, PC1 is hence an appropriate proxy of shell size because it reflects six measurements jointly rather than one only (Huntley et al., 2008). Log-transformed shell length was also computed as a second proxy of shell size (Huntley et al., 2008).

Two-source input mass balance model

The isotopic composition of a consumer tissue represents the isotopic values of the consumed foods (DeNiro and Epstein, 1978), weighted by the proportions of such dietary items (e.g., Phillips, 2012). With one isotope system (e.g., δ13C value of snail body) and two possible sources (e.g., δ13C values of CAM and C3 plants), a two-source input mass balance equation can be used to determine the proportion of each source that contributes to the δ13C values of the snail body (e.g., Phillips, 2012). This is mathematically expressed as follows:

$$\delta^{13}C_{\text{snail body}} = \%C3 \times \delta^{13}C_{\text{C3}} + \%CAM \times \delta^{13}C_{\text{CAM}}$$

where \%C3 + %CAM = 100

then \%CAM = $\left(\delta^{13}C_{\text{snail body}} - \delta^{13}C_{\text{C3}}\right) / \left(\delta^{13}C_{\text{CAM}} - \delta^{13}C_{\text{C3}}\right) \times 100$.

The aforementioned equation was employed to estimate the proportion of assimilated CAM plant in land snail body tissues from Lanzarote Island, assuming that C3 and CAM plants were the only food items ingested by snails in the sampling localities, and subtracting 1‰ from the body tissue to correct for the isotopic fractionation between consumer tissue and diet (DeNiro and Epstein, 1978). Bayesian models are ideal to calculate the proportional contribution of consumed foods to a mixture because they can contemplate multiple food items and isotopes simultaneously, and they consider isotopic variations of those resources and mixtures (e.g., Parnell et al., 2010). In this work, only two possible food resources were tested and isotopic variability of those foods was not quantified. Also, only one isotope system (δ13C) was explored. Hence, a simple two-source input model was selected over a Bayesian model for the present study.
Balakrishnan and Yapp (2004) developed a steady-state flux balance mixing model to better understand the δ¹³C and δ¹⁸O values of aragonitic shells of land snails. For δ¹³C values, the model relates the amount and isotopic composition of consumed plants, the amount and isotope values of bicarbonate in the hemolymph, and the diffusive flux of bicarbonate from the hemolymph. One other parameter is the out flux of bicarbonate from the hemolymph (fₒ) relative to the influx of CO₂ derived from plants (fᵢₐₓ), which is called ϕ (=fₒ/fᵢₐₓ) and varies with metabolic rate (see Balakrishnan and Yapp, 2004 for further details). Model calculations are constrained by the δ¹³C value of the shell and the organic tissue (plant and snail body), and the ambient temperature during calcification.

For δ¹⁸O values, the model uses the amount and isotopic composition of ambient rain, the amount and isotopic composition of water from the hemolymph, the diffusive flux of water from the hemolymph by evaporation, and the temperature dependent oxygen isotope fractionation between the hemolymph and aragonite (Balakrishnan and Yapp, 2004). Temperature, δ¹⁸O values of water and water vapor, and relative humidity are the most important factors controlling the δ¹⁸O values of the hemolymph and the shell (Balakrishnan and Yapp, 2004). Another parameter is the out flux of water from the hemolymph (fₒ) relative to the influx of imbibed water (fᵢₐₓ), which is called θ (=fₒ/fᵢₐₓ).

Balakrishnan and Yapp (2004) showed that it is appropriate to assume that water vapor is in isotope equilibrium with liquid water in this model (see also Yanes et al., 2011). Also, water of the hemolymph is assumed to be lost only by evaporation (i.e., θ = 0) (Balakrishnan and Yapp, 2004). This assumption is adopted here. Model calculations are constrained by measured values of temperature and the δ¹⁸O values of rainwater and shell carbonate.

Results

Leaves of the CAM plants A. lancerottense from Gayo and O. dilenii from Loma de San Andrés had δ¹³C values of −13.7‰ (n = 1) and −14.1 ± 0.5‰ (n = 2), respectively (Table 1; Fig. 2A). The C₃ plant E. balsamifera from Gayo had an average δ¹³C value of −25.5 ± 0.03‰ (n = 3) whereas the same plant species from Loma de San Andrés displayed an average value of −26.0 ± 0.8‰ (n = 2) (Table 1). These values agree with published values for the same species collected from Tenerife Island of the Canary Archipelago (Yanes et al., 2009).

The δ¹³C values of land snail body tissue (n = 58) ranged from −27.4‰ to −20.0‰ (Table 2; Figs. 2A–B). Snails from Gayo had significantly lower δ¹³C values than those from Loma de San Andrés (Fig. 2C). The average δ¹³C value of the body was −23.9 ± 1.0‰ for specimens from Gayo that were resting on the CAM plant A. lancerottense, and −21.6 ± 1.2‰ for individuals from Loma de San Andrés which were resting on the CAM plant O. dilenii (Fig. 2C). Snails resting on the C₃ plant E. balsamifera at Gayo and Loma de San Andrés showed average δ¹³C values of −25.1 ± 0.9‰ and −24.7 ± 1.2‰, respectively (Fig. 2C). The difference in δ¹³C values between sites was lower for snails collected on C₃ plants (0.4‰) than those collected from CAM plants (2.3‰). The δ¹³C values of snail tissues were always lower in Gayo than in Loma de San Andrés (Fig. 2C).

Table 1
Carbon isotopic composition of leaves of succulent plants from Lanzarote Island on which snails were resting and feeding.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Sample ID</th>
<th>Locality</th>
<th>Plant species</th>
<th>δ¹³C (PDB)</th>
<th>Plant type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LGA-plant-1</td>
<td>Gayo</td>
<td>Aeonium lancerottense</td>
<td>−13.7</td>
<td>CAM</td>
</tr>
<tr>
<td>2</td>
<td>LGA-plant-2</td>
<td>Gayo</td>
<td>Euphorbia balsamifera</td>
<td>−25.5</td>
<td>C₃</td>
</tr>
<tr>
<td>3</td>
<td>LGA-plant-3</td>
<td>Gayo</td>
<td>Euphorbia balsamifera</td>
<td>−25.5</td>
<td>C₃</td>
</tr>
<tr>
<td>4</td>
<td>LGA-plant-4</td>
<td>Gayo</td>
<td>Euphorbia balsamifera</td>
<td>−25.6</td>
<td>C₃</td>
</tr>
<tr>
<td>5</td>
<td>LLA-plant-1</td>
<td>Loma San Andrés</td>
<td>Opuntia dilenii</td>
<td>−13.7</td>
<td>CAM</td>
</tr>
<tr>
<td>6</td>
<td>LLA-plant-2</td>
<td>Loma San Andrés</td>
<td>Opuntia dilenii</td>
<td>−14.4</td>
<td>CAM</td>
</tr>
<tr>
<td>7</td>
<td>LLA-plant-3</td>
<td>Loma San Andrés</td>
<td>Euphorbia balsamifera</td>
<td>−26.5</td>
<td>C₃</td>
</tr>
<tr>
<td>8</td>
<td>LLA-plant-4</td>
<td>Loma San Andrés</td>
<td>Euphorbia balsamifera</td>
<td>−25.4</td>
<td>C₃</td>
</tr>
</tbody>
</table>
The δ^{13}C values of shell carbonate ranged from −11.4‰ to −5.5‰ (Table 2; Figs. 2A–B). The mean δ^{13}C value of the shell was −9.6 ± 1.1‰ for specimens collected on CAM plants from Gayo (L. cameronii) and −7.5 ± 1.5‰ for individuals collected on CAM plants from Loma de San Andrés (O. dilleni) (Fig. 2C). Snails on the C3 plant (PDB) E. balsamifera at Gayo and Loma de San Andrés had a mean δ^{13}C value of −10.4 ± 0.7‰ and −9.9 ± 0.6‰, respectively (Fig. 2C). The δ^{13}C values of the shell and the body tissue correlated positively (Figs. 2B–C). The natural range of shell δ^{13}C values (Fig. 3A) from this study (from −11.4‰ to −5.5‰) overlaps with published data (from −15.7‰ to +1.7‰) from other Canary Islands (Yanes et al., 2008, 2009), including other localities on Lanzarote Island, which varied from −8.0‰ to −4.4‰ (n = 5) (Yanes et al., 2008).

Calculations from the two-source input equation suggest that snails from Lanzarote Island assimilated in their body tissues from a maximum of −41‰ of CAM plant to a minimum of 0‰ (Fig. 4A). End member values of −25.8‰ for C3 plant and −14.1‰ for CAM plant were used in the calculations (Table 1). The δ^{13}C values of snail body indicate that
specimens at Gayo locality collected from the CAM plant *A. lancerottense* consumed, on average, ~7% of CAM plant (open circle in Fig. 4A), whereas snails from Loma de San Andrés that were resting on the CAM plant *O. dilenii* consumed ~27% of CAM plant, on average (open diamond in Fig. 4A). Snails collected on the C3 plant *E. balsamifera* consumed less than ~1% of CAM plant at both locales. Specimens with body δ¹³C values lower than −25.8‰ (end member average value for local succulent C3 plant) consumed plants not sampled in this study. Consequently, model calculations here are provisional because a limited number of food sources were available for isotopic analysis.

Shell and body δ¹³C values were compared in Figure 4B. Calculations from the steady-state flux balance mixing model for δ¹³C values by Balakrishnan and Yapp (2004) suggest that different specimens exhibited relatively comparable metabolic rates as most data plotted close to the curve for ϕ = 0.00 (Fig. 4B). In sharp contrast, when δ¹³C values of the shell are plotted against values of the plant where snails were collected (Fig. 4C), metabolic rates among individuals differed considerably. Thus, specimens from CAM plants plot closer to ϕ = 0.80, whereas snails that were resting on C3 plants plot closer to ϕ = 0.00 (Fig. 4C).

Shells ranged from 10.08 to 13.85 mm in length and from 13.75 to 19.93 mm in width (Table 2; Figs. 5A–B). Shell length and width decreased as the percentage of CAM plants increased in the diet (Table 2; Figs. 5A–F). Proxies for shell size (log [length] and PC1) correlated negatively with the proportion of CAM plant ingested by the snails (Figs. 5C–F).

**Figure 3.** Natural range of δ¹³C (A) and δ¹⁸O (B) values for of land snail shells from this study (black bars) compared to published values by Yanes et al. (2008: white bars) and Yanes et al. (2009: gray bars).

**Figure 4.** Carbon stable isotope models. (A) Calculation of the proportion of consumed CAM plant based on snail body δ¹³C values using a two-source input equation (see text). Open circle depicts the average body δ¹³C value of snails on CAM plants in Gayo whereas open diamond depicts the average value for snails on CAM plants in Loma de San Andrés. Gray band depicts the range of body δ¹³C values in this study. (B) Measured shell δ¹³C values plotted against measured body tissue δ¹³C values. (C) Measured shell δ¹³C values plotted against measured plant δ¹³C values on which snails were feeding. Lines in panels B and C depict the calculations of the carbon flux balance model by Balakrishnan and Yapp (2004), assuming that average temperature during calcification was 20°C and that plant tissue is the only source of carbon for the shell.
of the Canary Archipelago (Yanes et al., 2008, 2009). Published δ¹⁸O values of modern shells from other localities on Lanzarote (Yanes et al., 2008) exhibited a range of values (from −0.3‰ to +2.5‰; n = 5) which was significantly higher than the measured values in the present study (from −2.1‰ to +0.7‰; n = 58) (Fig. 3B). Calculations from the evaporative steady-state flux balance model for δ¹⁸O by Balakrishnan and Yapp (2004) suggest that living land snails from Lanzarote Island deposited shell during an average relative humidity of ~91%, ranging from ~89% to ~96% (Fig. 6). These predicted values for relative humidity assume that calcification occurred during air temperatures of ~20°C and that environmental water imbibed by the snails had a δ¹³C value of −3.5‰ (SMOW). Model calculations also assumed that snails lost water only through evaporation and that the rainfall and water vapor were in isotopic equilibrium (Balakrishnan and Yapp, 2004; Balakrishnan et al., 2005).

Discussion

Based on the average δ¹³C values of body tissues (−23.9 ± 1.7‰; n = 58), the land snails of this study consumed ~10% of CAM plants, on average. This agrees with the relative percentage of CAM plant in the landscape (personal field observations, 2010–2012). Specimens that

Figure 5. Relationship between snail diet type and adult shell size. (A–B) Average maximum shell length and width classified by the proportion of CAM plan ingested. (C–D) Relationship between the estimated proportion of consumed CAM plant and log of shell length. (E–F) Relationship between the estimated proportion of consumed CAM plant and PC1 of size data. In panels E and F, diamonds depict samples from Loma de San Andrés (LLA) whereas circles depict samples from Gayo (LGA). Open symbols depict snail samples collected from CAM plants whereas filled symbols depict individuals collected on C₅ plants.
were collected directly from CAM plants at both locales (n = 28) ingested significantly higher proportions of CAM plants (~17%, on average) than individuals that were resting on C₃ plants at both sites (n = 30) (~0.5%, on average). Interestingly, specimens resting on CAM plants from Gayo (~9.6 ± 1.1‰) had average shell δ¹³C values that were almost equivalent to individuals resting on C₃ plants from Loma de San Andrés (~9.9 ± 0.6‰). This suggests that snails resting on a plant type with a specific photosynthetic pathway do not necessarily consume it intensively. Our results indicate that in C₃/CAM mixed ecosystems, land snails follow a noticeable variable diet. One specimen, with a body δ³¹P value of ~20‰, appears to have ingested up to ~41% of CAM plant whereas many others from the same locale did not consume CAM plant at all (Table 2). This finding clearly stresses the need for collecting numerous specimens from the same locale to calculate a meaningful average value that represents reasonably well the overall diet of a snail assemblage (Balakrishnan et al., 2005; Yanes et al., 2009, 2011), especially in ecosystems where plants utilize different photosynthetic pathways. Also, when collecting recently dead snails in C₃/CAM mixed sites, it is prudent to collect specimens from different areas within the sampling locale, considering the highly variable diet of contemporaneous snails from the same site can follow (Fig. 4A). Another issue that may complicate this approach is that the δ³¹C values of C₃ plants can be several per mil variable within the same locale, especially in semiarid to arid locales (Yanes et al., 2009). This further reinforces the importance of collecting large sample sizes when studying fossil land snails, and points out the need for better constraining the carbon isotopic variability of different plant species in the region in forthcoming studies.

The δ¹³C values of the shell correlated positively with that of the body tissue (Figs. 2B–C), reflecting that the dietary information of the body, which depicts the signature of the plant diet (DeNiro and Epstein, 1978), is tracked in the shell. When δ¹³C values of snail shells are plotted against values of snail bodies (Fig. 4B), and using the evaporative-steady-state flux balance model by Balakrishnan and Yapp (2004), we observed that studied snails experienced fairly comparable metabolic rates because most specimens plotted closely to the curve for Φ = 0.00 (Fig. 4B). This means that in most snails, the ratio of input and output flux of bicarbonate (HCO₃⁻) into the body fluid was similar (Balakrishnan and Yapp, 2004). In contrast, when shell δ¹³C values are plotted against the values of the measured plants from which they were collected (Fig. 4C), individuals appeared to have experienced significantly different metabolic rates. Snails collected on C₃ plants plotted close to the curve for Φ = 0.00, whereas specimens on CAM plants plotted near the curve for Φ = 0.80 (Fig. 4C). This means that for snails on CAM plants, the output flux of bicarbonate from the snail body fluid was greater than the input flux (Balakrishnan and Yapp, 2004). This contradictory result with respect to that from Figure 4B is due to the fact that snails did not consume either C₃ or CAM plant alone, but a mixture of both, as shown by the two-source input equation (Fig. 4A). Hence, in field studies, the δ¹³C values of the snail body represent more accurately the signature of the snail diet than the plants where snails reside when they are collected.

Stott (2002) performed a laboratory experiment in which snails were fed either C₃ or C₄ plant. In addition, specimens that were exposed to CaCO₃ (with a δ¹³C value of ~3.6‰) showed similar shell δ¹³C values than individuals that had no access to CaCO₃. However, this laboratory study was conducted on a single species (Cornu aspersum) that exhibits a relatively large but rather thin shell. Subsequent field studies conducted on different species with thicker shells (e.g., T. geminata) that inhabit carbonate-rich areas have found that anomalously large offsets in δ¹³C values between shell and body tissue may be explained by the assimilation of limestone (Yanes et al., 2008). In the eastern Canary Islands, bioclastic eolian dunes exhibit a δ¹³C value of ~0.1 ± 1.1‰ (n = 10) (Yanes et al., 2008) whereas modern bulk soil carbonates of some areas of the island display a value of ~10.5 ± 0.8‰ (n = 3) (Yanes et al., 2013). Thus, considering the large range of carbon isotopic values of the CaCO₃, in the study area, it is plausible that assimilation of soil carbonate may have had an effect on the δ¹³C values of snail shells. Furthermore, radiocarbon analyses of live-collected snails have shown that snails assimilate dead carbon from their surroundings into their shells (Goodfriend and Hood, 1983; Goodfriend, 1987; Goodfriend et al., 1996; Yanes et al., 2013), thus illustrating the potential effect of local CaCO₃ on the shell δ¹³C value, but not on the body tissue δ¹³C value.

The potential effect of CaCO₃ ingestion may be recognized by the comparison of the δ¹³C values of the shell and body (Fig. 7). Discontinuous lines in Figure 7A represent the relationships between δ¹³C values of shell and body of cultured snails by Stott (2002), Zongxiu et al. (2007) and Chiba and Davison (2009). All these regression equations showed a slope value near 1 (Fig. 7A). Interestingly, snails from this study (continuous black line in Figs. 7A–B) showed a slope of 0.7 and snails collected by Yanes et al. (2008) from carbonate-rich areas of the eastern Canary Islands (continuous gray line in Figs. 7A–B) exhibited a slope of 0.5. These differences may be a response to variable proportion of carbonates ingested by snails. It is plausible that snails from Yanes et al. (2008) ingested significantly higher proportions of carbonates than snails studied here. However, these hypotheses remain to be tested quantitatively in future laboratory studies where different snail species are fed carbonates with differing δ¹³C values and plants with invariable δ¹³C values.

This contribution of carbonate into the shell can be inferred using the flux balance mixing model by Balakrishnan and Yapp (2004). The snail samples that are placed directly on or immediately above the curve for Φ = 0.00 (Fig. 7C) can be explained by the ingestion of carbonate sources with relatively high carbon isotope compositions (also, see explanation in Balakrishnan and Yapp, 2004). Data from this study (gray circles in Fig. 7C) are consistent with a lower contribution of carbonate than samples from Yanes et al. (2008) (open diamonds in Fig. 7C). This is not surprising given that samples collected by Yanes et al. (2008) came from bioclastic eolian dunes whereas snails studied here were taken from sites with visibly lower concentrations of CaCO₃. Despite the potential contributions of carbonates into the shell δ¹³C values, tentative estimates of the ingested C₃ against CAM vegetation can still be achieved (Yanes et al., 2011). The δ¹³C values of fossil shells that inhabited C₃/CAM mixed ecosystems should be valuable for paleoenvironmental studies, even if CaCO₃ is present and assimilated by snails. However, accurate quantitative estimates of consumed plants are not yet possible in carbonate-rich areas.
Conclusions

The helicid T. geminata from Lanzarote Island (Canary Archipelago) was collected directly from either C3 or CAM plants in C3/CAM mixed ecosystems. Field observations indicate Thela feeds on the succulent C3 plant E. balsamifera (Euphorbiaceae) and the CAM plants A. lancerottense (Crassulaceae) and O. dilenii (Cactaceae). The carbon isotope composition of the land snail shell and body tissue is a reasonably good proxy for succulent vegetation in semiarid settings. Carbon isotope values of the snail body (−23.9 ± 1.7‰; n = 58) indicate that specimens ingested, on average, ~10% of CAM plants, which is consistent with the observed natural abundance of CAM plants in the local environment. Outputs from a snail evaporative steady state flux balance model for δ13C values suggest that measured specimens here experienced similar metabolic rates. Also, snail body is a more accurate proxy for snail diet than plants where snails rest in field studies. This is because snails migrate among plants and feed from multiple plants within the ecosystem. The δ13C values of snail body correlated positively with those ingested on average, likely associated to higher ingestion of carbonates, whereas snails from this study depict a tighter scatter.


din the present study, adult snails that assimilated higher proportion of CAM plants were significantly smaller than adult snails that consumed C3 plants alone (Figs 5A–F). Snails that consumed less than ~15% of CAM plant (n = 44) were ~5% larger and wider than those that consumed more than ~15% of CAM plants (n = 14) (Fig. 5A–B). These intriguing results reveal that different diet qualities appear to influence significantly snail growth and ultimate adult shell size. Possibly, CAM plants are of lower caloric benefit than C3 plants, so growth rates may be reduced. Analogous findings were observed in culture experiments by Metref et al. (2003). Snails fed corn powder (C4 plant) were considerably smaller than those fed lettuce (C3 plant). This may reflect that snails had a higher capacity to assimilate C3 over C4 plants (Metref et al., 2003). Although other factors may account for the observed differences in shell size, it is possible that diet quality influences snail size to some extent.

The oxygen isotope composition of the shell (−0.1 ± 0.5‰; n = 58) was ~1% lower (Fig. 3B) than the measured values for live specimens collected from other localities in the eastern Canary Archipelago (Yanes et al., 2008). This suggests that the two localities from this study exhibited a wetter microclimate than those sampled by Yanes et al. (2008). This also stresses the relatively high sensitivity of the δ18O values of land snail shells to microclimatic conditions within a single region.

Calculations from the flux balance model by Balakrishnan and Yapp (2004), assuming that calcification occurs year round at temperatures of ~20°C and rainfall δ18O values of ~3.5‰ (SMOW), suggest that snails deposited shells at a relative humidity of ~91%, on average (Fig. 6). This predicted value during snail active periods agrees with previous estimates in the study region (Yanes et al., 2011), and with measured values of relative humidity using a hygrometer. Overall, snails that live in semiarid areas appear to need high relative humidity for shell growth, which compensates with low rainfall totals.

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