Quaternary Research 80 (2013) 596–605

Contents lists available at ScienceDirect

Quaternary Research

journal homepage: www.elsevier.com/locate/yqres

Paleoenvironmental implications of carbon stable isotope composition of land snail tissues

Yurena Yanes [a,b,*], María P. Asta [b], Miguel Ibáñez [c], María R. Alonso [c], Christopher S. Romanek [d]

[a] Department of Geology, University of Cincinnati, Cincinnati, OH 45221, USA
[b] Instituto Andaluz de Ciencias de la Tierra (CSIC-Universidad de Granada), Avenida Las Palmeras 4, 18100 Armilla, Granada, Spain
[c] Departamento de Biologia Animal, Universidad de La Laguna, E-38206 La Laguna, Tenerife, Islas Canarias, Spain
[d] Department of Earth and Environmental Sciences, University of Kentucky, Lexington, KY 40506, USA

ARTICLE INFO

Article history:
Received 16 January 2013
Available online 3 October 2013

Keywords:
Land snails
Stable isotopes
C_3 and CAM plants
Paleoenvironment
Lanzarote
Canary Islands

ABSTRACT

Land snail shell $\delta^{13}C$ 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 metabolic rates complicate these relationships. We evaluate if live-collected snails from Lanzarote (Canary Islands) reflect the abundance of C_3 and CAM plants. Snails were collected on either CAM or C_3 plants for isotope analysis of shell and body, and shell size. Respective shell and body $\delta^{13}C$ values of snails collected on CAM plants averaged -8.5 ± 1.7‰ and -22.8 ± 1.6‰, whereas specimens from C_3 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 C_3 plants. Conclusively: 1) snails consume CAM plants when they are available; 2) migration of snails among C_3 and CAM plants is a common phenomenon; and 3) C_3 plants may be a more energetic food for growth than CAM plants. This study shows that shell $\delta^{13}C$ values offer approximate estimates of plants in C_3–CAM mixed environments.

Introduction

Plants differ in their carbon isotopic composition ($\delta^{13}C$) as a consequence of variable carbon isotope fractionations pathways during photosynthesis (e.g., O'Leary, 1981; Farquhar et al., 1989). C_4 plants have significantly higher $\delta^{13}C$ values, from −15‰ to −11‰, than C_3 plants, which range between −35‰ and −20‰. CAM plants, which follow a crassulacean acid metabolism, use C_3 or C_4 pathways depending on the times of the day and exhibit $\delta^{13}C$ values between C_3 and C_4 plants (see review in Dawson et al., 2002). C_3 plants, which include most trees, shrubs and grasses that grow in cold seasons, are favored somewhat cooler and wetter conditions, and relatively higher atmospheric CO_2 concentrations (e.g., Quade and Cerling, 1995). In contrast, C_4 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 CO_2 than C_3 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 isotopic signature of fossil plant material (Goodfriend, 1990; Goodfriend and Ellis, 2000, 2002). The great majority of land snails are primary consumers, mostly feeding on living and decayed vascular plants (Speiser, 2001; ZongXiu et al., 2007). The $\delta^{13}C$ values of snail tissues, thus, reflect the signature of the diet (DeNiro and Epstein, 1978). Previous laboratory studies demonstrate that the $\delta^{13}C$ values of the snail body tissue and the aragonitic shell mainly reflect the $\delta^{13}C$ values of the consumed C_3 or C_4 plant (Stott, 2002; Metref et al., 2003; ZongXiu et al., 2007). Accordingly, the $\delta^{13}C$ values of the shell may be used to reconstruct past variations in the relative abundance of C_3 and C_4 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 C_3/C_4 plants derived from the isotopic signature of snail shells are complicated by other factors, such as the ingestion

* Corresponding author at: Department of Geology, University of Cincinnati, Cincinnati, OH 45221, USA.
E-mail address: yurena.yanes@uc.edu (Y. Yanes).

0033-5894/$ – see front matter © 2013 University of Washington. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yqres.2013.08.010

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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ values as a paleovegetation proxy in oceanic semiarid settings (i.e., do shell $\delta^{13}C$ 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) $\delta^{18}O$ 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

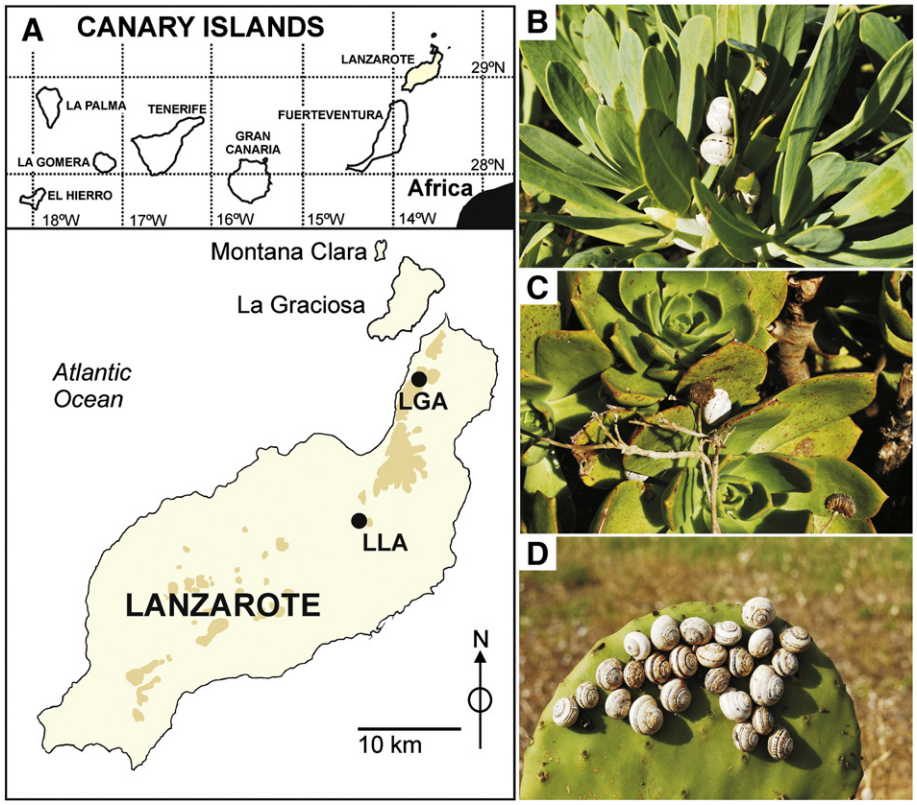

Figure 1. Geographical setting of sampling localities and photographs of snails and plants in the field. (A) Map of Lanzarote Island and sampling sites: Gayo (latitude: 29°10′10″, longitude: 13°30′40″, altitude: 450 m asl) and Loma de San Andrés (latitude: 29°02′12″ N, longitude: 13°36′48″, altitude: 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 C_3 plants) are present in Lanzarote. Overall, C_3 plants dominate the landscape, but C_4 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 plant species, one succulent C_3 plant, the endemic *Euphorbia balsamifera* (Fig. 1B), and two CAM plants, the endemic *Aeonium lancerottense* (Fig. 1C) and the introduced *Opuntia dilenii* (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 *Opuntia* (n = 14) and *Euphorbia* (n = 15). C_4 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 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 CO_2 produced after combustion was analyzed using a Finnigan Delta^{PLUS} 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 CO_2 gas by adding 0.1 ml of 104% H_3PO_4 at 25°C. The resulting CO_2 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:

$$\delta^{13}C \text{ or } \delta^{18}O = \left[\left(R_{sample}/R_{standard}\right)-1\right] \times 1000\ (‰)$$
$$\text{where } R = {}^{13}C/{}^{12}C \text{ or } {}^{18}O/{}^{16}O.$$

Statistical analyses

Statistical analyses were carried out using PAST 2.17 software (Hammer et al., 2001) considering statistical significance at $\alpha = 0.05$. 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., $\delta^{13}C$ value of snail body) and two possible sources (e.g., $\delta^{13}C$ values of CAM and C_3 plants), a two-source input mass balance equation can be used to determine the proportion of each source that contributes to the $\delta^{13}C$ values of the snail body (e.g., Phillips, 2012). This is mathematically expressed as follows:

$$\delta^{13}C_{snail\ body} = \%C_3 * \delta^{13}C_{C3} + \%CAM * \delta^{13}C_{CAM}$$
$$\text{where } \%C_3 + \%CAM = 100$$
$$\text{then, } \%CAM = \left[\left(\delta^{13}C_{snail\ body} - \delta^{13}C_{C3\ plant}\right)/\left(\delta^{13}C_{CAM\ plant} - \delta^{13}C_{C3\ plant}\right)\right] * 100.$$

The aforementioned equation was employed to estimate the proportion of assimilated CAM plant in land snail body tissues from Lanzarote Island, assuming that C_3 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 ($\delta^{13}C$) was explored. Hence, a simple two-source input model was selected over a Bayesian model for the present study.

Snail steady-state flux balance mixing models

Balakrishnan and Yapp (2004) developed a steady-state flux balance mixing model to better understand the $\delta^{13}C$ and $\delta^{18}O$ values of aragonitic shells of land snails. For $\delta^{13}C$ 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 (f_o) relative to the influx of CO_2 derived from plants (f_{in}), which is called ϕ ($=f_o/f_{in}$) and varies with metabolic rate (see Balakrishnan and Yapp, 2004 for further details). Model calculations are constrained by the $\delta^{13}C$ value of the shell and the organic tissue (plant and snail body), and the ambient temperature during calcification.

For $\delta^{18}O$ values, the model uses the amount and isotope composition of ambient rain, the amount and isotope 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, $\delta^{18}O$ values of water and water vapor, and relative humidity are the most important factors controlling the $\delta^{18}O$ values of the hemolymph and the shell (Balakrishnan and Yapp, 2004). Another parameter is the out flux of water from the hemolymph (f_o) relative to the influx of imbibed water (f_{in}), which is called θ ($=f_o/f_{in}$). 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., $\theta = 0$) (Balakrishnan and Yapp, 2004). This assumption is adopted here. Model calculations are constrained by measured values of temperature and the $\delta^{18}O$ 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 $\delta^{13}C$ values of -13.7‰ (n = 1) and -14.1 ± 0.5‰ (n = 2), respectively (Table 1; Fig. 2A). The C_3 plant *E. balsamifera* from Gayo had an average $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ values than those from Loma de San Andrés (Fig. 2C). The average $\delta^{13}C$ 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 C_3 plant *E. balsamifera* at Gayo and Loma de San Andrés showed average $\delta^{13}C$ values of -25.1 ± 0.9‰ and -24.7 ± 1.2‰, respectively (Fig. 2C). The difference in $\delta^{13}C$ values between sites was lower for snails collected on C_3 plants (0.4‰) than those collected from CAM plants (2.3‰). The $\delta^{13}C$ 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.

Sample #	Sample ID	Locality	Plant species	$\delta^{13}C$‰ (PDB)	Plant type
1	LGA-plant-1	Gayo	*Aeonium lancerottense*	−13.7	CAM
2	LGA-plant-2	Gayo	*Euphorbia balsamifera*	−25.5	C_3
3	LGA-plant-3	Gayo	*Euphorbia balsamifera*	−25.5	C_3
4	LGA-plant-4	Gayo	*Euphorbia balsamifera*	−25.6	C_3
5	LLA-plant-1	Loma San Andrés	*Opuntia dilenii*	−13.7	CAM
6	LLA-plant-2	Loma San Andrés	*Opuntia dilenii*	−14.4	CAM
7	LLA-plant -3	Loma San Andrés	*Euphorbia balsamifera*	−26.5	C_3
8	LLA-plant-4	Loma San Andrés	*Euphorbia balsamifera*	−25.4	C_3

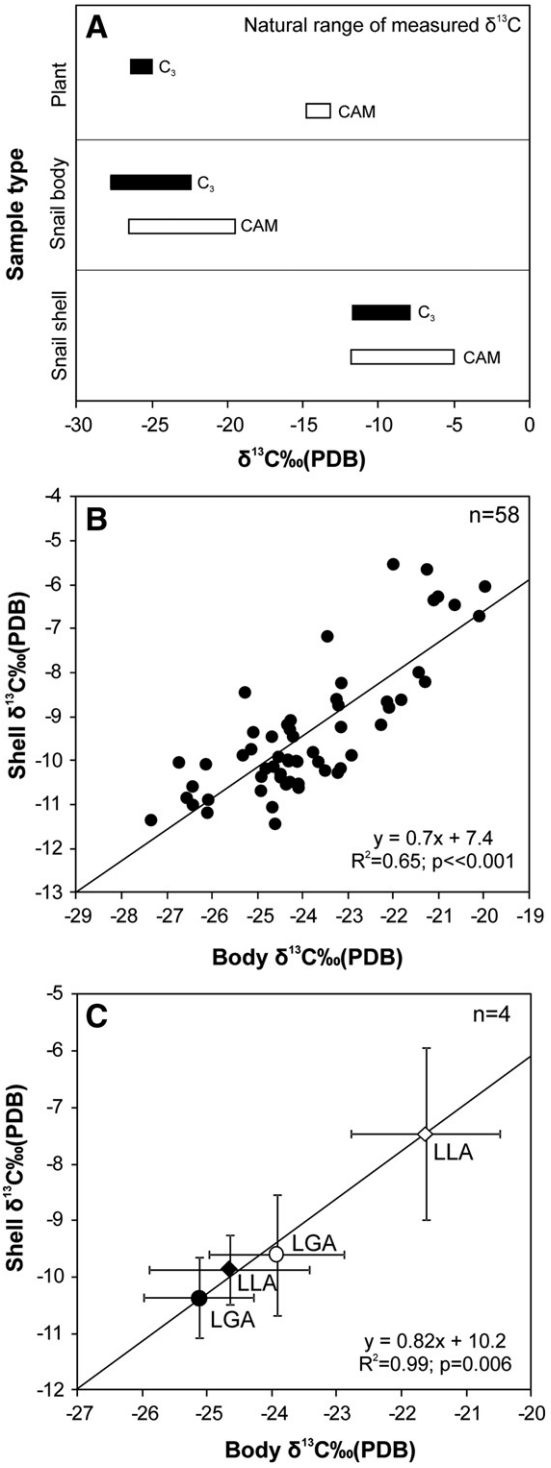

Figure 2. Measured $\delta^{13}C$ values of succulent plants and land snail shells and body tissues. (A) Natural range of measured $\delta^{13}C$ values for plants, shells and body tissues. Black bars depict C_3 samples whereas white bars depict CAM samples, or snails recovered from those types of plants. (B–C) Relationship between $\delta^{13}C$ values of shell and body. In panel C, diamonds depict average values from samples from Loma de San Andrés (LLA) whereas circles are for samples from Gayo (LGA). Open symbols are for snails collected from CAM plants whereas filled symbols are for individuals collected from C_3 plants. Solid lines in panels B and C depict the regression of the data.

Table 2
Isotopic composition of the helicid *Theba geminata* tissues from Gayo (LGA) and Loma de San Andrés (LLA) and adult snail body size.

		Body tissue	Aragonitic shell		Shell size								
Sample ID	Plant on which snails were resting	$\delta^{13}C$‰ (PDB)	$\delta^{13}C$‰ (PDB)	$\delta^{18}O$‰ (PDB)	$\Delta^{13}C_{(shell-body)}$	Length (mm)	Width (mm)	Two last spires height (mm)	Last spire height (mm)	Aperture height (mm)	Aperture width (mm)	Log (length)	PC 1
LGA-CAM-1	*Aeonium lancerottense*	−24.6	−11.4	0.3	13.2	11.87	15.37	10.37	7.81	6.66	7.31	1.074	−0.523
LGA-CAM-2	*Aeonium lancerottense*	−24.7	−9.4	−0.6	15.3	12.05	15.95	10.45	8.61	7.69	7.85	1.081	0.613
LGA-CAM-3	*Aeonium lancerottense*	−23.2	−8.2	−1.8	15.0	10.08	13.75	9.12	7.58	6.27	7.05	1.003	−3.057
LGA-CAM-4	*Aeonium lancerottense*	−23.7	−10.0	−0.1	13.7	12.62	15.61	10.91	8.66	7.27	7.52	1.101	0.575
LGA-CAM-5	*Aeonium lancerottense*	−24.5	−10.3	−0.1	14.2	13.27	18.38	11.78	9.93	8.15	9.27	1.123	4.167
LGA-CAM-6	*Aeonium lancerottense*	−23.5	−10.2	−0.2	13.3	11.52	14.81	11.27	7.69	6.53	7.09	1.061	−0.879
LGA-CAM-7	*Aeonium lancerottense*	−23.2	−10.3	0.2	12.9	11.32	14.58	9.98	8.16	7.18	6.93	1.054	−1.333
LGA-CAM-8	*Aeonium lancerottense*	−23.2	−10.2	0.6	13.0	11.14	14.93	9.86	9.20	6.93	7.51	1.047	−0.815
LGA-CAM-9	*Aeonium lancerottense*	−23.2	−8.7	−0.1	14.5	11.30	15.81	10.26	8.51	6.82	7.59	1.053	−0.178
LGA-CAM-10	*Aeonium lancerottense*	−25.2	−9.7	0.2	15.5	12.09	15.43	10.44	8.44	7.47	7.52	1.082	0.062
LGA-CAM-11	*Aeonium lancerottense*	−23.5	−7.2	−0.8	16.3	11.82	16.10	10.31	8.48	7.36	8.00	1.073	0.507
LGA-CAM-12	*Aeonium lancerottense*	−26.2	−10.1	−2.1	16.1	12.15	15.76	10.28	8.31	7.11	7.82	1.085	0.232
LGA-CAM-13	*Aeonium lancerottense*	−22.1	−8.8	−0.1	13.3	11.05	14.81	9.72	8.33	6.80	7.52	1.043	−1.240
LGA-CAM-14	*Aeonium lancerottense*	−24.3	−10.0	0.3	14.3	10.67	14.13	9.35	7.93	6.90	6.97	1.028	−2.248
LLA-CAM-1	*Opuntia dilenii*	−24.3	−10.5	−0.3	13.8	10.85	15.28	9.44	7.8	6.15	7.08	1.035	−1.524
LLA-CAM-2	*Opuntia dilenii*	−21.3	−8.2	−0.8	13.1	11.36	14.91	9.69	8.2	5.56	6.38	1.055	−1.744
LLA-CAM-3	*Opuntia dilenii*	−20.7	−6.4	−0.2	14.3	11.39	14.73	10.11	8.25	6.8	6.83	1.057	−1.256
LLA-CAM-4	*Opuntia dilenii*	−20.1	−6.7	−0.1	13.4	11.64	15.91	10.41	8.37	6.49	7.13	1.066	−0.177
LLA-CAM-5	*Opuntia dilenii*	−20.0	−6.0	−0.2	14.0	10.42	14.86	9.4	8.18	6.48	7	1.018	−1.852
LLA-CAM-6	*Opuntia dilenii*	−21.5	−8.0	−0.1	13.5	10.92	14.91	9.6	7.89	6.16	7.03	1.038	−1.688
LLA-CAM-7	*Opuntia dilenii*	−21.8	−8.6	−0.2	13.2	10.91	14.58	9.28	7.32	5.81	6.32	1.038	−2.487
LLA-CAM-8	*Opuntia dilenii*	−22.0	−5.5	0.1	16.5	11.8	15.15	10.34	8.08	5.79	6.48	1.072	−1.116
LLA-CAM-9	*Opuntia dilenii*	−23.3	−8.6	−0.3	14.7	10.65	14.55	9.68	7.87	6.3	6.33	1.027	−2.209
LLA-CAM-10	*Opuntia dilenii*	−21.3	−5.6	0.1	15.7	11.46	16.1	10.29	8.27	6.83	7.43	1.059	−0.009
LLA-CAM-11	*Opuntia dilenii*	−21.1	−6.3	0.3	14.8	11.32	14.83	10.01	8.23	6.12	6.54	1.054	−1.511
LLA-CAM-12	*Opuntia dilenii*	−21.0	−6.3	−0.1	14.7	10.27	14.61	9.16	7.60	6.19	6.66	1.012	−2.498
LLA-CAM-13	*Opuntia dilenii*	−22.3	−9.2	−0.4	13.1	10.56	14.43	9.33	7.77	5.89	6.63	1.024	−2.486
LLA-CAM-14	*Opuntia dilenii*	−22.2	−8.6	−0.2	13.6	10.65	14.3	9.37	7.78	6.07	6.87	1.027	−2.405
LGA-C3-1	*Euphorbia balsamifera*	−24.7	−11.0	−0.4	13.7	12.79	16.56	11.31	9.03	6.82	7.96	1.107	1.568
LGA-C3-2	*Euphorbia balsamifera*	−24.9	−10.7	−0.4	14.2	13.06	19.93	11.13	9.11	7.00	8.46	1.116	4.194
LGA-C3-3	*Euphorbia balsamifera*	−24.1	−10.6	0.0	13.5	13.84	19.54	12.09	9.69	7.92	8.93	1.141	5.095
LGA-C3-4	*Euphorbia balsamifera*	−26.4	−11.0	−0.1	15.4	12.60	17.63	11.03	9.13	7.40	8.32	1.100	2.423
LGA-C3-5	*Euphorbia balsamifera*	−24.8	−10.2	0.5	14.6	12.83	16.79	11.04	9.28	7.53	8.06	1.108	1.924
LGA-C3-6	*Euphorbia balsamifera*	−25.3	−8.4	−0.6	16.9	12.46	18.27	11.25	9.30	7.51	8.29	1.096	2.952
LGA-C3-7	*Euphorbia balsamifera*	−26.6	−10.8	−0.3	15.8	13.85	19.90	11.81	9.66	7.83	8.44	1.141	5.074
LGA-C3-8	*Euphorbia balsamifera*	−26.4	−10.6	0.4	15.8	12.21	16.55	10.97	8.95	7.34	8.15	1.087	1.373
LGA-C3-9	*Euphorbia balsamifera*	−24.9	−10.3	0.2	14.6	13.04	18.50	11.29	8.88	7.52	8.20	1.115	3.229
LGA-C3-10	*Euphorbia balsamifera*	−24.6	−9.9	−0.1	14.7	12.33	17.21	10.96	9.10	7.74	8.32	1.091	2.070
LGA-C3-11	*Euphorbia balsamifera*	−24.5	−10.4	0.3	14.1	11.33	15.71	9.94	8.28	6.70	8.00	1.054	−0.310
LGA-C3-12	*Euphorbia balsamifera*	−25.1	−9.3	0.7	15.8	12.85	16.56	10.81	8.62	7.42	7.63	1.109	1.360
LGA-C3-13	*Euphorbia balsamifera*	−24.1	−10.5	0.0	13.6	12.68	17.38	11.20	8.89	7.37	7.95	1.103	2.155
LGA-C3-14	*Euphorbia balsamifera*	−24.4	−10.5	0.2	13.9	12.59	17.03	10.93	8.66	7.24	7.90	1.100	1.673
LGA-C3-15	*Euphorbia balsamifera*	−26.1	−11.2	−0.1	14.9	12.75	17.30	11.23	9.48	7.74	8.35	1.106	2.507
LLA-C3-1	*Euphorbia balsamifera*	−22.9	−9.9	0.2	13.0	10.88	15.19	9.93	7.96	6.36	7.08	1.037	−1.312
LLA-C3-2	*Euphorbia balsamifera*	−24.3	−9.3	−0.2	15.0	12.04	15.45	10.52	8.58	6.48	7.52	1.081	−0.125
LLA-C3-3	*Euphorbia balsamifera*	−23.2	−9.2	0.2	14.0	12.06	15.62	10.58	8.7	7	7.26	1.081	0.103
LLA-C3-4	*Euphorbia balsamifera*	−24.3	−9.1	0.4	15.2	11.9	15.56	10.25	8.46	6.89	7.54	1.076	−0.122
LLA-C3-5	*Euphorbia balsamifera*	−25.3	−9.9	−0.2	15.4	10.92	15.4	9.81	7.63	6.04	6.79	1.038	−1.444
LLA-C3-6	*Euphorbia balsamifera*	−24.1	−10.0	0.0	14.1	11.3	16.18	10.11	8.44	6.68	7.03	1.053	−0.196
LLA-C3-7	*Euphorbia balsamifera*	−24.3	−10.0	−0.5	14.3	11.27	14.86	9.07	8.02	6.09	6.9	1.052	−1.786
LLA-C3-8	*Euphorbia balsamifera*	−26.7	−10.0	−0.2	16.7	13.29	18.06	12.02	9.19	6.74	7.76	1.124	3.028
LLA-C3-9	*Euphorbia balsamifera*	−24.4	−9.2	−0.2	15.2	11.99	15.84	10.46	8.32	6.04	6.9	1.079	−0.260
LLA-C3-10	*Euphorbia balsamifera*	−24.7	−10.1	−0.3	14.6	11.81	15.24	10.37	8.2	6.35	6.67	1.072	−0.810
LLA-C3-11	*Euphorbia balsamifera*	−24.2	−9.4	0.0	14.8	11.26	14.37	9.87	8.27	6.19	6.66	1.052	−1.841
LLA-C3-12	*Euphorbia balsamifera*	−26.1	−10.9	−0.5	15.2	11.84	15.02	9.97	8	6.25	6.86	1.073	−1.108
LLA-C3-13	*Euphorbia balsamifera*	−27.4	−11.3	−0.5	16.1	11.07	14.91	9.82	8.12	6.48	6.59	1.044	−1.547
LLA-C3-14	*Euphorbia balsamifera*	−23.8	−9.8	0.1	14.0	11.76	14.43	10	8.01	6.4	6.57	1.070	−1.593
LLA-C3-15	*Euphorbia balsamifera*	−24.2	−10.0	−0.2	14.2	11.44	14.88	10.23	8.44	6.37	6.68	1.058	−1.192

The $\delta^{13}C$ values of shell carbonate ranged from −11.4‰ to −5.5‰ (Table 2; Figs. 2A–B). The mean $\delta^{13}C$ value of the shell was −9.6 ± 1.1‰ for specimens collected on CAM plants from Gayo (*A. lancerottense*) and −7.5 ± 1.5‰ for individuals collected on CAM plants from Loma de San Andrés (*O. dilenii*) (Fig. 2C). Snails on the C_3 plant *E. balsamifera* at Gayo and Loma de San Andrés had a mean $\delta^{13}C$ value of −10.4 ± 0.7‰ and −9.9 ± 0.6‰, respectively (Fig. 2C). The $\delta^{13}C$ values of the shell and the body tissue correlated positively (Figs. 2B–C). The natural range of shell $\delta^{13}C$ 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 C_3 plant and −14.1‰ for CAM plant were used in the calculations (Table 1). The $\delta^{13}C$ 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 C_3 plant *E. balsamifera* consumed less than ~1% of CAM plant at both locales. Specimens with body $\delta^{13}C$ values lower than −25.8‰ (end member average value for local succulent C_3 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 $\delta^{13}C$ values were compared in Figure 4B. Calculations from the steady-state flux balance mixing model for $\delta^{13}C$ values by Balakrishnan and Yapp (2004) suggest that different specimens exhibited relatively comparable metabolic rates as most data plotted close to the curve for $\phi = 0.00$ (Fig. 4B). In sharp contrast, when $\delta^{13}C$ 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 $\phi = 0.80$, whereas snails that were resting on C_3 plants plot closer to $\phi = 0.00$ (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

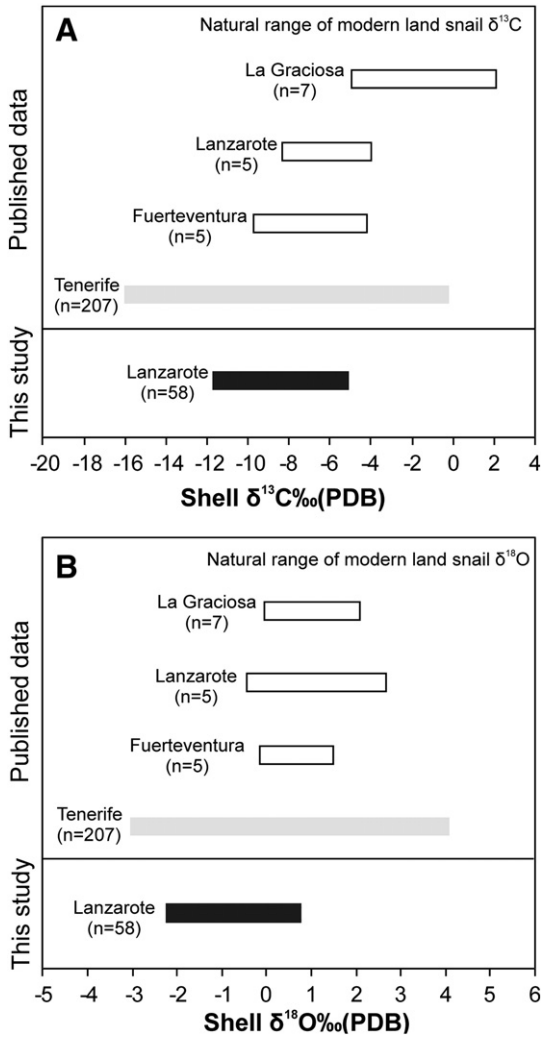

Figure 3. Natural range of $\delta^{13}C$ (A) and $\delta^{18}O$ (B) values for of land snail shells from this study (black bars) compared to published values by Yanes et al. (2008: white bars) and Yanes et al. (2009: gray bars).

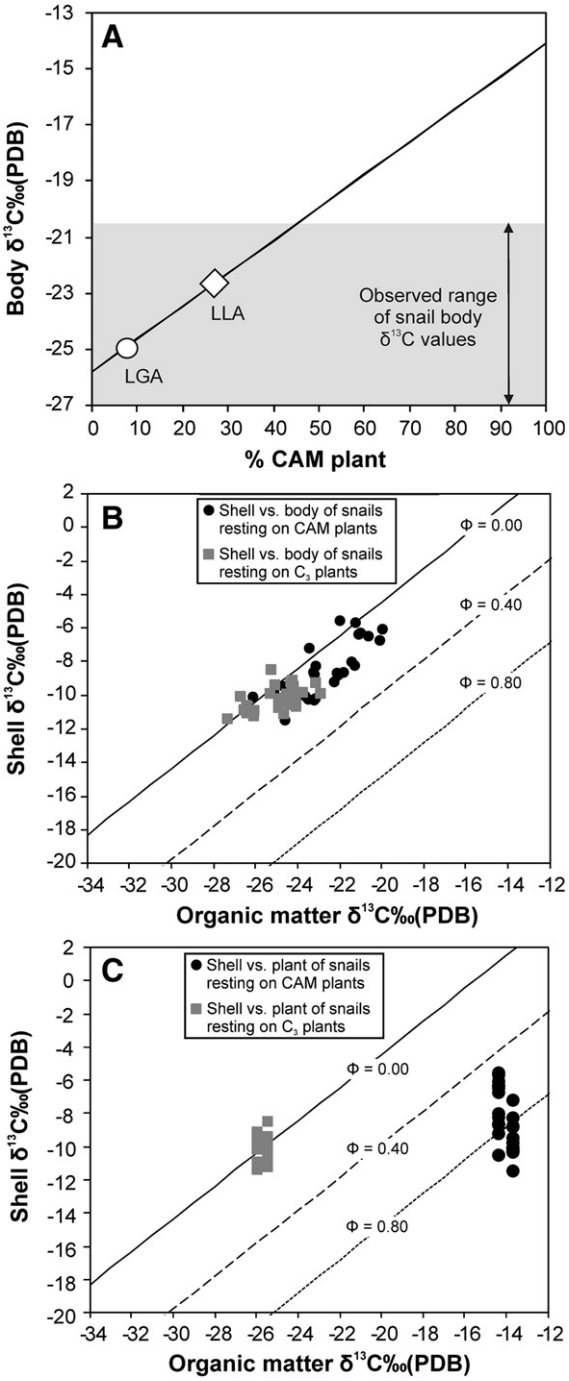

Figure 4. Carbon stable isotope models. (A) Calculation of the proportion of consumed CAM plant based on snail body $\delta^{13}C$ values using a two-source input equation (see text). Open circle depicts the average body $\delta^{13}C$ value of snails on CAM plants in Gayo whereas open diamond depicts the average value for snails on CAM plants in Loma de San Andrés. Gray band depicts the range of body $\delta^{13}C$ values in this study. (B) Measured shell $\delta^{13}C$ values plotted against measured body tissue $\delta^{13}C$ values. (C) Measured shell $\delta^{13}C$ values plotted against measured plant $\delta^{13}C$ 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.

(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).

Shell $\delta^{18}O$ values ($n = 58$) ranged from −2.1‰ to +0.7‰ (Fig. 3B) and they overlap the published range of $\delta^{18}O$ values (from −2.9‰ to +3.9‰) for snails from Tenerife, Fuerteventura and Lanzarote islands

of the Canary Archipelago (Yanes et al., 2008, 2009). Published $\delta^{18}O$ 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 $\delta^{18}O$ 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 $\delta^{18}O$ 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 $\delta^{13}C$ 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

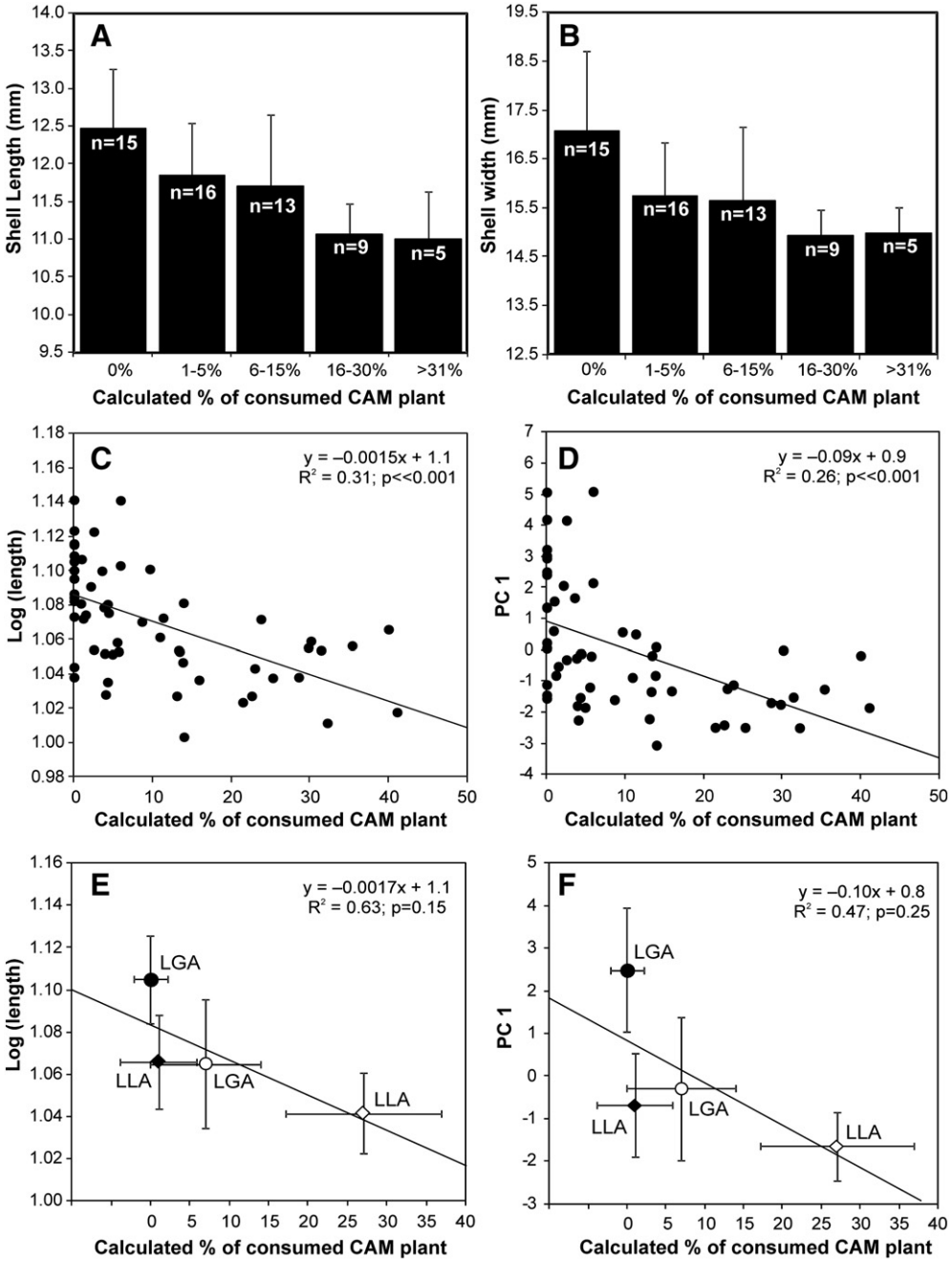

Figure 5. Relationship between snail diet type and adult shell size. (A–B) Average maximum shell length and width classified by the proportion of CAM plan ingested. (C–D) Relationship between the estimated proportion of consumed CAM plant and log of shell length. (E–F) Relationship between the estimated 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 C_3 plants.

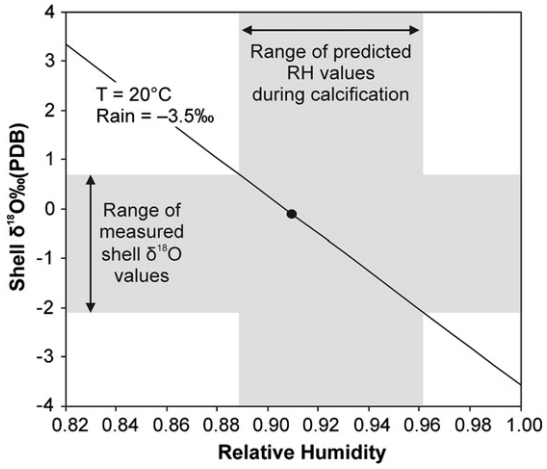

Figure 6. Calculated curves of shell $\delta^{18}O$ as a function of relative humidity (RH) for a temperature of 20°C and a rainfall $\delta^{18}O$ value of −3.5‰ (PDB) using Balakrishnan and Yapp (2004) flux balance mixing model. Horizontal gray band depicts the range of measured shell $\delta^{18}O$ values collected alive in Lanzarote Island. Vertical gray band depicts the range of RH values under which snails are predicted to have deposited shell. Filled dot depicts the average measured shell $\delta^{18}O$ value in Lanzarote Island.

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 C_3 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 $\delta^{13}C$ values that were almost equivalent to individuals resting on C_3 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 C_3/CAM mixed ecosystems, land snails follow a noticeable variable diet. One specimen, with a body $\delta^{13}C$ 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 C_3/CAM mixed sites, it is prudent to collect specimens from different areas within the sampling locale, considering the highly variable diet that contemporaneous snails from the same site can follow (Fig. 4A). Another issue that may complicate this approach is that the $\delta^{13}C$ values of C_3 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\phi = 0.00$ (Fig. 4B). This means that in most snails, the ratio of input and output flux of bicarbonate (HCO_3^-) into the body fluid was similar (Balakrishnan and Yapp, 2004). In contrast, when shell $\delta^{13}C$ 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 C_3 plants plotted close to the curve for $\phi = 0.00$, whereas specimens on CAM plants plotted near the curve for $\phi = 0.80$ (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 C_3 or CAM plant alone, but a mixture of both, as shown by the two-source input equation (Fig. 4A). Hence, in field studies, the $\delta^{13}C$ 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 C_3 or C_4 plant. In addition, specimens that were exposed to $CaCO_3$ (with a $\delta^{13}C$ value of −3.6‰) showed similar shell $\delta^{13}C$ values than individuals that had no access to $CaCO_3$. 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $CaCO_3$ in the study area, it is plausible that assimilation of soil carbonate may have had an effect on the $\delta^{13}C$ 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., 1999; Yanes et al., 2013), thus illustrating the potential effect of local $CaCO_3$ on the shell $\delta^{13}C$ value, but not on the body tissue $\delta^{13}C$ value.

The potential effect of $CaCO_3$ ingestion may be recognized by the comparison of the $\delta^{13}C$ values of the shell and body (Fig. 7). Discontinuous lines in Figure 7A represent the relationships between $\delta^{13}C$ 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 or carbonates with higher $\delta^{13}C$ values 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 $\delta^{13}C$ values and plants with invariable $\delta^{13}C$ 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 $\phi = 0.00$ (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 $CaCO_3$. Despite the potential contributions of carbonates into the shell $\delta^{13}C$ values, tentative estimates of the ingested C_3 against CAM vegetation can still be achieved (Yanes et al., 2011). The $\delta^{13}C$ values of fossil shells that inhabited C_3/CAM mixed ecosystems should be valuable for paleoenvironmental studies, even if $CaCO_3$ is present and assimilated by snails. However, accurate quantitative estimates of consumed plants are not yet possible in carbonate-rich areas.

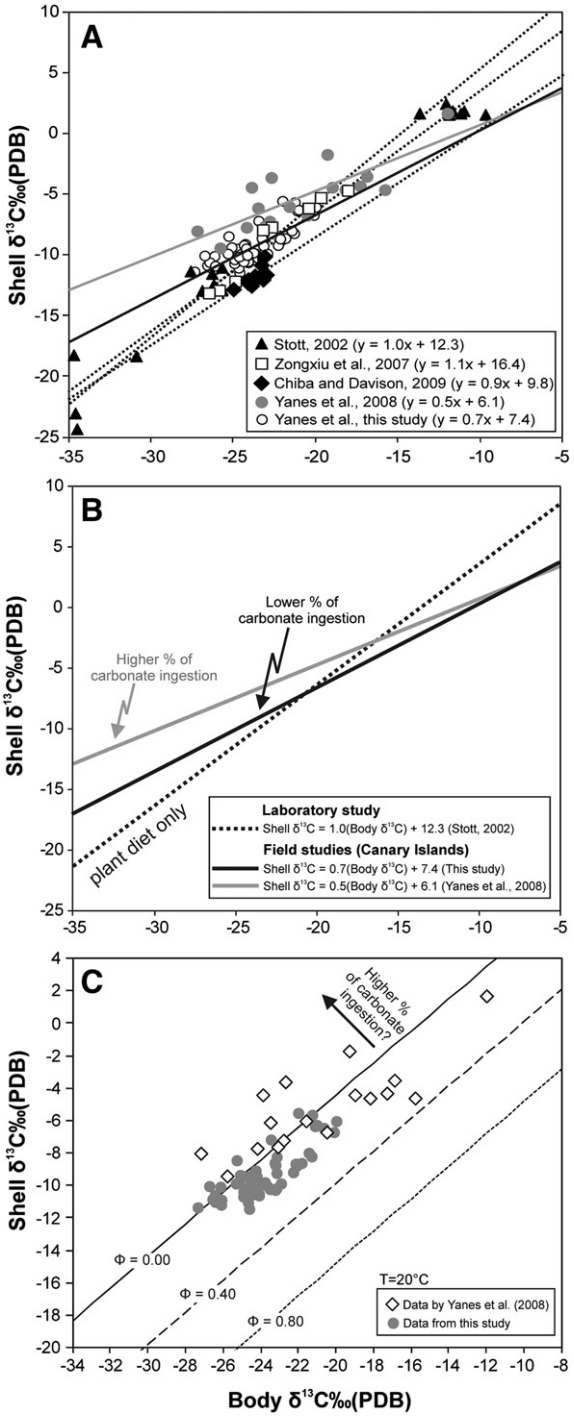

Figure 7. (A) Relationship between the $\delta^{13}C$ values of land snail shell and body tissue from published laboratory experiments (discontinuous lines) and field studies from the Canary Islands (continuous lines). (B) Relationship between the $\delta^{13}C$ values of shell and body from Stott (2002) (discontinuous black line), which depicts the relationship expected if limestone ingestion has a negligible effect into the shell, compared to field studies from the eastern Canary Islands by Yanes et al. (2008) (continuous gray line) and the present study (continuous black line). Note that slopes of regression equations differ probably due to variable proportion of limestone assimilation in the shell. (C) Measured $\delta^{13}C$ values of shell carbonate plotted against $\delta^{13}C$ values of body tissue. Lines depict the calculations of the flux balance model by Balakrishnan and Yapp (2004). Note that many snails studied by Yanes et al. (2008) (open diamonds) plotted substantially above the line for $\Phi = 0.00$, likely associated to higher ingestion of carbonates, whereas snails from this study depict a tighter scatter.

Snail growth rates and ultimate adult size depend on many ecological (e.g., population density, competition, predation pressure, illness) and environmental (e.g., humidity, carbonate availability, soil pH, food quality) factors (Goodfriend, 1986; Perry and Arthur, 1991; Hausdorf, 2006; Huntley et al., 2008). In the present study, adult snails that assimilated higher proportion of CAM plants were significantly smaller than adult snails that consumed C_3 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 ($n = 14$) (Figs. 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 C_3 plants, so growth rates may be reduced. Analogous findings were observed in culture experiments by Metref et al. (2003). Snails fed corn powder (C_4 plant) were considerably smaller than those fed lettuce (C_3 plant). This may reflect that snails had a higher capacity to assimilate C_3 over C_4 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 $\delta^{18}O$ 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 $\delta^{18}O$ 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.

Conclusions

The helicid *T. geminata* from Lanzarote Island (Canary Archipelago) was collected directly from either C_3 or CAM plants in C_3/CAM mixed ecosystems. Field observations indicate *Theba* feeds on the succulent C_3 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 $\delta^{13}C$ 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 $\delta^{13}C$ values of snail body correlated positively with those from the shell, reinforcing the suggestion that they both record similar plant-diet information. Although accurate estimates of the proportion of C_3 against CAM plants are not possible using shell $\delta^{13}C$ values alone due to potential limestone assimilation in the shell, realistic approximate estimates can be achieved. Pending work in the region includes (1) quantifying the effects of $\delta^{13}C$ values of $CaCO_3$ into the shell, (2) analyzing additional plant items potentially consumed by snails, and (3) quantifying the isotopic variability of differing plant sources.

Acknowledgments

The Spanish Ministry of Economy and Competitiveness (Mineco) research grant CGL2011-29898 to Y.Y. funded this research. The Spanish Mineco supports M.P.A. through the *Juan de la Cierva* program. Special

thanks go to Daniel Fuente-Friend for assistance during fieldwork and to Crayton J. Yapp (SMU) for thoughtful comments on the earlier versions of this manuscript. Additional thanks go to the editors Alan Gillespie and Bob Booth, and to André C. Colonese and an anonymous reviewer for critical revisions that improved the quality and clarity of this work.

References

Balakrishnan, M., Yapp, C.J., 2004. Flux balance model for the oxygen and carbon isotope compositions of land snail shells. Geochimica et Cosmochimica Acta 68, 2007–2024.

Balakrishnan, M., Yapp, C.J., Theler, J.L., Carter, B.J., Wyckoff, D.G., 2005. Environmental significance of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios of modern land-snail shells from the southern Great Plains of North America. Quaternary Research 63, 15–30.

Baldini, L.M., Walker, S.E., Bruce, R., Baldini, J.U.L., Crowe, D.E., 2007. Isotope ecology of the modern land snails Cerion, San Salvador, Bahamas: preliminary advances toward establishing a low-latitude island palaeoenvironmental proxy. Palaios 22, 174–187.

Chiba, S., Davison, A., 2009. Associations between the shell stable carbon isotope and vegetation in modern and fossil land snails Mandarina cichijimana on Chichijima of the Ogasawara Islands. Paleontological Research 13, 151–157.

Clementz, M.T., 2012. New insight from old bones: stable isotope analysis of fossil mammals. Journal of Mammalogy 93, 368–380.

Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, PlH, Tu, K.P., 2002. Stable isotopes in plant ecology. Annual Review of Ecology and Systematics 33, 507–559.

DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42, 495–506.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40, 503–537.

Fernández-Palacios, J.M., Whittaker, R.J., 2008. The Canaries: an important biogeographical meeting place. Journal of Biogeography 35, 379–387.

Goodfriend, G.A., 1986. Variation in land snail shell form and size and its causes: a review. Systematic Zoology 35, 204–223.

Goodfriend, G.A., 1987. Radiocarbon age anomalies in shell carbonate of land snails from semi-arid areas. Radiocarbon 29, 159–167.

Goodfriend, G.A., 1990. Rainfall in the Negev Desert during the Middle Holocene, Based on ^{13}C of Organic Matter in Land Snail Shells. Quaternary Research 34, 186–197.

Goodfriend, G.A., Ellis, G.L., 2000. Stable carbon isotope record of middle to late Holocene climate changes from land snail shells at Hinds Cave. Texas. Quaternary International 67, 47–60.

Goodfriend, G.A., Ellis, G.L., 2002. Stable carbon and oxygen isotope variations in modern *Rabdotus* land snail shells in the southern Great Plains, USA, and their relation to environment. Geochimica et Cosmochimica Acta 66, 1987–2002.

Goodfriend, G.A., Hood, D.G., 1983. Carbon isotope analysis of land snail shells: implications for carbon sources and radiocarbon dating. Radiocarbon 25, 810–830.

Goodfriend, G.A., Ellis, G.L., Toolin, L.J., 1999. Radiocarbon age anomalies in land snail shells from Texas: ontogenetic, individual and geographic patterns of variation. Radiocarbon 41, 149–156.

Hammer, O., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4 (1).

Hausdorf, B., 2006. Is the interspecific variation of body size of land snails correlated with rainfall in Israel and Palestine? Acta Oecologica 30, 374–379.

Huntley, J.W., Yanes, Y., Kowalewski, M., Castillo, C., Delgado-Huertas, A., Ibáñez, M., Alonso, M.R., Ortiz, J.E., Torres, T., 2008. Testing limiting similarity in Quaternary terrestrial gastropods. Paleobiology 34, 378–388.

Jahren, A.H., 2004. The carbon stable isotope composition of pollen. Review of Palaeobotany and Palynology 132, 291–313.

Kerney, M.P., Cameron, R.A.D., 1979. A Field Guide to the Land Snails of Britain and North-West Europe. Collins, London.

Koch, P.L., Diffenbaugh, N.S., Hoppe, K.A., 2004. The effects of late Quaternary climate and pCO_2 change on C_4 plant abundance in the south-central United States. Palaeogeography Palaeoclimatology Palaeoecology 207, 331–357.

Metref, S., Rousseau, D.D., Bentaleb, I., Labonne, M., Vianey-Liaud, M., 2003. Study of the diet effect on $\delta^{13}C$ of shell carbonate of the land snail *Helix aspersa* in experimental conditions. Earth and Planetary Science Letters 211, 381–393.

Nelson, D.M., Sheng Hu, F., Michener, R.H., 2006. Stable-carbon isotope composition of *Poaceae* pollen: an assessment for reconstructing C_3 and C_4 grass abundance. The Holocene 16, 819–825.

O'Leary, M.H., 1981. Carbon isotope fractionation in plants. Phytochemistry 20, 553–567.

Parnell, A., Inger, R., Bearhop, S., Jackson, A.L., 2010. Source partitioning using stable isotopes: coping with too much variation. PloS One 5, e9672. http://dx.doi.org/10.1371/journal.pone.0009672.

Perry, R., Arthur, W., 1991. Shell size and population density in large helicid land snails. The Journal of Animal Ecology 60, 409–421.

Phillips, D.L., 2012. Converting isotope values to diet composition: the use of mixing models. Journal of Mammalogy 93, 342–352.

Quade, J., Cerling, T.E., 1995. Expansion of C_4 grasses in the Late Miocene of Northern Pakistan: evidence from stable isotopes in paleosols. Palaeogeography Palaeoclimatology Palaeoecology 115, 91–116.

Sarakinos, H.C., Johnson, M.L., Vander Zanden, M.J., 2002. A synthesis of tissue-preservation effects on carbon and nitrogen stable isotope signatures. Canadian Journal of Zoology 80, 381–387.

Speiser, B., 2001. Food and feeding behavior. In: Barker, G.M. (Ed.), The Biology of Terrestrial Mollusk. CABI, pp. 259–288.

Stott, L.D., 2002. The influence of diet on the $\delta^{13}C$ of shell carbon in the pulmonate snail *Helix aspersa*. Earth and Planetary Science Letters 195, 249–259.

Yanes, Y., Delgado, A., Castillo, C., Alonso, M.R., Ibáñez, M., De la Nuez, J., Kowalewski, M., 2008. Stable isotope ($\delta^{18}O$, $\delta^{13}C$, and δD) signatures of recent terrestrial communities from a low-latitude, oceanic setting: endemic land snails, plants, rain, and carbonate sediments from the eastern Canary Islands. Chemical Geology 249, 377–392.

Yanes, Y., Romanek, C.S., Delgado, A., Brant, H.A., Noakes, J.E., Alonso, M.R., Ibáñez, M., 2009. Oxygen and carbon stable isotopes of modern land snail shells as environmental indicators from a low-latitude oceanic island. Geochimica et Cosmochimica Acta 73, 4077–4099.

Yanes, Y., Yapp, C.J., Ibáñez, M., Alonso, M.R., De la Nuez, J., Quesada, M.L., Castillo, C., Delgado, A., 2011. Pleistocene–Holocene environmental change in the Canary Archipelago as inferred from stable isotopes of land snail shells. Quaternary Research 65, 658–669.

Yanes, Y., García-Alix, A., Asta, M.P., Ibáñez, M., Alonso, M.R., Delgado, A., 2013. Late Pleistocene–Holocene environmental conditions in Lanzarote (Canary Islands) inferred from calcitic and aragonitic land snail shells and bird bones. Palaeogeography Palaeoclimatology Palaeoecology 378, 91–102.

ZongXiu, L., ZhaoYan, G., NaiQin, W., Bing, X., 2007. Diet control on carbon isotopic composition of land snail shell carbonate. Chinese Science Bulletin 52, 388–394.