

Calibration of the stable isotope composition and body size of the arid-dwelling land snail *Sphincterochila candidissima*, a climatic archive abundant in Mediterranean archaeological deposits

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Abstract

Land snails recovered from archeological deposits may be used to deduce climatic conditions during prehistoric occupation because their aragonitic skeletons are usually well-preserved and document valuable climatic information in the form of isotope codes. Since the snail *Sphincterochila candidissima* is common in archeological sites along the western Mediterranean but has been minimally investigated, the present work examines the relationship between the oxygen ($\delta^{18}\text{O}$) and carbon ($\delta^{13}\text{C}$) stable isotopes of modern specimens and relevant meteorological data. Individuals of *Sphincterochila* were regularly live-collected throughout 1 year (from September 2013 to October 2014) in Tarragona, NE Spain, and the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values were measured at the shell lip or aperture, which depicts the last growth episode closest to specimens' collection date. Shell margin $\delta^{18}\text{O}$ values varied from +3.1‰ in September to −0.8‰ in April. Average shell margin $\delta^{18}\text{O}$ values per collection date positively correlated with monthly averaged rainwater $\delta^{18}\text{O}$, and negatively correlated with monthly averaged maximum relative humidity, while other variables (i.e. temperature and precipitation amount) did not explain monthly–submonthly isotopic variations in the shell lip. Shell margin $\delta^{13}\text{C}$ values ranged from −11‰ in September to −4.8‰ in March, pointing to detectable variations in snails' diet between seasons. Interestingly, snail body size positively correlated with shell margin $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values, suggesting that larger snail size is reached under drier conditions. This work shows that *S. candidissima* from the western Mediterranean is a valid paleoprecipitation $\delta^{18}\text{O}$ and/or paleohumidity proxy in the region at the monthly–submonthly scale. Although shell margin $\delta^{18}\text{O}$ recorded fall and spring climate conditions only, limiting therefore their use to estimate the season of harvest, snails that grow year-round (e.g. at subtropical–tropical regions) may be used usefully for such analyses.

Keywords

land snails, last growth episode, seasonal climate, shell middens, Spain, stable isotopes

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Introduction

Large (edible) land snails have been heavily exploited as a food resource by humans since the late Paleolithic. This activity is manifested in numerous archeological sites across the western Mediterranean region, where land snail shells are well-preserved and often concentrated in high densities (Bosch et al., 2015; Fernández-Lopez-de-Pablo et al., 2014; Hill et al., 2015; Lubell, 2004a, 2004b). Apart from subsistence strategies and food handling processes, the shells from these 'prehistoric meals' can be used to gain insight into the environmental conditions or climatic context during ancient human occupation (Balakrishnan et al., 2005; Colonese et al., 2007, 2010a, 2010b, 2011, 2013a, 2013b; Paul and Mauldin, 2013; Prendergast et al., 2016a; Stevens et al., 2012; Yanes et al., 2011, 2012, 2013a, 2013b, 2014). Land snail shells are entirely made of aragonite, and the oxygen ($\delta^{18}\text{O}$) and carbon ($\delta^{13}\text{C}$) stable isotope composition of the shell record some aspects of the environment. For example, shell $\delta^{18}\text{O}$ values appear to track at least four atmospheric variables during calcification including rainwater $\delta^{18}\text{O}$, water vapor $\delta^{18}\text{O}$, relative humidity (RH), and temperature (Balakrishnan and Yapp, 2004). The shell $\delta^{13}\text{C}$ values seem to primarily represent the consumed and

assimilated plant $\delta^{13}\text{C}$ values (Metref et al., 2003; Stott, 2002), possibly in combination with some ingestion of limestone (Balakrishnan and Yapp, 2004; Goodfriend et al., 1999; Liu et al., 2007; Prendergast et al., in press; Yanes et al., 2008; Zhang et al., 2014), especially in the case of large body size snails with thick shells. Considering that (1) multiple environmental factors operate jointly, often difficult to discriminate, and (2) not all land snail species behave equally, the interpretation of the stable isotope composition of snail shells remains rather challenging. The oxygen isotope composition of the snail shell is

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therefore particularly difficult to interpret. One way to overcome this challenge is to conduct calibration studies of the target snail species using living specimens collected from localities with well-constrained climate.

Land snail species of the genus *Sphincterochila* have been recovered in outstanding quantities from a large number of western and central Mediterranean archeological sites (Aparicio et al., 2015; Fernández-Lopez-de-Pablo and Gabriel, 2016; Fernández-Lopez-de-Pablo et al., 2011; Hutterer et al., 2014; Mannino and Mazzanti, 2013; Yanes et al., 2013a). The archeological shells are almost always exceptionally preserved and due to their large and thick shells, *Sphincterochila* is an excellent candidate for both annual-average and seasonal paleoclimatic inferences. However, studies that have examined the environmental meaning of the isotopic composition of this species are minimal (Yanes et al., 2013a), and calibration studies using living specimens to evaluate the isotopic variability of *Sphincterochila candidissima* at monthly–submonthly resolution remain to be undertaken.

Archeologists working on anthropogenic shell assemblages often attempt to infer some features of the subsistence strategies followed by the prehistoric human groups that occupied the target locality. In marine shells, scientists have used the $\delta^{18}\text{O}$ values of the last growth episode, which principally depicts the water temperature closest to the organism's collection date, to identify the season of shellfish collection and therefore, the season of site occupation (Andrus, 2011; Andrus and Thompson, 2012; Colonese et al., 2009; Hallman et al., 2009; Mannino et al., 2007; Prendergast et al., 2016b; Surge and Barrett, 2012). This type of research has never been conducted for land snail shell assemblages, in part because it has never been evaluated if adult snail shells record in the last growth episode the climatic conditions that dominate when snails are being harvested. Thus, it is as yet unexplored if we can objectively use the isotopic composition of the land snail shell margin to infer the approximate season of land snail collection.

This study investigates the seasonal variability of the oxygen and carbon isotope values of living specimens of *S. candidissima* collected in Tarragona (NE Spain) throughout a year. We evaluate whether or not the target species track climatic variations at the monthly–submonthly resolution for the last growth episode, and contrast isotopic data with relevant climatic variables accessible from local meteorological stations. This research is the first to investigate the potential use of land snail shell margin isotope data for estimating the approximate season of snail collection.

Material and methods

Sphincterochila candidissima

The target species belongs to the family Sphincterochilidae, which is a widely spread group in the western Mediterranean basin. All Sphincterochilidae species are adapted to arid–semiarid conditions. Moreno-Rueda (2007, 2008, 2014) and Moreno-Rueda and Collantes-Martin (2007) conducted field studies of *S. candidissima* in Sierra Elvira (Granada, Southern Spain). His field observations suggest that this species hibernates during the winter months to avoid cold temperatures by burying themselves under the soil surface. Moreover, the species tends to aestivate during the summer months to elude hot soil temperatures by climbing onto plants. Main activity period for *S. candidissima* was observed to be in the spring. This species is also capable of surviving extremely arid conditions by slowing down the rate of water loss through evaporation. *Sphincterochila* species exhibit a lifespan that ranges from 1 to several years. Growth rates likely vary across seasons, depending on moisture and temperature levels. *Sphincterochila* is broadly considered a generalized herbivore abundant in limestone areas.

Climate of Tarragona

Tarragona, located in the coast of northeast Spain at 41°06'N, 1°14'E and 68 m a.s.l. (Figure 1), has a Mediterranean climate, with mild cool winters and warm summers. Climate data were adapted from the Spanish Government Meteorological Agency website (<http://www.aemet.es>). Average annual air temperature is ~16°C, ranging from 9.0°C in January to 24.6°C in August (Figure 2). The area receives about 500 mm of annual precipitation. Average air temperature and precipitation during fall (September to November) and spring (March to May) is ~16°C and ~324 mm, respectively. Average annual RH is ~68%, ranging from 63% in July to 73% in October. The maximum RH value in the fall and spring seasons is ~90%, on average. Accordingly, fall and spring seasons, when *Sphincterochila* seems to be primarily active, tend to be the wettest seasons while winter and summer, when snails may hibernate or aestivate, are relatively dry.

Rainwater oxygen isotope values in the nearby coastal city of Barcelona were adopted from Araguas-Araguas and Diaz-Teijeiro (2005) and data available at the International Atomic Energy Agency website (www.iaea.org). The average annual rainwater $\delta^{18}\text{O}$ value is $-5.0 \pm 1.0\text{‰}$ (SMOW) (1-sigma), varying between $-7.0 \pm 2.5\text{‰}$ (SMOW) in November and $-2.4 \pm 2.5\text{‰}$ (SMOW) in June. Rainwater $\delta^{18}\text{O}$ values during fall and spring seasons are -5.0‰ (SMOW), on average (Figure 2). The vegetation in the region is dominated by C_3 -type plants. The most abundant plant species were Mediterranean shrubs (i.e. *Rosmarinus officinalis*, *Quercus coccifera*, *Pistacia lentiscus*) and tree forms (i.e. *Pinus halepensis*, *Ceratonia siliqua*) (personal field observations, 2013–2014).

Field sample collection

For this project, we planned a sampling strategy that has been commonly employed for marine gastropods (e.g. Colonese et al., 2009; Prendergast et al., 2013) but has never been conducted for land snails in a systematic manner (but see Goodfriend et al., 1989). A total of 44 adult specimens of *S. candidissima* were live-collected nine times throughout 1 year (from September 2013 to October 2014) near the city of Tarragona, NE Spain (Figure 1). All specimens were randomly collected in two sampling localities of Tarragona, distanced ~0.7 km from each other (site 1: 41°07'45"N, 1°15'28"E, 62 m a.s.l.; site 2: 41°08'6"N, 1°15'39"E; 106 m a.s.l.). Snails were gathered within a radius of ~100 m in both areas. These two sites were equivalent in terms of climate, vegetation, and soil type, and land snail assemblages present.

As expected, living specimens were only found in the fall and spring seasons, when snails from mid-latitudes are primarily active and calcification takes place. Individuals that are thought to have been aestivating or hibernating during the summer or winter, respectively, were never found despite our field sampling efforts. Collected snails were rapidly submerged in ~80% ethanol right after collection for its preservation. This sampling locality was selected because (1) relevant meteorological data are available, (2) *S. candidissima* living populations are abundant and accessible throughout many months of the year, and (3) Pleistocene–Holocene shell middens rich in this species are common in eastern Spain.

Body size analysis

The shell dimensions of all specimens collected in the field were measured to assess potential variations in body size throughout the year. Six standard measurements for globose shells were collected using procedures established by Kerney and Cameron (1979), including maximum shell length, width, height of the two last spires, height of the last spire, aperture height, and aperture width. All shell measurements were obtained from the field-collected 44 individuals using an electronic caliper to the nearest 0.02 mm.

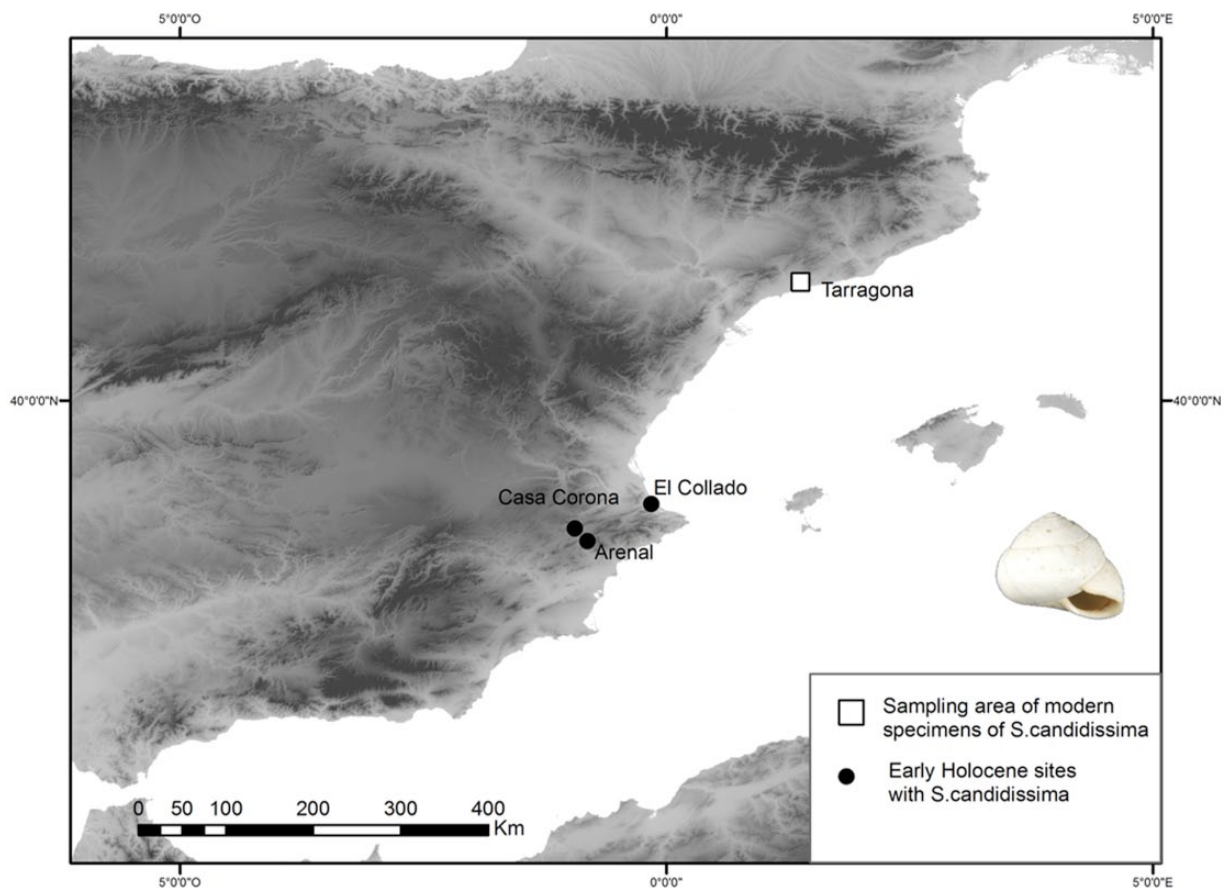


Figure 1. Map indicating the geographical location of Tarragona, NE Spain (open quadrat) where snails were live-collected between 2013 and 2014. A photograph of the target land snail species (*Sphincterochila candidissima*) is also shown above legend. Note that some relevant late Paleolithic and Mesolithic archeological sites rich in shells of *S. candidissima* are abundant in eastern Spain (filled circles).

Laboratory analyses

Shells were cleaned in distilled water and ultrasonication, and dried at room temperature overnight. The shell margin, which should record the environmental conditions during the last growth episode closest to snail's collection date, was milled slowly using a manual dremel drill with a 5-mm-diameter bit. Growth lines were not visible, probably because of the continuous fast-growing mode of snail shells. Because the same researcher collected the same amount of carbonate for each shell, sampling bias are minimized. Although the time-averaging represented in the shell margin samples analyzed here is uncertain, considering that this species is short-lived and the amount of carbonate collected was very small (~150 µg), we assume that each sample represents less than ~1 month of growth.

Oxygen and carbon isotope values of the shell margin were measured using an automated carbonate preparation device (KIEL-III) coupled to a gas-ratio mass spectrometer (Finnigan MAT 252) at the University of Arizona. Powdered samples were reacted with dehydrated phosphoric acid under vacuum at 70°C. The isotope ratio measurement was calibrated using repeated measurements of NBS-19 and NBS-18. Precision was $\pm 0.1\text{‰}$ for $\delta^{18}\text{O}$ and $\pm 0.08\text{‰}$ for $\delta^{13}\text{C}$ (1-sigma). All stable isotope results are reported in δ notation relative to the international standard Pee Dee Belemnite (PDB). The δ values are defined as:

$$\delta^{13}\text{C} \text{ or } \delta^{18}\text{O} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000 (\text{‰})$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{18}\text{O}/{}^{16}\text{O}$.

Statistical analyses

All statistical analyses were performed using *PAST 3.12* software (Hammer et al., 2001) considering statistical significance at $\alpha = 0.05$. Linear regression analyses were carried out using the average values per collection date ($n = 9$). Note that snails were sometimes collected twice in a single month (a few weeks apart), which allowed us to conduct submonthly evaluations (see Table 1). A multiple regression analysis was also computed combining rain $\delta^{18}\text{O}$ and maximum RH values.

Results

Shell margin $\delta^{18}\text{O}$ values averaged $+0.7 \pm 0.8\text{‰}$ ($n = 44$) -1 sigma, and varied from $+3.1\text{‰}$ in September to -0.8‰ in April, so a total of 3.9‰ isotopic variability was documented between months (Table 1). Shell margin $\delta^{18}\text{O}$ values were up to $5.7 \pm 0.6\text{‰}$ higher than observed local rainfall $\delta^{18}\text{O}$. The positive offset between shell and rain $\delta^{18}\text{O}$ was relatively constant throughout the year. The average shell margin $\delta^{18}\text{O}$ values per collection date ($n = 9$) correlated positively with monthly averaged rainwater $\delta^{18}\text{O}$ values from the region ($R^2 = 0.42$; $p = 0.05$; $n = 9$; Figure 3a), and negatively correlated with monthly averaged maximum RH values ($R^2 = 0.58$; $p = 0.018$; $n = 9$; Figure 3b). A multiple regression analysis slightly improved the linear relationship between the dependent (shell $\delta^{18}\text{O}$) and these two independent (rain $\delta^{18}\text{O}$ and max. RH) variables ($R^2 = 0.67$; $p = 0.036$; $n = 9$). No significant relationships were observed with other relevant climatic parameters for snails like average RH, air temperature, or precipitation amount. Thus, snail shell margin $\delta^{18}\text{O}$ values exhibit a strong relationship with local maximum RH values during the snail collection date, but none with the average RH values of that month shown in Figure 2b.

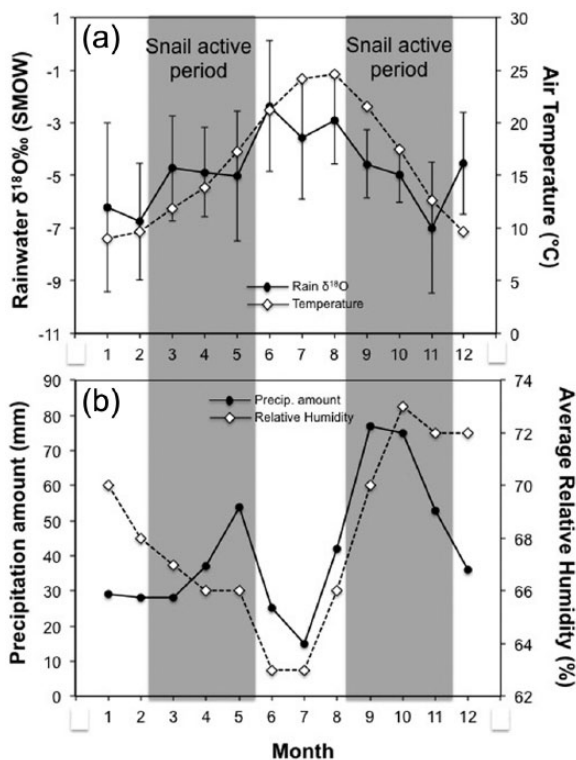


Figure 2. Current climate of Tarragona, NE Spain: (a) seasonal variation of rainwater oxygen isotope values and air temperature; (b) seasonal variation of the precipitation amount and average relative humidity values. Shaded areas depict the months of the year when snails are primarily active and grow shell (personal field observations, 2013–2014). Climate data were adapted from the Spanish Government Meteorological Agency website (<http://www.aemet.es>). Rainwater oxygen isotope values were taken from Araguas-Araguas and Diaz-Tejedor (2005) and the International Atomic Energy Agency website (www.iaea.org).

Shell margin $\delta^{13}\text{C}$ values varied from -11.0‰ in September to -4.8‰ in March, so up to 6.2‰ isotopic variability was documented throughout the year (Table 1). Shell margin $\delta^{13}\text{C}$ values showed an average value of $-7.6 \pm 1.9\text{‰}$ ($n = 44$) and correlated positively with shell margin $\delta^{18}\text{O}$ values ($R^2 = 0.47$; $p = 0.05$; $n = 9$; Figure 3c).

The average maximum shell length of adult *S. candidissima* specimens from Tarragona averaged 17.5 ± 1.6 mm ($n = 44$), ranging from 20.5 to 13.3 mm (Table 1). No definite body size differences were observed between seasons. However, adult snails collected in the fall of 2013 were significantly smaller (average shell length = 15.5 ± 0.8 mm) than individuals found the following fall of 2014 (average shell length = 17.7 ± 0.8 mm). Specimens from the spring of 2014 (average shell length = 18.0 ± 1.1 mm) were statistically equivalent in terms of shell dimensions than those encountered in the fall of the same year (Figure 4a). Interestingly, average shell margin $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values for each of the nine collection dates throughout the year positively correlated with snail shell maximum length ($R^2 = 0.41$; $p = 0.062$; $n = 9$; for $\delta^{18}\text{O}$) and ($R^2 = 0.78$; $p = 0.001$; $n = 9$; for $\delta^{13}\text{C}$) (Figure 4b and c). Thus, larger individuals exhibited the highest $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values in the shell margin whereas smaller specimens always showed the lowest shell margin $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values.

Discussion

Shell margin oxygen isotope values

Shell margin $\delta^{18}\text{O}$ values across seasons imitated the fluctuations in local monthly averaged rain $\delta^{18}\text{O}$ values (Figure 3d), which

reinforces that adult shells of the arid-dwelling *S. candidissima* from the region can be used as a convincing proxy for precipitation $\delta^{18}\text{O}$, even at monthly–submonthly resolution. On average, shell margin $\delta^{18}\text{O}$ was $\sim 5.7\text{‰}$ higher than rain $\delta^{18}\text{O}$, an ^{18}O -enrichment consistent with other snail species in Europe and the Mediterranean (Goodfriend et al., 1989; Lecolle, 1985). This shell–rain ^{18}O offset was relatively constant throughout the sampled year. Balakrishnan and Yapp (2004) interpret this offset as the evaporative loss of water from the snail body fluid. Because this offset seems to be constant throughout the year ($\delta^{18}\text{O}_{\text{shell-rain}} = 5.7 \pm 0.6\text{‰}$), *S. candidissima* shell margin $\delta^{18}\text{O}$ values should still reflect the signature of the local rain $\delta^{18}\text{O}$.

The slope of the regression equation obtained in this study between rain and shell margin $\delta^{18}\text{O}$ values of *S. candidissima* at the submonthly timescale (Figure 3a: $\delta^{18}\text{O}_{\text{p}} = 0.49 * \delta^{18}\text{O}_{\text{s}} + 3.1$; $R^2 = 0.42$) was comparable to other published regression equations using average annual rain and entire-shell $\delta^{18}\text{O}$ values of different snail species combined. For instance, Zanchetta et al. (2005) measured multiple snail taxa across the Italian Peninsula ($\delta^{18}\text{O}_{\text{p}} = 0.54 * \delta^{18}\text{O}_{\text{s}} - 5.7$; $R^2 = 0.50$), Lecolle et al. (1985) analyzed numerous species around Europe ($\delta^{18}\text{O}_{\text{p}} = 1.2 * \delta^{18}\text{O}_{\text{s}} - 5.9$; $R^2 = 0.93$), and Prendergast et al. (2015) measured specimens from Northern Africa ($\delta^{18}\text{O}_{\text{p}} = 0.95 * \delta^{18}\text{O}_{\text{s}} - 5.2$; $R^2 = 0.58$). In all these case studies, including the present work here, the slope varies between ~ 0.5 and 1, suggesting that, on average, for every $\sim 1\text{‰}$ (SMOW) increase in rain $\delta^{18}\text{O}$, snail shell $\delta^{18}\text{O}$ increases ~ 0.5 – 1‰ (PDB). The remarkable similarity in the slope of these regression equations indicates that both variables (rain and shell $\delta^{18}\text{O}$) exhibit similar rate of variation at both average annual and submonthly resolution. The intercept of the regression equation of this study, however, is notably different than previously published work in the Mediterranean region, suggesting that the species used here displays a significantly different offset between rain and shell $\delta^{18}\text{O}$ than other land snail taxa. This therefore may be explained by significantly different ecologies among species and also emphasizes the need to develop site-specific and species-specific calibration studies. In contrast to other recent snail studies (Prendergast et al., 2015; Zanchetta et al., 2005), *Sphincterochila* $\delta^{18}\text{O}$ values did not correlate with precipitation amount, which further reinforces the need for calibrating the target species using living individuals from the region before analyzing fossil or archeological shells for paleoclimatic inferences. Considering that the rainwater $\delta^{18}\text{O}$ values vary seasonally, primarily in response to air temperature seasonal fluctuations, it seems possible to use the shell margin $\delta^{18}\text{O}$ values of land snails recovered from archeological sites to estimate, at least roughly, the approximate season of snail collection. However, these kinds of studies appear to be more plausible in lower-latitude areas where snails may grow continuously year-round and shell margin $\delta^{18}\text{O}$ values track rain $\delta^{18}\text{O}$ values across all seasons, rather than just fall and spring seasons as documented here. This hypothesis remains to be tested in future studies in subtropical–tropical areas.

Both land snails and slugs are highly sensitive to dehydration via water loss through evaporation under dry conditions. While in moist environments, temperature seems to be the limiting factor for snails, in dry environments, moisture is the major controlling factor for snail active periods (Moreno-Rueda, 2007; Moreno-Rueda and Collantes-Martin, 2007). Hence, it is expected that snail active periods and therefore most of shell growth, would occur at night, when RH is the highest, especially in arid environments. The snail flux balance model of Balakrishnan and Yapp (2004), combined with the aragonite–water oxygen isotope fractionation equation of Grossman and Ku (1986), was used to infer RH values from the shell margin $\delta^{18}\text{O}$ values (Figure 5). The model posits that temperature, $\delta^{18}\text{O}$ values of water and water vapor, and RH are the most important variables in the accurate determination of the body fluid and the shell $\delta^{18}\text{O}$ value

Table 1. Snail body size and oxygen and carbon stable isotope values of the shell margin of live-harvested adult individuals of *Sphincterochila candidissima* (Gastropoda: Sphincterochilidae) from Tarragona, NE Spain.

Shell ID	Date of collection	A (mm)	B (mm)	C (mm)	D (mm)	E (mm)	F (mm)	$\delta^{13}\text{C}$ (PDB)	$\delta^{18}\text{O}$ (PDB)
SC-TAR-01	8 September 2013	17.37	19.39	14.29	10.92	8.17	8.18	-9.4	0.6
SC-TAR-02	8 September 2013	16.43	20.45	13.97	10.95	8.74	8.43	-8.7	-0.4
SC-TAR-03	8 September 2013	16.45	19.75	13.40	10.47	8.28	8.15	-10.9	1.2
SC-TAR-04	8 September 2013	14.36	19.01	12.94	9.58	8.26	8.16	-9.3	1.0
SC-TAR-05	8 September 2013	13.27	17.05	11.30	8.67	6.97	7.09	-11.0	0.6
SC-TAR-06	23 October 2013	16.30	20.11	13.89	10.73	8.83	8.49	-9.6	0.2
SC-TAR-07	17 November 2013	15.13	18.94	13.24	10.08	7.93	8.03	-10.2	0.0
SC-TAR-08	17 November 2013	14.28	19.71	12.36	9.88	7.93	8.23	-10.4	-0.1
SC-TAR-09	17 November 2013	14.90	18.83	13.03	10.51	8.29	8.18	-10.8	-0.1
SC-TAR-10	30 March 2014	18.42	20.57	14.68	10.66	8.88	9.54	-5.3	0.2
SC-TAR-11	30 March 2014	18.38	20.90	15.36	10.97	9.23	9.33	-5.0	0.6
SC-TAR-12	30 March 2014	18.03	21.27	14.68	11.05	9.45	9.70	-4.9	0.5
SC-TAR-13	30 March 2014	18.45	20.91	14.60	10.83	8.40	9.11	-4.8	0.9
SC-TAR-14	30 March 2014	17.48	20.72	14.69	11.22	9.82	9.28	-5.8	1.4
SC-TAR-15	30 March 2014	17.69	21.65	15.08	11.57	8.95	9.24	-6.4	0.7
SC-TAR-16	30 March 2014	17.59	20.96	14.97	11.23	9.09	9.24	-6.3	0.8
SC-TAR-17	22 April 2014	19.84	22.16	15.90	12.01	9.89	9.39	-6.3	1.5
SC-TAR-18	22 April 2014	19.22	20.89	16.65	11.81	9.57	9.42	-5.3	1.4
SC-TAR-19	22 April 2014	18.24	19.99	15.16	11.00	9.06	9.05	-6.6	0.1
SC-TAR-20	22 April 2014	19.28	22.39	16.56	11.94	9.86	10.06	-8.1	-0.8
SC-TAR-21	22 April 2014	18.75	21.38	15.52	11.38	10.32	9.84	-8.2	1.4
SC-TAR-22	22 April 2014	19.36	21.02	15.98	11.56	9.42	9.61	-6.6	1.0
SC-TAR-23	22 April 2014	18.73	21.49	15.30	11.75	10.16	9.83	-5.8	1.0
SC-TAR-24	9 May 2014	19.47	22.37	16.19	12.24	9.98	10.25	-8.8	1.5
SC-TAR-25	9 May 2014	16.93	20.31	13.95	10.84	8.46	8.48	-10.1	0.9
SC-TAR-26	9 May 2014	19.03	18.19	14.42	9.79	8.21	8.03	-7.6	1.1
SC-TAR-27	9 May 2014	15.89	18.83	13.29	10.31	8.43	7.99	-10.4	0.0
SC-TAR-28	9 May 2014	14.89	18.88	13.52	9.76	7.52	7.68	-10.3	-0.5
SC-TAR-29	9 May 2014	15.80	17.83	12.73	9.46	7.54	7.48	-9.7	-0.5
SC-TAR-30	9 May 2014	15.89	17.87	12.89	8.19	7.54	7.55	-9.0	0.8
SC-TAR-31	15 September 2014	20.49	21.65	16.20	12.00	9.72	9.44	-6.2	1.4
SC-TAR-32	15 September 2014	18.43	20.34	15.21	11.57	9.99	8.38	-6.0	1.8
SC-TAR-33	15 September 2014	18.64	21.84	15.68	11.33	9.54	9.67	-5.4	2.3
SC-TAR-34	15 September 2014	16.94	19.96	14.14	10.57	8.76	8.43	-6.4	1.5
SC-TAR-35	28 September 2014	17.45	19.55	14.61	10.59	8.69	8.10	-6.4	3.1
SC-TAR-36	28 September 2014	16.91	20.11	14.37	10.61	7.66	7.91	-6.4	1.2
SC-TAR-37	28 September 2014	16.87	19.63	14.06	10.58	8.59	8.10	-6.1	0.7
SC-TAR-38	28 September 2014	16.39	20.61	13.47	10.36	8.20	8.51	-7.7	0.1
SC-TAR-39	28 September 2014	17.68	19.84	14.85	11.03	8.77	8.86	-8.6	1.8
SC-TAR-40	5 October 2014	18.75	22.10	15.66	12.12	9.73	9.83	-7.6	1.3
SC-TAR-41	5 October 2014	17.13	21.15	14.66	11.30	8.60	8.67	-6.6	0.7
SC-TAR-42	5 October 2014	16.77	19.37	14.49	10.25	7.92	8.54	-5.9	0.7
SC-TAR-43	5 October 2014	18.30	22.02	16.00	11.19	10.00	9.33	-6.1	-0.2
SC-TAR-44	5 October 2014	16.58	21.49	14.09	10.75	8.61	8.74	-8.6	-0.6

A: maximum shell length; B: maximum shell width; C: height of two last spires; D: height of last spire; E: aperture height; F: aperture width; PDB: Pee Dee Belemnite.

(Balakrishnan and Yapp, 2004). Another important parameter is the flux of liquid water output from the body fluid (f_o) relative to the flux of liquid water ingested by the snail (f_{in}) during shell formation. This ratio is called θ , which is defined as $\theta = (f_o/f_{in})$. Balakrishnan and Yapp (2004) showed that it is appropriate to assume that ambient water vapor is in isotope equilibrium with the liquid water. Furthermore, for $\theta < 0.40$, an assumption that snail body fluid is lost only by evaporation (i.e. $\theta = 0$) is a good approximation and introduces very little error in model calculations (Balakrishnan and Yapp, 2004). Thus, a value of $\theta = 0$ is adopted here.

The average shell margin $\delta^{18}\text{O}$ value of *Sphincterochila* individuals collected in the fall and spring seasons in Tarragona showed an average value of $+0.7 \pm 0.8\text{‰}$ versus PDB ($n = 44$). If collected snails grew their shell lip at average atmospheric conditions for the fall and spring seasons, that is, air temperature of

$\sim 16^\circ\text{C}$ and rainwater $\delta^{18}\text{O}$ values of $\sim -5\text{‰}$ (SMOW), then, the model predicts that snails were active at times when RH was, on average, $\sim 89\%$. The predicted RH value from the snail model is a value very close to the observed average maximum RH value ($\sim 90\%$) for those same months combined. Accordingly, the shell $\delta^{18}\text{O}$ values of *S. candidissima* shells seem to credibly record maximum RH values in the context of the flux balance model by Balakrishnan and Yapp (2004), which is constrained by independent climate data.

Model calculations were also used to predict RH values throughout the sampled months. The calculated RH values per collection date, assuming that calcification occurred under atmospheric conditions operating during the same collection date, positively correlated with observed maximum RH values ($R^2 = 0.61$; $p = 0.016$; $n = 9$). The significant relationship between calculated and observed RH

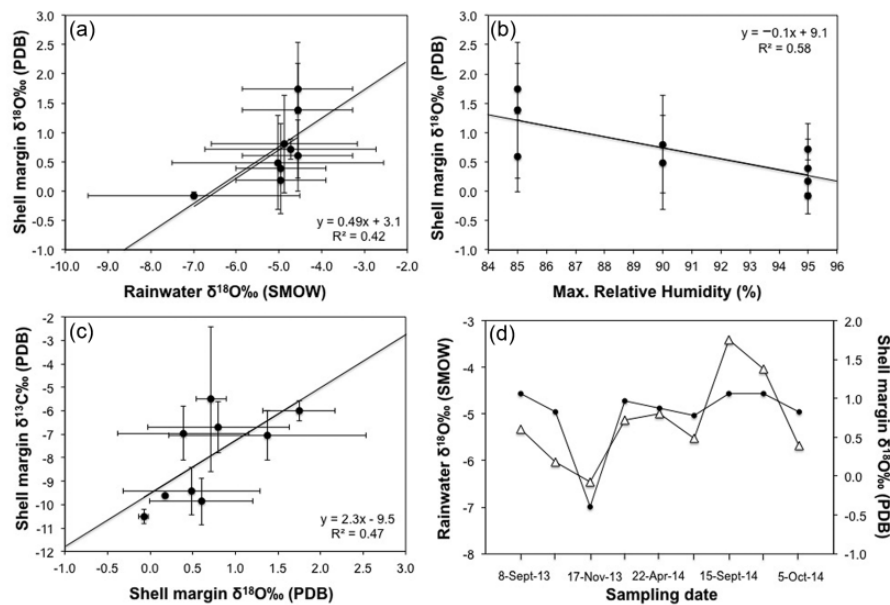


Figure 3. Relationship between shell margin $\delta^{18}\text{O}$ values and local climate data. Filled symbols represent average values per collection date whereas whiskers represent the standard deviation of the data: (a) relationship between $\delta^{18}\text{O}$ values of snail shell margin and precipitation; (b) relationship between $\delta^{18}\text{O}$ values of snail shell margin and local maximum relative humidity; (c) relationship between shell margin $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values; (d) average rainwater $\delta^{18}\text{O}$ (open triangles) and average shell margin $\delta^{18}\text{O}$ (filled circles) per sampling date throughout a year.

values through seasons further reinforces the usefulness of the model by Balakrishnan and Yapp (2004) to predict moisture conditions during snail calcification when other climatic variables (shell $\delta^{18}\text{O}$, rainwater $\delta^{18}\text{O}$, and air temperature) are known or can be deduced independently. Overall, the shell $\delta^{18}\text{O}$ values of *S. candidissima* appear to respond primarily to variations in rain $\delta^{18}\text{O}$ and maximum RH values, with higher shell $\delta^{18}\text{O}$ values associated with somewhat drier conditions (i.e. higher rain $\delta^{18}\text{O}$ values and/or lower RH) while lower values are linked to wetter times (i.e. lower rain $\delta^{18}\text{O}$ values and/or higher RH). Future studies in the region will evaluate the validity of shell margin $\delta^{18}\text{O}$ values from Pleistocene–Holocene shell middens to attempt to reconstruct atmospheric conditions at the time when snails were harvested by ancient human groups.

Shell margin carbon isotope values

The $\delta^{13}\text{C}$ values of land snail shells are primarily a function of the consumed and assimilated plants, as shown in cultured experiments (Metref et al., 2003; Stott, 2002; Zhang et al., 2014). Considering that there is an isotopic offset between shell and diet of $\sim 17\text{‰}$ for this species (Yanes et al., 2013a), and that the average shell margin $\delta^{13}\text{C}$ value for Tarragona specimens is $\sim -7.6\text{‰}$, sampled snails should have ingested (at the time of collection) resources with an average $\delta^{13}\text{C}$ value of $\sim -24.6\text{‰}$, ranging from $\sim -21.8\text{‰}$ for snails with a shell $\delta^{13}\text{C}$ value of -4.8‰ to $\sim -28.0\text{‰}$ for individuals with a shell $\delta^{13}\text{C}$ value of -11.0‰ . Accordingly, we can conclude that measured specimens followed a pure C_3 plant diet throughout the year, which is reasonable considering that the sampling area is dominated by Mediterranean C_3 -type plants.

Even though it has been shown that many small land snail taxa do not seem to incorporate significant amounts of limestone in the shell (Pigati et al., 2004, 2010), for many large body size species with access to limestone, the ingestion of foreign carbonates can be significant and detectable in the shell via radiocarbon and stable carbon isotope analysis (Goodfriend et al., 1999; Yanes et al., 2008). *S. candidissima* is known to be abundant in soils and areas rich in calcium carbonate, and considering the large size and thickness of its white shell, ingestion of carbonate as a source of calcium for shell growth is probably important (Yanes et al.,

2013a), possibly accounting for the ~ 10 – 30% of snail shell carbon isotope budget (Goodfriend et al., 1999; Yanes et al., 2008). Although the shell $\delta^{13}\text{C}$ values are overwhelmingly dominated by the $\delta^{13}\text{C}$ values of consumed plants, the effect of limestone ingestion is possibly also recorded in the shell of *Sphincterochila* as suggested by carbon flux balance mixing models for the same species in other localities in Spain (see Figure 3 in Yanes et al., 2013a). However, because limestone $\delta^{13}\text{C}$ values were not available for the present study, further quantitative evaluations are not yet possible. Accordingly, higher $\delta^{13}\text{C}$ values in *S. candidissima* shells should reflect C_3 plants enriched in ^{13}C , possibly at times of drier conditions when plants are more water-stressed (Dawson et al., 2002; Farquhar et al., 1989; Prendergast et al., in press), perhaps in combination with increased limestone intake. In contrast, lower shell $\delta^{13}\text{C}$ values are related to C_3 plants with lower $\delta^{13}\text{C}$ values, likely in response to wetter conditions, and/or lower limestone intake. Even though some studies have speculated that shell $\delta^{13}\text{C}$ values can also be impacted by atmospheric CO_2 (e.g. ZongXiu et al., 2007), a published flux balance mixing model suggests that the $\delta^{13}\text{C}$ values of well-mixed atmospheric CO_2 should not significantly impact the snail carbon isotope budget because of the small difference in the partial pressure of the CO_2 in the lungs of the snail and the open atmosphere (Balakrishnan and Yapp, 2004).

Considering that oxygen and carbon stable isotopes of *S. candidissima* positively correlate (Figure 3c), which is unusual in other edible pulmonate snail species (see Figure 6 in Yanes et al., 2013a), both isotope systems appear to be directly or indirectly affected by moisture. Thus, *Sphincterochila* shell $\delta^{13}\text{C}$ values may be used as an additional indirect proxy for moisture, although this might not be the case for most land snail species.

Relationship between stable isotopes and body size

In the present work, we observed a positive correlation between *Sphincterochila* shell size and both oxygen and carbon stable isotopes (Figure 4), which suggests that body size of this species may respond to both climate and diet. It is generally anticipated that snail body size is significantly influenced by climate. However, the extent and direction to which climate influences snail ultimate body size is unclear. In arid regions, moisture is the

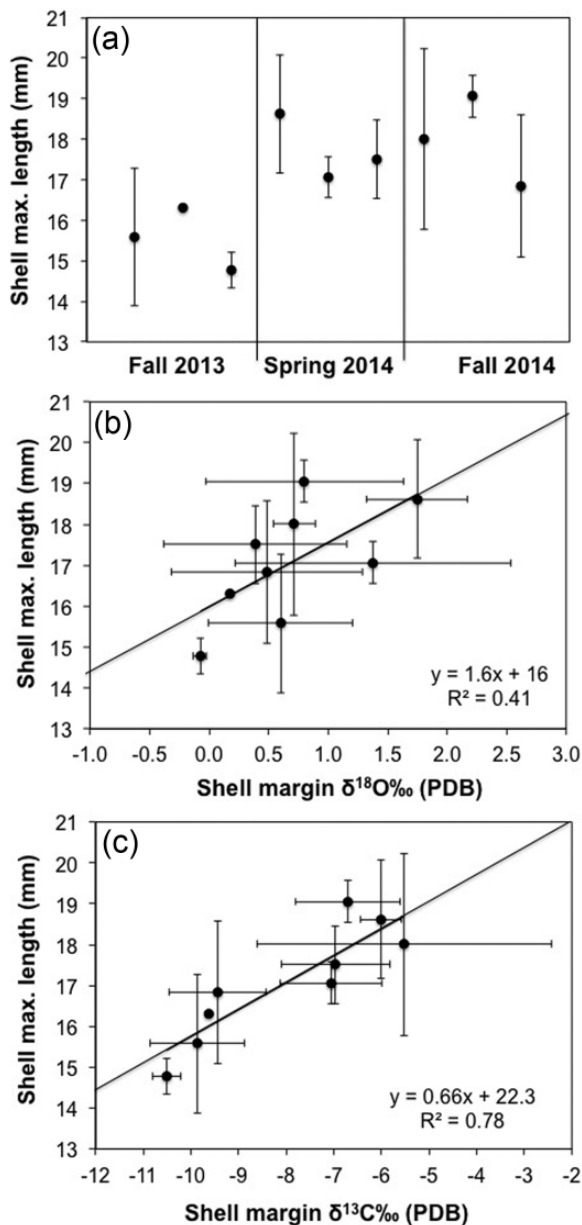


Figure 4. (a) Body size of *Sphincterochila candidissima* collected throughout a year in Tarragona, NE Spain. (b) Relationship between shell margin $\delta^{18}\text{O}$ values and body size. (c) Relationship between shell margin $\delta^{13}\text{C}$ values and body size. Filled circles represent average values per collection date whereas whiskers represent the standard deviation of the data.

dominant limiting factor for land snails. While some studies have observed that snails reach larger sizes in desert areas as a survival mechanism to minimize the risk of water loss because it decreases the surface-to-volume ratio (Nevo et al., 1983), other studies have observed that snail size tends to increase with increasing moisture because snails are able to grow and be active for longer periods (Goodfriend, 1986). A third possibility is that snail size does not exhibit a linear relationship with rainfall (Hausdorf, 2006).

Considering that shell $\delta^{18}\text{O}$ values of *Sphincterochila* primarily reflect moisture conditions, with higher values mainly associated with higher rain $\delta^{18}\text{O}$ values and lower RH (Figure 3), we can infer that *S. candidissima* individuals reach larger size during drier times. Therefore, *Sphincterochila* ultimate body size may function as an ecological mechanism to survive drier conditions by lowering the surface-to-volume ratio and thus, reduce the risk of water loss by evaporation. The same observations were made by Yanes et al. (2012) for the edible land snail *Cepaea nemoralis*

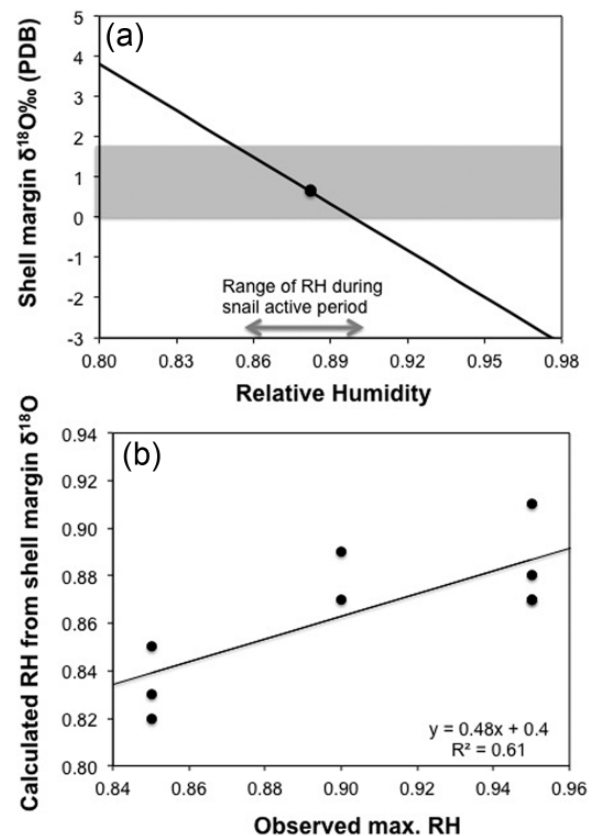


Figure 5. (a) Calculations of the shell margin $\delta^{18}\text{O}$ values as a function of RH using the snail evaporative steady-state flux balance model by Balakrishnan and Yapp (2004). Filled circle depicts the average shell margin $\delta^{18}\text{O}$ value of all 44 analyzed snails in this study. (b) Relationship between calculated RH from the snail shell margin $\delta^{18}\text{O}$ values and observed maximum RH values at the snail collection date. RH: relative humidity.

(Gastropoda: Helicidae) from coastal sites in Asturias and Cantabria, northern Spain.

The positive relationship between snail body size and shell $\delta^{13}\text{C}$ values (Figure 4) also suggest that adult individuals that ingested more water-stressed plants, which are usually enriched in ^{13}C (Dawson et al., 2002; Farquhar et al., 1989), and/or higher proportion of limestone, which displays significantly higher $\delta^{13}\text{C}$ values than C_3 plants (Yanes et al., 2013a), tend to reach larger dimensions in the study area.

All in all, the results of this study support the hypothesis that adult specimens of *S. candidissima* from Spain tend to exhibit larger dimensions under drier conditions, possibly as a survival mechanism to reduce the surface-to-volume ratio. Correspondingly, shell size of adult individuals may be used as an additional proxy for moisture conditions in the western Mediterranean. Future studies in the region will analyze body size and isotope composition of the shell jointly using archeological specimens to further evaluate this hypothesis using ancient shelly materials. Although this approach does not seem to be valid for all snail species (Hausdorf, 2006), the shell size of *S. candidissima* and other edible species like the European snail *Cepaea nemoralis* may be used as a complementary proxy for humidity, with larger individuals pointing to somewhat drier conditions.

Archaeological implications

Although earlier archaeological work generally focused on zooarcheological remains (e.g. bones, marine shells) other than land snails, recent investigations illustrate that (1) land snails have

been consistently exploited by many prehistoric foragers (Bosch et al., 2015; Fernández-Lopez-de-Pablo et al., 2014; Hill et al., 2015; Lubell, 2004a, 2004b); and that (2) the stable isotope composition of archeological shells can credibly reflect average (dominant) climatic conditions during relevant cultural periods (e.g. Colonese et al., 2007, 2010a, 2010b, 2011, 2013a, 2013b; Paul and Mauldin, 2013; Prendergast et al., 2016a; Stevens et al., 2012; Yanes et al., 2011, 2012, 2013a, 2013b, 2014). Studies that assess the season of shell harvest are also relevant for archeologists because they inform about season of site occupation, human mobility, and subsistence patterns (e.g. Prendergast et al., 2016b). Our results demonstrate that shell margin $\delta^{18}\text{O}$ values of *S. candidissima* from northeastern Spain ($\sim 41^\circ \text{N}$) convincingly record the dominant conditions at the time of snail collection, that is, at monthly–submonthly resolution (Figure 3a, b and d). However, because the target species seems to only grow during fall and spring seasons (only half of the months of the year), estimates of season of collection during prehistoric times seem limited in this case study.

Another difficulty is whether or not snails harvested by human foragers were collected while specimens were active, hibernating/aestivating, or both. If snails were collected at times when snails did not secrete shell, then, inferences of season of shell collection may be biased. Since *S. candidissima* remains inactive during the winter and summer seasons, and consequently, does not grow shell, it could be argued that the archaeological interest of this species to determine the season of collection is of limited use. Our current knowledge about cultural exploitation and density of *S. candidissima* suggests that humans likely harvested snails during snail active periods (fall and spring). Ethno-biological references about the human collection of land snails in Mediterranean Spain indicates that the collection takes place after rain events, usually in April and May, when snails are most active (Fajardo et al., 2009). In addition, field observations in Mediterranean Spain suggest that *S. candidissima* displays significantly higher abundance after rain events, and therefore, they are easier to find and collect while they are active rather than when they are aestivating/hibernating (personal field observations, 2013–2014). We therefore assume that ancient human groups likely harvested snails more easily while they were active.

The fact that shell margin $\delta^{18}\text{O}$ values reflect dominant conditions at the time of snail collection, even for fall and spring seasons only, is a very encouraging result and calls for additional investigation. This sampling approach to determine season of shell harvest may be more effective in studies from lower-latitude regions where snails may grow continuously and uninterruptedly year-round.

This study also illustrates that body size of *S. candidissima* snails can be used as a qualitative proxy for moisture in the region, given the documented associations between shell margin isotope composition and shell dimensions (Figure 4b and c). This observation could be explored and applied to archeological studies that use the same species. However, if past environments were significantly different than at present-day, body size of archeological shells may differ from those of today (Yanes et al., 2012). In those cases, the combination of body size and stable isotopes from the same shells will be critical to determine if these relationships hold in the past.

Conclusion

This is the first study to investigate the isotopic composition of the last growth episode (shell lip) of adult land snails and its relationship to climate throughout a year. Arid-dwelling *S. candidissima* was collected during 2013–2014 in Tarragona, NE Spain. The results show that shell margin $\delta^{18}\text{O}$ values primarily record local rainwater $\delta^{18}\text{O}$ values and maximum RH conditions,

reinforcing that *S. candidissima* can certainly be used as a valid paleoprecipitation $\delta^{18}\text{O}$ and/or paleohumidity proxy at both annual and monthly–submonthly resolution. The positive relationship between shell $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and shell size suggests that specimens of *Sphincterochila* tend to reach larger size under drier conditions, probably as a mechanism to reduce the risk of water loss associated with lower surface-to-volume ratio. The size of this species, hence, may be used as a complementary paleohumidity proxy. The shell margin $\delta^{13}\text{C}$ values varied significantly throughout the year suggesting significant variations in snail diet and limestone ingestion. The observed strong correlation between shell $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values, not common for other edible snail species, suggests that both isotope systems are influenced by moisture directly or indirectly.

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