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Holocene paleoenvironment (∼7200–4000 cal BP) of the Los Castillejos archaeological site (SE Spain) inferred from the stable isotopes of land snail shells

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ABSTRACT

Neolithic, Copper Age and modern land snail shells from the Los Castillejos archeological site (37°20′N), SE Iberian Peninsula, were analyzed for $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios to estimate the paleoenvironmental conditions during shell deposition. Modern and ancient $\delta^{13}C$ values ranged from −9.2‰ to −7.7‰ and from −13.6‰ to −5.7‰, respectively, and they differed among taxa. $\delta^{13}C$ values were similar for Neolithic ($\delta^{13}C = -8.1 \pm 2.3$‰), Copper Age ($\delta^{13}C = -8.4 \pm 0.6$‰) and modern ($\delta^{13}C = -8.4 \pm 0.5$‰) herbivorous *Cernuella* specimens. Alternatively, fossil shells of the herbivorous *Ferussacia folliculum* ($\delta^{13}C = -9.9 \pm 1.2$‰) and the omnivorous *Rumina decollata* ($\delta^{13}C = -11 \pm 1.2$‰) showed substantially lower $\delta^{13}C$ values than modern specimens, which may suggest lower water stress during the early to mid Holocene than today. The $\delta^{18}O$ values from modern specimens ranged from −1.1‰ to +0.6‰ while the $\delta^{18}O$ values from fossil specimens ranged from −6.7‰ to +0‰ and they differed among species. *Cernuella* exhibited shell $\delta^{18}O$ values that increased from the Neolithic ($\delta^{18}O = -2.3 \pm 1.8$‰) and Copper Age ($\delta^{18}O = -1.7 \pm 0.6$‰) to the present ($\delta^{18}O = -0.3 \pm 0.5$‰). The $\delta^{18}O$ values of fossil shells of *F. folliculum* ($\delta^{18}O = -3.7 \pm 1.0$‰) and *R. decollata* ($\delta^{18}O = -4.0 \pm 1.6$‰) were also lower than modern shells. Calculations of a published snail-flux balance mixing model for $\delta^{18}O$ values indicate that early to mid Holocene shells precipitated during times when relative humidity was greater than today. The SE Iberian Peninsula was noticeably wetter ∼7200 cal BP, and experienced drier conditions thereafter. The results are consistent with other regional paleoclimatic proxies and reinforce the potential use of land snail shells as paleoenvironmental archives.

1. Introduction

Many archaeological sites around the world contain abundant and well-preserved land snail shells (e.g., Goodfriend and Ellis, 2000; Lubell, 2004; Balakrishnan et al., 2005b; Colonese et al., 2007, 2010a, b). The stable isotopic composition of land snail shells records potentially the environmental conditions at the time when individuals were active and deposited shell material. The carbon isotope composition ($\delta^{13}C$) of the shell is primarily a function of the consumed plant carbon and consequently, shell $\delta^{13}C$ values may be used to document the paleo-vegetation of a landscape, which in turn may provide paleoclimatic information such as the aridity level of an ecosystem (e.g., Balakrishnan et al., 2005a, b; Baldini et al., 2007; Goodfriend and Ellis, 2000, 2002; Stott, 2002; Metref et al., 2003; Colonese et al., 2007, 2010a, b; Yanes et al., 2008, 2009, 2011). This assumes that snails ingest the surrounding plants in the proportion that they are available in the habitat. Previous laboratory experiments (Stott, 2002; Metref et al., 2003) have shown that the $\delta^{13}C$ values of the shell are positively correlated with the $\delta^{13}C$ values of the shell organic and snail body tissue, which are both derived from ingested plants. However, this relationship is complicated in carbonate-rich areas where snails may ingest large and variable proportions of limestone as a source of calcium to grow their shells (e.g., Goodfriend and Hood, 1983; Goodfriend, 1987; Goodfriend and Gould, 1996; Goodfriend et al., 1999; Yanes et al., 2008). However, recent radiocarbon studies indicate that some minute terrestrial gastropod species appear to

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ingest negligible amounts of limestone (Pigati et al., 2004, 2010). Moreover, it has been suggested that atmospheric CO_2 may influence significantly the $\delta^{13}C$ value of the shell (e.g., Goodfriend and Hood, 1983), but calculations from a recent snail-flux balance model proposed by Balakrishnan and Yapp (2004) indicate that atmospheric CO_2 should not affect significantly the snail shell at normal ambient concentration of CO_2.

Environmental factors that most affect the oxygen isotopic composition ($\delta^{18}O$) of the shell include rain water $\delta^{18}O$ values, relative humidity, atmospheric water vapor $\delta^{18}O$ values, and temperature (Yapp, 1979; Balakrishnan and Yapp, 2004). Accordingly, several field studies have shown a systematic relationship between the $\delta^{18}O$ values of the local rain water and the aragonite shell (e.g., Lécolle, 1985; Zanchetta et al., 2005). Moreover, the bulk of previous studies indicate that the $\delta^{18}O$ values of shells are consistently enriched in ^{18}O by several per mil with respect to the local rain water, which is best explained by a flux balance model in which shell is deposited in oxygen isotope equilibrium with body fluid that has experienced ^{18}O enrichment as a consequence of body water loss through evaporation (Balakrishnan and Yapp, 2004).

In the present study, numerous ancient land snail shells from the Los Castillejos archaeological site (SE Spain) were analyzed isotopically to estimate paleoenvironmental conditions between ~7200 and 4000 cal BP. Live-collected snail specimens were also analyzed to estimate a modern snail isotopic baseline for the study area. The results presented here are the first isotopic data of fossil and modern land snails from the Iberian Peninsula. Data were evaluated using a published mass evaporative steady-state flux balance mixing model (Balakrishnan and Yapp, 2004) and contrasted with other regional paleoclimatic proxies.

2. Geographical, archaeological and chronological context

Los Castillejos (latitude: 37°20′02″N, longitude: 4°00′05″W), located at 900 m above sea level (m a.s.l.) near Montefrío (Granada, Spain), contains abundant and well-preserved human-related animal and vegetal remains that are useful to reconstruct the prehistory of the SE Iberian Peninsula (Fig. 1A). The study area was an attractive site for human occupation due to its variety of habitats, food sources and water availability. Agriculture was well established in SE Spain during the Copper Age, where C_3 grains, including naked wheat (*Triticum aestivum/durum*) and barley (*Hordeum vulgare ssp. nudum*), were the main crops (Rovira, 2007; Aguilera et al., 2008). Native wild vegetation is vastly dominated by C_3 plants, such as evergreen oak (*Quercus ilex L.*), kermes oak (*Quercus coccifera L.*) and several herbaceous species, including rosemary, halt and juniper (Rovira, 2007; Aguilera et al., 2008). There is no evidence of the natural presence of C_4 and CAM plants in Montefrío. Current land use near the site includes cultivation of olive, wheat and barley (Aguilera et al., 2008).

The archeological remains of Los Castillejos are preserved in stratigraphic layers with no evidence of disturbance (Fig. 1B). An archaeomagnetic study of ceramic remains (Nachasova et al., 2007), numerous AMS and conventional radiocarbon analyses ($n = 36$) of charcoal and mainly fossil cereal grains (e.g., Cámara et al., 2005; Cámara-Serrano et al., in press; Martínez et al., 2009), and ten TL dates for the oldest phases (unpublished data), indicate that the age of the site ranges from ~7200 to 4000 cal BP (Table 1; see also the Appendix in Supplementary Material), including the Neolithic (~7000–5000 cal BP) and Copper Age (~5000–4000 cal BP) periods. The bottom of the Los Castillejos deposits (from level I to IV in Fig. 1B) represents successive layers of Neolithic times, with decreasing decorated pottery and space use changes from a communal area with numerous hearths to real residential units. The upper part of the deposit (from level V to VII in Fig. 1B) represents the Copper Age, with round huts in which some copper tools have been found (e.g., Cámara et al., 2005; Cámara-Serrano et al., in press). Mixed farming was important throughout the studied period (Riquelme, 1996; Rovira, 2007). Technological and economic strategy changes occurred around 6000 cal BP (level III) as seen in the pottery (Cámara-Serrano et al., in press), flaked lithic industry (Martínez et al., 2009), and farming intensity as well as the appearance of new plant crops such as flax and *Papaverum somniferum* (Aguilera et al., 2008). Land snail shell material was recovered from multiple layers from layer I to VII (see Fig. 1B).

3. Climatic setting

Southern Spain is influenced by Atlantic low pressure systems and by Mediterranean disturbances and it experiences extreme

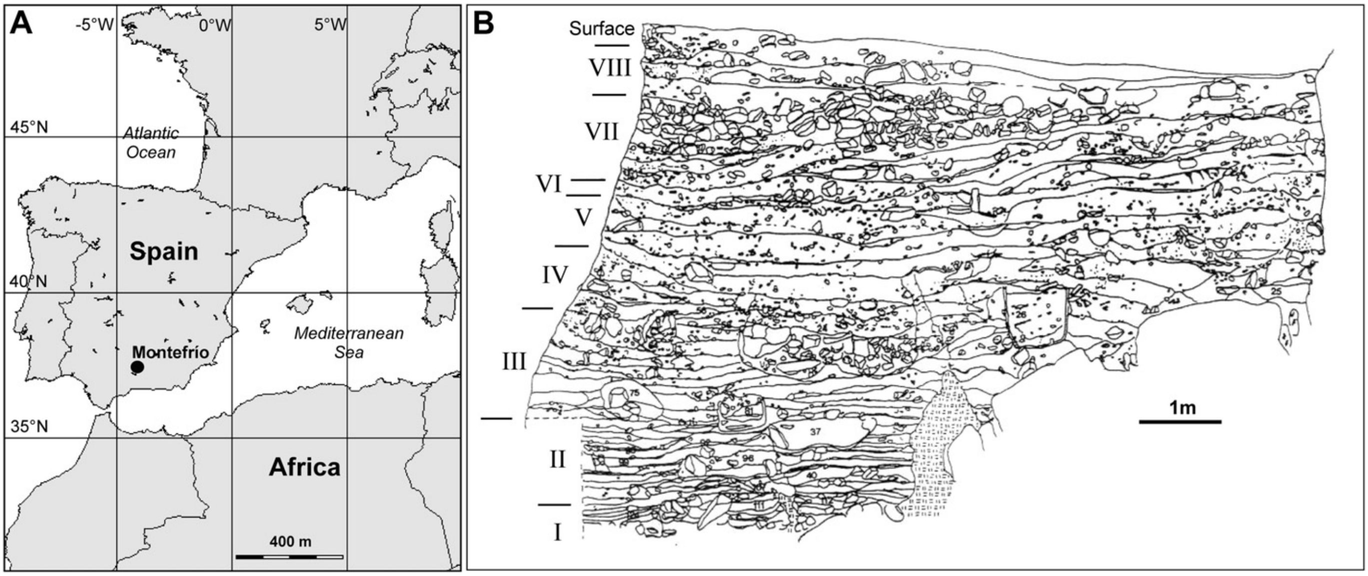

Fig. 1. Study area. (A) Geographical location of the Montefrío locality, Granada, Spain. (B) Stratigraphy of the Los Castillejos archeological site indicating the main archeological layers on the left (adapted from Afonso-Marrero et al., 1995).

Table 1
Average carbon and oxygen isotopic composition of land snail shells from the Los Castillejos Archeological site, Montefrío, Granada (SE Spain), separately by species.

Age (cal BP)	Age period	Species	Feeding habit	n	δ^{13}C‰(PDB)		δ^{18}O‰(PDB)	
Modern	Modern	*Cernuella virgata*	Herbivorous	15	−8.4	±0.5	−0.3	±0.4
4270 ± 80	Copper Age	*Cernuella* sp.	Herbivorous	2	−8.4	±0.6	−1.7	±0.5
5540 ± 47	Neolithic	*Cernuella* sp.	Herbivorous	2	−5.7	±0.0	−2.1	±0.3
7010 ± 91	Neolithic	*Cernuella* sp.	Herbivorous	3	−9.0	±1.9	−3.0	±1.2
7150 ± 84	Neolithic	*Cernuella* sp.	Herbivorous	2	−9.0	±3.2	−1.6	±0.0
4070 ± 61	Copper Age	*Ferussacia folliculum*	Herbivorous	15	−9.4	±0.8	−3.8	±0.6
4270 ± 80	Copper Age	*F. folliculum*	Herbivorous	5	−9.0	±0.4	−3.2	±0.2
4360 ± 52	Copper Age	*F. folliculum*	Herbivorous	5	−8.9	±0.4	−3.2	±0.2
5120 ± 121	Neolithic	*F. folliculum*	Herbivorous	15	−9.5	±0.4	−3.5	±0.9
5160 ± 99	Neolithic	*F. folliculum*	Herbivorous	35	−9.7	±1.5	−4.0	±1.1
5540 ± 47	Neolithic	*F. folliculum*	Herbivorous	30	−9.8	±1.1	−3.6	±0.7
5710 ± 42	Neolithic	*F. folliculum*	Herbivorous	36	−9.9	±1.4	−3.7	±1.2
6060 ± 88	Neolithic	*F. folliculum*	Herbivorous	30	−10.3	±1.2	−4.0	±1.1
7010 ± 91	Neolithic	*F. folliculum*	Herbivorous	17	−10.5	±1.0	−3.4	±0.7
5160 ± 99	Neolithic	*Rumina decollata*	Omnivorous	2	−12.1	±1.3	−4.1	±1.0
5710 ± 42	Neolithic	*R. decollata*	Omnivorous	5	−12.0	±0.8	−4.7	±1.2
6061 ± 88	Neolithic	*R. decollata*	Omnivorous	4	−11.0	±0.9	−3.5	±1.9
7010 ± 91	Neolithic	*R. decollata*	Omnivorous	4	−11.1	±1.2	−5.0	±1.3
7040 ± 88	Neolithic	*R. decollata*	Omnivorous	4	−11.2	±1.8	−3.0	±1.7
7041 ± 88	Neolithic	*R. decollata*	Omnivorous	5	−10.0	±0.7	−2.6	±2.0
7150 ± 84	Neolithic	*R. decollata*	Omnivorous	6	−10.8	±1.3	−3.8	±1.0
7240 ± 47	Neolithic	*R. decollata*	Omnivorous	5	−10.5	±0.7	−5.4	±1.0

n: number of shells analyzed.

seasonal contrasts. Montefrío (Granada, SE Spain), located at 900 m a.s.l., exhibits a Continental Mediterranean climate, with marked cool wet winters and hot dry summers (Fig. 2A). Climatic data recorded between 2001 and 2010 from two meteorological stations closest to Montefrío, including Iznalloz (808 m a.s.l.) and Loja (445 m a.s.l.), were used to evaluate the current environmental conditions in the study area (Junta de Andalucía: http://www.juntadeandalucia.es). Mean annual temperature is 15.4 °C and it ranges from 6.6 °C in the winter season to 26.1 °C in the summer months (Fig. 2A–B). Precipitation varies from as little as 9.7 mm during the summer months to 704.2 mm during the winter season (Fig. 2A). The maximum monthly relative humidity value averages 78.9%, ranging from 58.3% in summer to 89.2% in winter (Fig. 2B). Precipitation and maximum relative humidity correlate positively ($r_s = 0.87$; $p = 0.0003$; $n = 12$). Temperature negatively correlates with both precipitation ($r_s = -0.80$; $p = 0.0016$; $n = 12$) and maximum relative humidity ($r_s = -0.99$; $p < 0.001$; $n = 12$).

The oxygen isotope composition of local rain water from Granada was studied by Delgado-Huertas et al. (1991) between 1988 and 1991. The δ^{18}O values of rain are quite variable throughout the year, ranging from −15.9‰ (SMOW) in February to −1.9‰ (SMOW) in March (Fig. 2C). The weighted average δ^{18}O value of the local rain water is −7.5‰ (SMOW).

4. Methods

4.1. Sampling strategy and land snail species

Modern adult individuals ($n = 15$) of *Cernuella virgata* (Da Costa, 1778), an air-breading pulmonate gastropod, were live-collected randomly from Montefrío in October, 2010.

Fossil adult shells ($n = 258$) were gathered from layers I to VII (see Fig. 1B) by excavating about 30–50 cm into the exposed surface to avoid potential contamination and reworked material. A total of six species were identified: *Cernuella* sp., *Ferussacia folliculum* (Schröter, 1784), *Rumina decollata* (Linnaeus, 1758), *Truncatellina cylindrica* (Férussac, 1807), *Vitrea crystallina* (Müller, 1774) and *Cecilioides acicula* (Müller, 1774). These species have a widespread Mediterranean distribution and are herbivorous, feeding on green and/or dead plant material, with the exception of *R. decollata*, which is omnivorous (e.g., Kerney and Cameron, 1979). They display an epifaunal lifestyle, living on rocks, plants or under leaf litter, mostly on calcareous soils, except for *C. acicula*, which is subterranean, burrowed well below the soil surface among plant roots (e.g., Kerney and Cameron, 1979). Because modern specimens of *C. acicula* may inhabit the same niche as fossil specimens, data from this species ($n = 8$) are not considered for paleoenvironmental interpretation as a precautionary measure. Moreover, only two shells of *V. crystallina* and six shells of *T. cylindrica* were available for isotopic analyses. These species are ignored as well due to their small sample sizes. Nevertheless, data for these three disregarded species are reported in the Appendix (see supplementary material). As a result, data from the epifaunal herbivorous *Cernuella* ($n = 24$) and *F. folliculum* ($n = 188$) species, together with data from the epifaunal omnivorous *R. decollata* ($n = 35$) species, were used in the present study.

Ancient shells were evaluated for diagenetic alteration prior to stable isotope analysis. First a visual evaluation was conducted using a binocular microscope to evaluate for evidence of macroscopic alteration such as fragmentation, deformation or corrosion. X-ray diffraction analysis on five randomly selected shells that were finely ground, showed that the shells retained their original aragonite composition, suggesting that diagenetic alteration was unlikely and therefore, this material was suitable for geochemical analysis.

4.2. Activity periods of land snails

Land snail activity is controlled by complex internal and external factors that vary across sampling sites and species. Dependence on air temperature and moisture make land snails concentrate their active periods during certain months of the year, when a balance between water availability and temperature is reached (Iglesias et al., 1996 and references therein). Although many snails are active at fairly low temperatures (between 0 and 10 °C), especially in dry localities where dew and fog are their main water source (Örstan, 2010), optimum temperatures for activity and growth range from 10 °C to 27 °C (e.g., Cook, 2001). Similarly, snails require ambient relative humidity values above 70% to minimize the risk of desiccation (e.g., Cook, 2001). While tropical-subtropical environments commonly have mild climates that permit snail activity throughout the year (i.e., no hibernation periods and short estivation episodes), in more temperate to high latitude locales, with more extreme seasonal climates, snail activity is reduced to a few

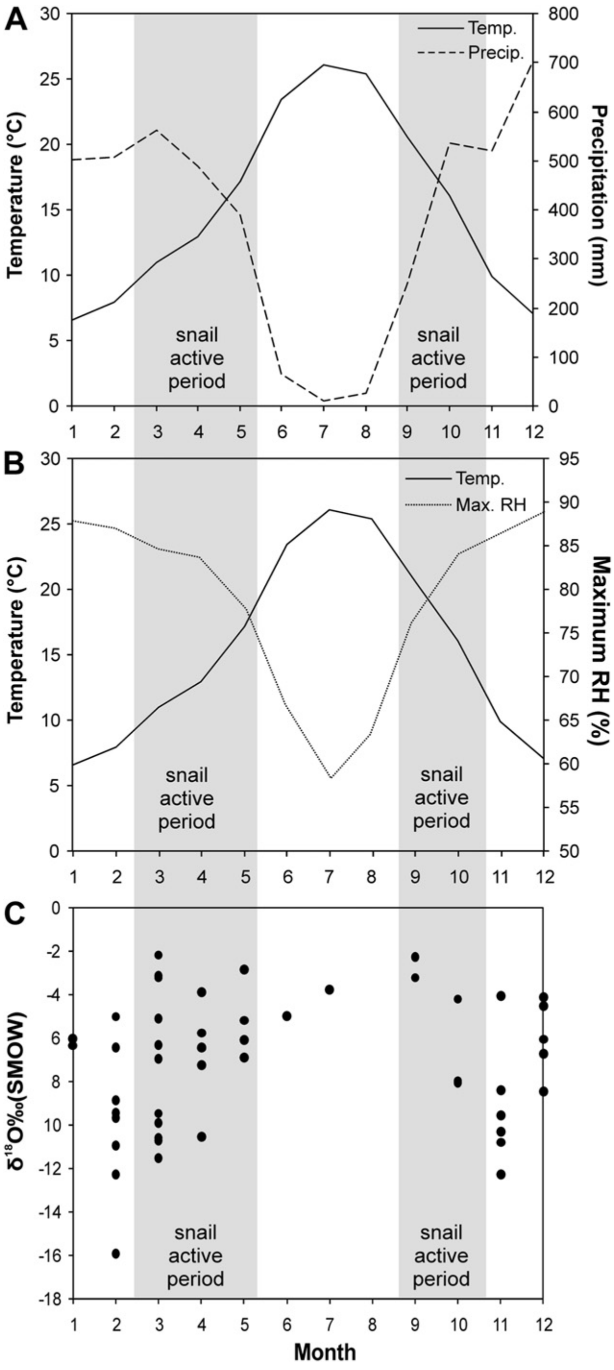

Fig. 2. Current climatic conditions of the study area. (A) Temperature (°C) and Precipitation (mm). (B) Temperature (°C) and maximum Relative Humidity (%). Climate values recorded between 2001 and 2010 were taken from the meteorological stations of Loja and Iznalloz, Granada, Southern Spain at the "Junta de Andalucía" webpage (http://www.juntadeandalucia.es). (C) Oxygen isotope composition of rain water from Granada, Southern Spain. Rain water was collected by Delgado-Huertas et al. (1991) between 1988 and 1991. Gray bands represent the theoretical periods of activity of land snails in the study area (March to May and September to October: Iglesias et al., 1996).

months out of the year. In the mid-temperate climate of Galicia (NW Iberian Peninsula), Iglesias et al. (1996) observed that the helicid *Cornu asperum* experienced two periods of inactivity yearly: ∼4 months of hibernation (from November to February) and ∼3 months of estivation (from June to August). They also documented longer dormancy episodes in adult individuals than juveniles. In Granada, at ∼900 m a.s.l., a Mediterranean type climate prevails with cold humid winters and hot dry summers (Fig. 2). Accordingly, snails should hibernate for three to four months per year (e.g., from November to February) and estivate during the summer (e.g., from June to August), similar to the active periods of snails in Galicia (Iglesias et al., 1996). Such a possible scenario for adult snail annual activity in Montefrío is assumed as a working hypothesis (see Fig. 2).

4.3. Stable isotope analyses

Land snail shells were prepared and analyzed in the Savannah River Ecology Laboratory, University of Georgia (USA). Each entire shell was cleaned ultrasonically in deionized water to remove any adhering particles, and pulverized by hand with an agate mortar and pestle. Organic matter was removed by placing the powdered sample in a bath of 3% sodium hypochlorite (NaOCl) for 48 h at room temperature (∼22 °C). Subsequently, the powder was rinsed three times with deionized water by repeated centrifugations and dried in an oven at 40 °C overnight (e.g., Yanes et al., 2008, 2009, 2011). The entire shell was used in the analysis as a proxy for average environmental conditions over the life of the organism rather than pieces of shell material that may only capture a seasonal component of growth. Average values should be representative of the dominant conditions because numerous contemporaneous specimens are analyzed per time-interval. Also, most species studied here are short-lived (annual to biannual) and many of them are minute, so an entire shell is needed for the analysis.

Shell carbonate powder (∼150 μg) 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 0.5 ml of 100% phosphoric acid (H_3PO_4) at 25 °C. The resulting CO_2 was analyzed isotopically after 24 h using the GasBench II and a Finnigan Delta^{plus} XL isotope ratio mass spectrometer (IRMS), which was operating in a continuous flow mode. All stable isotope results are reported in δ notation relative to the international standard Pee Dee Belemnite (PDB) for carbonate and SMOW for water. The δ values are defined as:

$$\delta X = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \times 1000(‰)$$

where $X = {}^{13}C$ for $R = {}^{13}C/{}^{12}C$, or $X = {}^{18}O$ for $R = {}^{18}O/{}^{16}O$. Carbon and oxygen isotope values were calibrated against the international standard NBS-19 and in-house standards. The precision of the analyses was better than ±0.1‰ (1σ standard deviation) for carbon and oxygen isotopes based on the repeated measurement ($n = 30$) of the NBS-19 and in-house standards. Replicate analyses of aliquots of powdered snail shells had an overall precision of ±0.2‰ for $\delta^{18}O$ and $\delta^{13}C$.

4.4. Statistical treatment

Statistical analyses were performed using PAST 1.38b software (Hammer et al., 2001), assuming a significance level of $\alpha = 0.05$. Non-parametric (rank-based) tests were computed to test samples for statistical differences. Spearman correlation was used to test whether or not two variables showed a monotonic relationship. The Kruskal–Wallis test was used to estimate whether or not groups of samples showed similar median distribution values.

5. Results

Live-collected adult snails of *C. virgata* displayed an average $\delta^{13}C$ value of −8.4 ± 0.5‰ ($n = 15$) with a relatively narrow range from −9.2‰ to −7.7‰ (see the Appendix in Supplementary Material), while fossil shell $\delta^{13}C$ values of three species exhibited an average of −10.0 ± 1.4‰ ($n = 232$) with a rather large range of −13.6‰ to −5.7‰ (see the Appendix in Supplementary Material). Thus, ancient shells displayed a wider range of δ^{13}C values than their modern counterparts. The δ^{13}C values of Neolithic shells differed significantly among species (Kruskal–Wallis, $p < 0.001$; Fig. 3A). Specimens of *Cernuella* exhibited considerably higher shell δ^{13}C values than shells of *F. folliculum* and *R. decollata* (Table 1; Fig. 3A). Fossil *Cernuella* shells displayed comparable δ^{13}C values from ~7200 cal BP to the present (Fig. 4A), whereas Neolithic and Copper Age shells of *F. folliculum* (Fig. 4B) and Neolithic shells of *R. decollata* (Fig. 4C) exhibited significantly lower δ^{13}C values than recent *Cernuella* shells (Table 1; Fig. 4A–C).

Modern specimens showed an average δ^{18}O value of −0.3 ± 0.4‰ ($n = 15$) with a range of −1.1‰ to +0.6‰ (see the Appendix in Supplementary Material), while fossil shell δ^{18}O values exhibited an average of −3.7 ± 1.1‰ ($n = 232$) with a comparatively large range from −6.7‰ to +0‰ (see the Appendix in Supplementary Material). The δ^{18}O values of the shell differed significantly among the three land snail species (Kruskal–Wallis, $p < 0.001$). Neolithic *Cernuella* exhibited significantly higher δ^{18}O values than Neolithic *F. folliculum* and *R. decollata* (Table 1; Fig. 3B). *Cernuella* shells showed a progressive increase in δ^{18}O values from −2.4 ± 0.9‰ for the Neolithic population to −1.7 ± 0.5‰ for the Copper Age group to −0.3 ± 0.4‰ for the modern specimens (Fig. 4D). Neolithic-Copper Age specimens of *F. folliculum* (δ^{18}O = −3.7 ± 1.0‰; Fig. 4E) and Neolithic shells of *R. decollata* (δ^{18}O = −4.0 ± 1.6‰; Fig. 4F) recorded considerably lower δ^{18}O values than modern *Cernuella* counterparts as well (Fig. 4D–F). Overall, fossil land snail shells generally showed substantially lower δ^{13}C and δ^{18}O values during the early to mid Holocene than today (Table 1; Fig. 4).

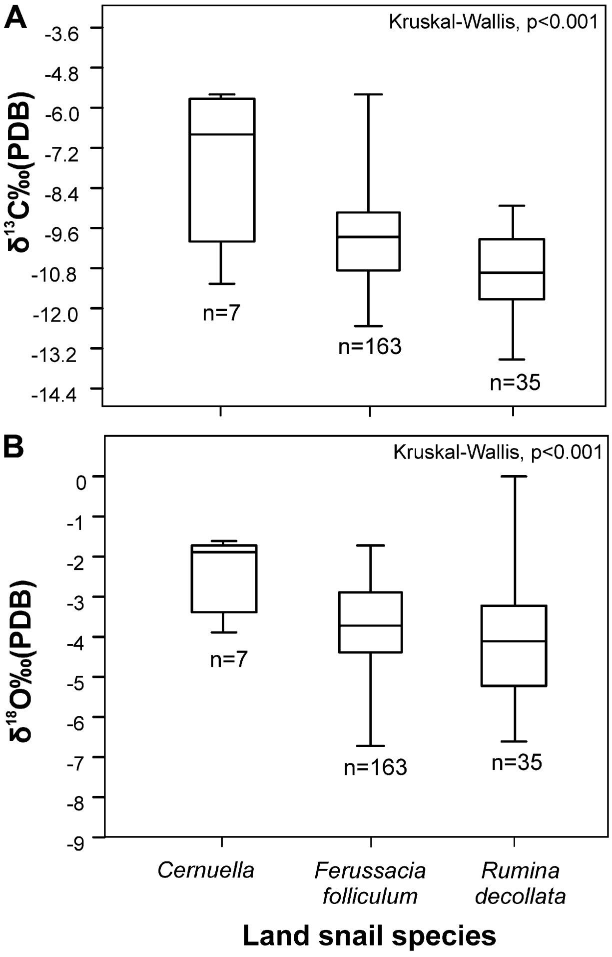

Fig. 3. Comparison of the carbon and oxygen isotope values of Neolithic land snail species recovered from the Los Castillejos archeological site, Montefrío (Granada, SE Spain). Boxes represent the 25–75% quartiles. Horizontal line inside the box is the median value. Whiskers show the minimum and maximum values. n indicates the number of shells analyzed.

6. Discussion

6.1. Paleoenvironmental significance of shell δ^{13}C values

Experimental and field approaches indicate that the δ^{13}C values of land snail shell are offset from the organic tissues by ~12–16‰ (Stott, 2002; Metref et al., 2003; ZongXiu et al., 2007; Yanes et al., 2008). Accordingly, the δ^{13}C values (from −13.6‰ to −5.7‰) of measured land snail shells indicate that the natural landscape in the study area was clearly dominated by C_3 plants over the last ~7200 years, whereas C_4 plants were rare, if present.

Although shells of *Cernuella* ($n = 24$) indicate that the carbon isotopic signature of the surrounding vegetation in Montefrío appears to have not changed substantially from ~7200 cal BP to the present (Table 1; Fig. 4A), the δ^{13}C values of *R. decollata* (δ^{13}C = −11.0 ± 1.2‰; $n = 35$) and *F. folliculum* (δ^{13}C = −9.9 ± 1.2‰; $n = 188$) were substantially lower during the Neolithic-Copper Age period than those of modern *Cernuella* (δ^{13}C = −8.4 ± 0.5‰, $n = 15$; Fig. 4A–C). Even though further research is needed to assure that local modern *F. folliculum* and *R. decollata* shells from Montefrío (Granada) record significantly higher δ^{13}C values than ancient shells of the same taxa, live-collected shells of *R. decollata* from Béznar (Granada), at 538 m a.s.l., display an average δ^{13}C value of −9.8 ± 0.5‰ ($n = 4$; unpublished data), which is significantly higher than Neolithic *R. decollata* shells from Montefrío (Mann–Whitney *U* test, $p = 0.046$).

There is a lack of evidence for C_4 plants in the study area. Although variations in the proportion of C_4 plant consumption cannot be completely ruled out, it appears to have been a minor controlling factor of the δ^{13}C value of local land snail shells. The higher δ^{13}C values of modern *Cernuella* shells compared to fossil *F. folliculum* and *R. decollata* shells (Fig. 4B–C) may be explained primarily by lower water stress conditions (i.e., greater water use efficiency in C_3 plants: Farquhar et al., 1989; Dawson et al., 2002) during the early to mid Holocene than today. This is consistent with the study of local C_3 domestic cereal grains recovered by Araus et al. (1997a, b) and Aguilera et al. (2008), who concluded that artificial irrigation practices were improbable during the Neolithic-Copper Age period and therefore, crops should have grown under significantly natural wetter conditions than today. Moreover, local wetter conditions during the early to mid Holocene have been inferred from pollen records in multiple sections from Granada, which exhibited generally higher abundances of several pine and oak species and a lower presence of arid-associated herbs and shrubs, with subsequent aridification from the mid Holocene to the present (e.g., Fernández et al., 2007; Carrión et al., 2007, 2010a,b).

The variations in the δ^{13}C values of the vegetation recorded in the snail shell can be overprinted by the ingestion of carbonate-rich sediments. The study area contains Jurassic marine limestone with a δ^{13}C value likely close to ~0‰, to which snails have been exposed. Because the amount of carbonates ingested by the snails vary among individuals (e.g., Goodfriend and Hood, 1983; Goodfriend, 1987; Goodfriend et al., 1999) it is not possible to quantify this potential contribution to the shell. Pigati et al. (2004, 2010) observed that various minute species of land snails generally incorporate negligible amounts of limestone into the shell. The species studied here can be classified in two groups based on their shell size. *F. folliculum* (width = 2.5–3.5 mm, height = 6–9 mm) is a minute species while *R. decollata* (width = 10–14 mm,

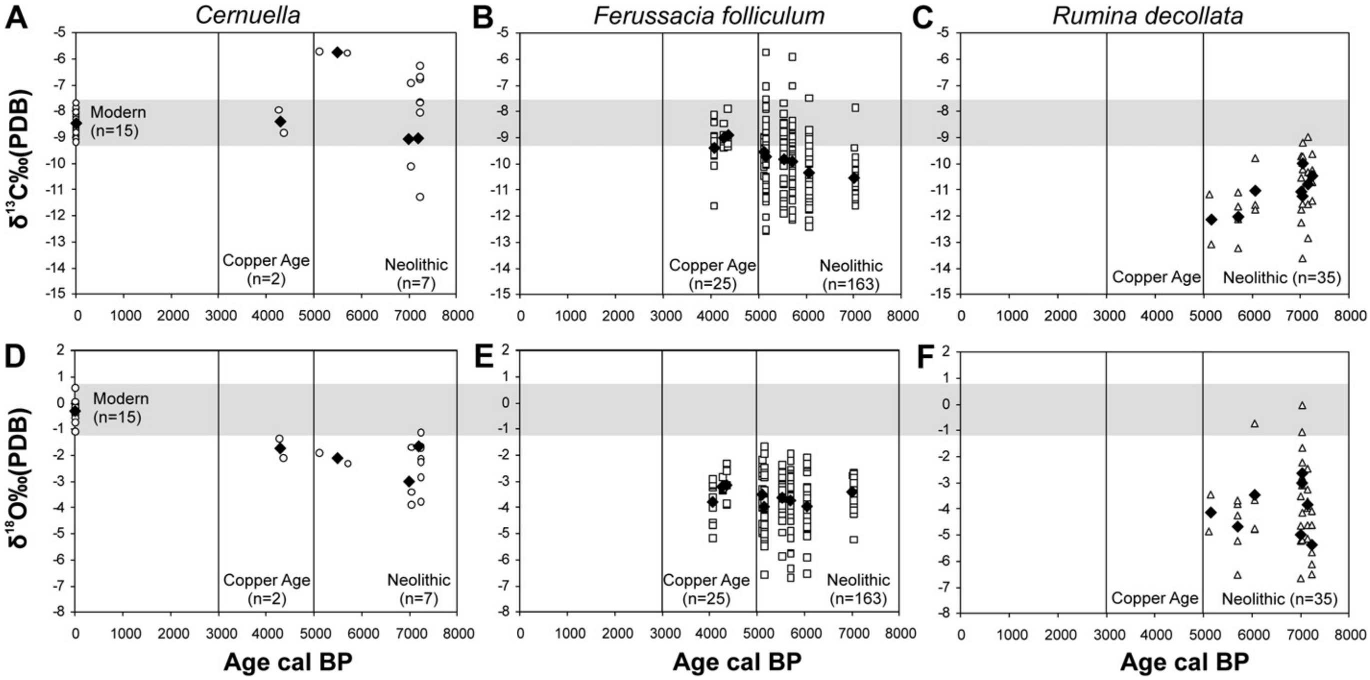

Fig. 4. Stable isotope composition of shells from the Los Castillejos archeological site, Montefrío (Granada, SE Spain) plotted separately by species. (A–C) Raw data (open symbols) and mean values (filled diamonds) of shell $\delta^{13}C$ values through time. (D–F) Raw data (open symbols) and mean values (filled diamonds) of shell $\delta^{18}O$ values through time. Gray band represents the range of modern carbon and oxygen isotope values of *Cernuella* shells.

height = 25–40 mm) and *Cernuella* (width = 12–16 mm, height = 9–13 mm) are comparatively large taxa. Consequently, preferential ingestion of limestone by large taxa cannot explain the observed difference in average $\delta^{13}C$ values between fossil *R. decollata* and *F. folliculum* shells and modern *Cernuella* shells (Fig. 4A–C).

Even though further research on modern individuals at Montefrío is needed to better constrain the dietary habits across species, the $\delta^{13}C$ values of land snail shells appear to suggest somewhat wetter conditions during the early to mid Holocene than today in the SE Iberian Peninsula. The results presented here also reinforce the contention that the $\delta^{13}C$ values of different land snail species from the Iberian Peninsula yield potentially valuable paleoecological information regarding species-specific feeding strategies (see also Colonese et al., 2010b).

6.2. *Environmental significance of recent shell $\delta^{18}O$ values*

The $\delta^{18}O$ values of live-collected shells (avg. $\delta^{18}O = -0.3 \pm 0.4‰$) are ~7‰ higher than the local weighted rain $\delta^{18}O$ value. This is consistent with previous field studies which have observed that land snail shell $\delta^{18}O$ values are higher than rain by ~4–8‰ (e.g., Goodfriend et al., 1989; Goodfriend and Ellis, 2002). Empirical and theoretical data indicate that such offsets are explained by a flux balance model where the shell precipitates in oxygen isotope equilibrium with body fluids that are fractionated due to body water loss via evaporation (Balakrishnan and Yapp, 2004). Accordingly, the $\delta^{18}O$ values of modern shells were evaluated by using the evaporative steady-state flux balance model by Balakrishnan and Yapp (2004). The model predicts the $\delta^{18}O$ values of the shell through the quantitative relationship between the amount and the $\delta^{18}O$ values of the input liquid water, the water from the snail body, the diffusive flux of water from the body through evaporation, and the temperature dependence oxygen isotope fractionation between the snail body water and aragonite (Grossman and Ku, 1986). Relative humidity (RH) and the $\delta^{18}O$ values of water vapor and the flux of liquid water output relative to the flux of liquid water input (θ) are also considered (Balakrishnan and Yapp, 2004). Model calculations assume that water vapor is in isotopic equilibrium with liquid water input and that body water is lost via evaporation ($\theta = 0$) (see Balakrishnan and Yapp, 2004 for further details).

The average temperature during the months when snails are expected to be active ($T = 16$ °C) and the weighted rain $\delta^{18}O$ values during snail growth ($\delta^{18}O = -7.5‰$ vs. SMOW) based on published data from Granada city (Delgado-Huertas et al., 1991), were used for model calculations, together with measured $\delta^{18}O$ values of modern shells from the Montefrío locality (Fig. 5A).

Model calculations for modern individuals are shown in Fig. 5A, where the gray band represents the range of measured shell $\delta^{18}O$ values and the filled circle shows the average value. The model output suggests that live-collected specimens from Montefrío precipitated their shell during average RH of ~0.83 (Fig. 5A). This value is approximately equivalent to the measured maximum RH during the months of snail activity (~0.82) recorded by the two meteorological stations closest to Montefrío (Fig. 2B). The equivalence of local maximum RH values and the predicted RH value indicates that the flux balance model by Balakrishnan and Yapp (2004) estimates reasonably well the current atmospheric conditions in SE Spain. Hence, this model was used to constrain the Holocene paleoenvironmental conditions in the study area.

6.3. *Paleoclimatic inferences of early to mid Holocene in SE Spain*

Regional published paleoclimatic proxies were used to approximate values of paleotemperature and the $\delta^{18}O$ value of ancient rain in SE Spain. Speleothem proxies from the eastern Mediterranean region have documented that the early to mid Holocene $\delta^{18}O$ values of rain were ~1‰ lower than modern values (e.g., Bar-Matthews et al., 1999, 2000). In contrast, western Mediterranean proxies generally exhibit a subdued average variation in rain $\delta^{18}O$ values due to the influence of North Atlantic waters, which appears to

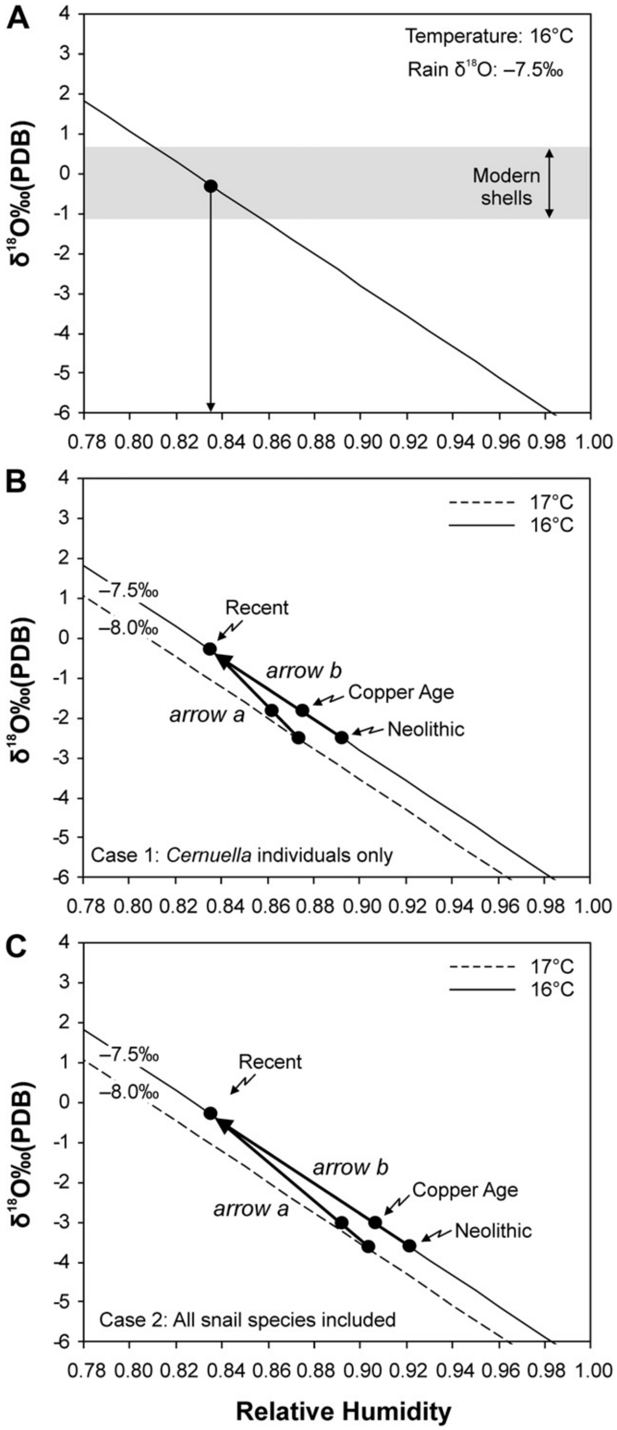

Fig. 5. Calculations of shell $\delta^{18}O$ values using the evaporative steady-state flux balance model of Balakrishnan and Yapp (2004), assuming isotopic equilibrium between rain water and water vapor. (A) Modern $\delta^{18}O$ values of snails from Montefrío, Granada. Gray band represents the range of values for live-collected *Cernuella* specimens. Weighted average $\delta^{18}O$ values of local rain water (−7.5‰ vs. SMOW: Delgado-Huertas et al., 1991) and average temperature at the time of snails' activity (16 °C) were used for model calculations. (B–C) Arrow "a" represents a possible paleoclimatic scenario where increasing $\delta^{18}O$ values of the shell are the result of decreasing RH, increasing rain $\delta^{18}O$ values from −8.0‰ to −7.5‰ and decreasing temperature from 17 °C to 16 °C (see text). Arrow "b" represents an alternative paleoclimatic scenario for increasing $\delta^{18}O$ values of the shell as a result of a progressive decrease in relative humidity, assuming steady temperature and rain $\delta^{18}O$ values. In plot B, only *Cernuella* specimens are considered. In plot C, all three land snail species are considered.

have not changed substantially over the last 10,000 years.The $\delta^{18}O$ values of early to mid Holocene benthic foraminifera from deep sea cores of the western Mediterranean region are ~0.5‰ lower than modern specimens (see Table 5 in Rohling and De Rijk, 1999). This indicates that ocean waters in the region were ~0.5‰ lower in $\delta^{18}O$ during the early to mid Holocene than today. Assuming that local rain water mimicked such ocean changes, the $\delta^{18}O$ values of rain were possibly ~0.5‰ lower in the study area during the Neolithic-Copper Age period than the present.

Sea surface temperature based on alkenone paleotemperature estimates from deep sea cores collected off the Iberian Peninsula in the North Atlantic and western Mediterranean suggest that temperatures were ~1 °C higher during the early to mid Holocene than currently (Cacho et al., 2001; Sbaffi et al., 2001; Bard, 2002; Marchal et al., 2002;).

According to the aforementioned paleoclimatic data, early to mid Holocene conditions in the western Mediterranean region appear to have been characterized by rain $\delta^{18}O$ values that were possibly ~0.5‰ lower than today (subdued compared to the eastern Mediterranean region) and temperatures that were ~1 °C higher than the present. Thus, for model calculations, early-mid Holocene (~7200–4000 cal BP in this study) temperature at the time of snail activity was assumed to be ~17 °C and the $\delta^{18}O$ value of rain was assumed to be −8.0‰ on average (Fig. 5B–C). On the other hand, observed modern average ambient temperature and $\delta^{18}O$ values for rain are ~16 °C and −7.5‰, respectively (Fig. 5A). These early-mid Holocene and modern paleoclimatic values were used in the model calculations.

Significant differences in $\delta^{18}O$ values of the shell were observed among the species studied (Fig. 3B). Accordingly, two case scenarios are evaluated. The first considers fossil and modern *Cernuella* specimens alone (Fig. 5B), whereas the second considers all three snail species (Fig. 5C).

Arrow "a" in Fig. 5B represents a possible trajectory of increasing shell $\delta^{18}O$ values as a function of declining RH from ~0.87 to ~0.83, declining temperature from ~17 °C–~16 °C and rising rain water $\delta^{18}O$ values from −8.0‰ to −7.5‰. An alternative paleoclimatic scenario is shown by arrow "b", where increasing shell $\delta^{18}O$ values from the early Holocene to the present are a function of declining RH from ~0.89 to ~0.83, assuming that temperature and rain $\delta^{18}O$ values remained steady throughout the Holocene in the study region. Both model-constrained paleoclimatic scenarios indicate that relative humidity decreased during the time when the snails grew their shells (Fig. 5B). However, the boundary conditions represented by arrow "a" are the most feasible scenario based on published regional paleoclimatic proxies.

When all land snail species are considered jointly (Fig. 5C), arrow "a" represents the early Holocene-present increasing $\delta^{18}O$ value for the shell, assuming that the $\delta^{18}O$ value for rain increased from −8.0‰ to −7.5‰ and temperature declined from ~17 °C–~16 °C. Under these conditions, average RH should have declined from ~0.91 in the Neolithic period to ~0.83 at present (Fig. 5C). Alternatively, if rain $\delta^{18}O$ values and temperature remained constant throughout the Holocene in the study area, increasing shell $\delta^{18}O$ values can be explained by a decline in RH from ~0.93 in the Neolithic period to ~0.83 today, as shown by arrow "b" in Fig. 5C. Considering that relative humidity is correlated with precipitation amount in the study area (Fig. 2), rainfall was likely enhanced during the Neolithic-Copper Age compared to the present day for the SE Iberian Peninsula.

Other land snail oxygen isotopic proxies from the Mediterranean region have documented similar trends over the Holocene. Land snail shells from Grotta della Serratura around 7300 cal BP (Colonese et al., 2010a), and Grotta di Latronico 3 (both in Southern Italy) around 8200–8800 cal BP (Colonese et al., 2010b) had significantly lower $\delta^{18}O$ values than those from today in response to enhanced rainfall. Additionally, early to mid Holocene land snail shells from the northern Negev desert (southern Israel) also displayed lower $\delta^{18}O$ values than modern specimens as a result of the increasing frequency of storms in the area (Goodfriend, 1991). Furthermore, multiple paleoclimatic proxies from North Africa have documented that conditions were considerably wetter during the early to mid Holocene than the present (e.g., Gasse, 2000; deMenocal et al., 2000; Tjallingii et al., 2008). In addition, lake multi-proxies from the Iberian Peninsula have also documented enhanced precipitation during the early Holocene (e.g., Morellón et al., 2009 and references therein).

Considered collectively, the environmental conditions inferred from the measured $\delta^{18}O$ values of land snails in this study are in agreement with other published regional and local proxies, emphasizing the utility of land snail shells for paleoenvironmental studies in the Iberian Peninsula. This study also highlights the potential use of $\delta^{18}O$ values of land snail shells to estimate relative humidity variations in ancient atmospheres of the Mediterranean region.

7. Conclusions

The Los Castillejos archeological site at Montefrío, Granada (SE Spain), contains Neolithic and Copper Age (~7200–~4000 cal BP) land snail shells from multiple species. Fossil and modern shell $\delta^{13}C$ values (−13.6‰ to −5.7‰) indicate that snails consumed mostly C_3 plants (and minor amounts of local limestone) while C_4 plant consumption was mostly negligible. Most fossil shells generally showed significantly lower $\delta^{13}C$ values than modern shells, suggesting wetter conditions and lower water stress during the early to mid Holocene than the present. However, further research on modern specimens of multiple species from the SE Iberian Peninsula is needed to better constrain the breadth of dietary habits possible. Shell $\delta^{18}O$ values from all species increased significantly from the Neolithic period to the present, primarily as a response to a decline in relative humidity, which suggests the SE Iberian Peninsula was wetter (and rainfall was probably enhanced) during the early to mid Holocene than today. The approach presented here is consistent with other published regional and local paleoclimatic proxies, and reinforces the potential use of land snail shells in paleoenvironmental studies.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.quaint.2011.04.031.

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