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Stable isotope composition of middle to late Holocene land snail shells from the Marroquíes archeological site (Jaén, southern Spain): Paleoenvironmental implications

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ABSTRACT

The Marroquíes archeological site, located in the metropolitan area of Jaén (37°46′), southern Spain, contains land snail shells which have been preserved jointly with other human and zooarcheological remains. New radiocarbon analyses carried out on pristine domestic animal and human bones confirmed that these remains belong to the Copper Age interval (~4470–3880 cal BP; $n = 8$). Land snail entire shells of two species (one herbivorous and one omnivorous) were analyzed for $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios to estimate the paleoenvironmental conditions prevailing during the middle to late Holocene in the southernmost part of Europe. The $\delta^{13}C$ values of fossil shells ranged from −13.8‰ to −8.1‰ ($n = 15$), whereas modern specimens ranged from −10.8‰ to −8.6‰ ($n = 20$). The fact that ancient and modern shells generally exhibited similar $\delta^{13}C$ values suggests that the $\delta^{13}C$ values of the vegetation have remained relatively stable during ~4470–3880 cal BP and the present. Snail species did not differ in $\delta^{13}C$ values despite their differing dietary habits. The $\delta^{18}O$ values of fossil shells ranged from −4.9‰ to −1.2‰ ($n = 15$), whereas modern specimens ranged from −2.8‰ to +0.9‰ ($n = 20$). Fossil shells were, on average, ~2‰ lower in $\delta^{18}O$ values than modern shells. Calculations from a snail evaporative steady-state flux balance model suggest that shells at ~4470–3880 cal BP precipitated under appreciably higher relative humidity conditions than today, whereas rain $\delta^{18}O$ values and air temperatures at the soil–air interface during snail active period were possibly similar. Although samples from this study represent a time-period in which the well-documented late Holocene aridification trend in southern Spain already started, snails suggest conditions at ~4470–3880 cal BP were yet noticeably wetter than at present. This study shows that land snail shells preserved in Holocene archeological sites offer valuable paleoenvironmental information in their isotope codes.

1. Introduction

Zooarcheological remains are valuable retrospective archives not only for inferring past socio-economic strategies followed by ancient human groups, but also for reconstructing past environmental/climatic conditions. Land snail shells are frequently abundant and well-preserved in many archeological sites; however, their study is still considerably underrepresented in the published literature. Some studies have revealed that land snails have been an important food resource for many civilizations (e.g., Lubell, 2004a,b; Gutiérrez-Zugasti, 2011). Other studies observed that even when no evidence of human exploitation, land snail shells preserved naturally (non-human induced) in archeological sites lived simultaneously with other archeological remains and therefore, are useful paleoenvironmental bioindicators (e.g., Colonese et al., 2007, 2010a,b, 2011; Yanes et al., 2011a). Human-exploited or not, land snails recovered from archeological sites are alternative environmental records which can reinforce or add new insights into past continental climates. One of the best direct geochemical proxies employed to reconstruct past environmental conditions from land snails is the study of stable isotope signatures recorded in their aragonitic shells. The carbon stable isotope values ($\delta^{13}C$) of the shell largely reflect the $\delta^{13}C$ values of the consumed plants (Stott, 2002; Metref et al., 2003). Because most land snails are generalized herbivorous (i.e., snails are assumed to consume plants indiscriminately in the proportion of their availability in the

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landscape), they have been used to infer variations in the relative abundance of C_3, C_4 and CAM plants (e.g., Goodfriend and Ellis, 2000, 2002; Balakrishnan et al., 2005a,b; Baldini et al., 2007; Yanes et al., 2008, 2009, 2011b). In landscapes where C_3 plants are dominant, for instance Europe, where less than 5% of plant species are C_4 (Collins and Jones, 1986), shell $\delta^{13}C$ values may reflect water stress conditions in C_3 plants (e.g., Colonese et al., 2007, 2010a,b, 2011; Yanes et al., 2011a). The oxygen stable isotope composition ($\delta^{18}O$) of land snail shells records the rain $\delta^{18}O$ values and the air temperature (e.g., Lécolle, 1985; Goodfriend, 1991; Zanchetta et al., 2005; Baldini et al., 2007; Yanes et al., 2008, 2009), in addition to the water vapor $\delta^{18}O$ values and the relative humidity (Yapp, 1979; Goodfriend et al., 1999; Balakrishnan and Yapp, 2004). However, a recent clumped isotope study on modern land snails suggests that calcification appears to occur at body temperatures several degrees Celsius higher than observed ambient temperatures in mid to high latitudes, e.g., caused by the sunlight-warm effect (Zaarur et al., 2011). Despite inherent difficulties, the $\delta^{18}O$ values of fossil shells have been increasingly employed to reconstruct environmental conditions of ancient atmospheres at the soil–air interface during the snail calcification period (see recent publications in Balakrishnan et al., 2005b; Colonese et al., 2007, 2010a,b, 2011; Kehrwald et al., 2010; Yanes et al., 2011a,b).

In the Iberian Peninsula, the southernmost part of Europe, many archeological sites contain rich accumulations of land snails yet poorly studied (but see Gutiérrez-Zugasti, 2011; Yanes et al., 2011a). In particular, the Marroquíes archeological site, situated in Jaén (Andalucía, Southern Spain), contains well-preserved land snail shells which represent a useful material available for exploring paleoenvironmental conditions in the southern Iberian Peninsula. This area is interesting from a paleoclimatic perspective because is affected by interactions between atmospheric and oceanic circulation systems from the North Atlantic and Mediterranean regions, and the indirect effects of the African and Asian monsoons (e.g., Moreno et al., 2005; Roberts et al., 2011). Holocene continental paleoenvironmental conditions in southern Spain have been primarily studied from lake sediments (e.g., Anderson et al., 2011; Jiménez-Moreno and Anderson, 2012) and pollen records (e.g., Carrión et al., 2001, 2010). Therefore, new and complementary continental proxies from the study area are yet necessary to improve understanding in the local and regional Holocene environmental change, which in turn, is important for the global outlook. Thus, this paper presents an additional proxy using the isotopic fingerprint of ancient land snail shells, a continental proxy vastly under-exploited in the study area (but see Yanes et al., 2011a).

The present study firstly analyzed various bone samples by AMS radiocarbon dating to corroborate the chronology of the Marroquíes archeological site, and secondly, studied the carbon and oxygen stable isotope composition of both modern and fossil land snail shells to infer paleoenvironmental conditions during the middle Holocene in southern Iberian Peninsula. The isotopic results are explained using a snail evaporative steady-state flux balance mixing model and compared with other well-established regional paleoclimatic proxies.

2. Background

2.1. Geographical and environmental context

Jaén is a city of Andalucía, Southern Spain, sited at 37°46′N (Fig. 1) and at an elevation of 573 m above sea level (a.s.l.). The locality exhibits a Mediterranean type climate with wet and cool winters and warm and dry summers (Fig. 2A). Climatic data was obtained from the meteorological station of Jaén managed by the Junta de Andalucía between 2008 and 2011 recording period (www.juntadeandalucia.es). Mean monthly temperatures range from 7.5 ± 2.7 °C in January to 27.9 ± 2.0 °C in July. Mean temperature during snail active period (from March to May, and from September to November) is ~16 °C. Total monthly precipitation varied from ~30 mm in August to ~464 mm in December (Fig. 2A). Precipitation during the snail active seasons is ~214 mm. Maximum relative humidity ranges from 63 ± 10% in July to 94 ± 11% in November (Fig. 2B). Average maximum relative humidity at the time when snails are assumed to be most active is ~89%.

Delgado-Huertas et al. (1991) studied the oxygen isotope composition of rain water from Granada (~90 km south from Jaén) between 1988 and 1991. The mean monthly $\delta^{18}O$ values of rain varied from −9.7‰ (SMOW) in February to −2.5‰ (SMOW) in September (Fig. 2B). The weighted annual mean $\delta^{18}O$ value of the rain in Granada is −7.5‰ (SMOW). The mean $\delta^{18}O$ value of rain during the periods at which snails are mainly active in Jaén is ~−6‰ (SMOW).

2.2. Archeological context

The Marroquíes archeological site of Jaén embraces late Neolithic and mostly Copper Age cultivate, dwelling and necropolis zones organized in an overall circular disposition surrounded by walls and ditches, occupying a maximum surface of over ~1–2 km² (e.g., Zafra-de-la-Torre et al., 1999, 2003). This spectacular ancient human settlement contains abundant cultural and architecture remains, human and domestic animal bones (e.g., Riquelme-Cantal, 2010), and charcoal derived primarily from several C_3 species such as *Fraxomis* and *Olea* (Olaceae), *Quercus* (Fagaceae) and *Arbustus* (Ericaceae) (e.g., Rodríguez-Ariza, 2005, 2011). Although the Marroquíes site was known since the 1950s (Espantaleón-Jubes, 1957, 1960), new areas of this site were discovered (and subsequently destroyed) during the intense urban development of Jaén since the 1990s (e.g. Lizcano et al., 2004). Different areas of the Marroquíes site have been recently excavated during the construction of a tram system in Jaén between 2008 and 2011. This newly studied material includes domestic huts, fortification ditches and human burials (Fig. 1B–E), all of them containing remains of domestic animals (i.e., dog, pig, ovicaprid, cow and horse) and terrestrial gastropods. While human and cattle remains have received considerable attention from the scientific community, ancient mollusks from the Marroquíes site have never been studied. Land snails do not exhibit evidence of human gathering or consumption (e.g., specific break patterns or gathering/consuming tool-derived marks on shells), and therefore, they are considered to have been preserved naturally, without anthropogenic involvement.

3. Material and methods

3.1. Sample

Eight bones (five human and three animal bones) recovered from three collective graves from Subestación (Fig. 1B–C), Paseo Estación (Fig. 1D–E) and García Triviño zones were selected for radiocarbon dating of the bone collagen (Table 1). Radiocarbon analyses were carried out by AMS in the Angström Laboratory of the University of Uppsala (Sweden). Radiocarbon dates were calibrated using the CalPal programme and the HULU 2007 calibration curve (Weninger and Jöris, 2008; Weninger et al., 2008).

Fossil land snails were found completely buried at two dwelling areas of Subestación and Paseo Estación zones jointly with other archeological remains. No definite stratigraphic layers were

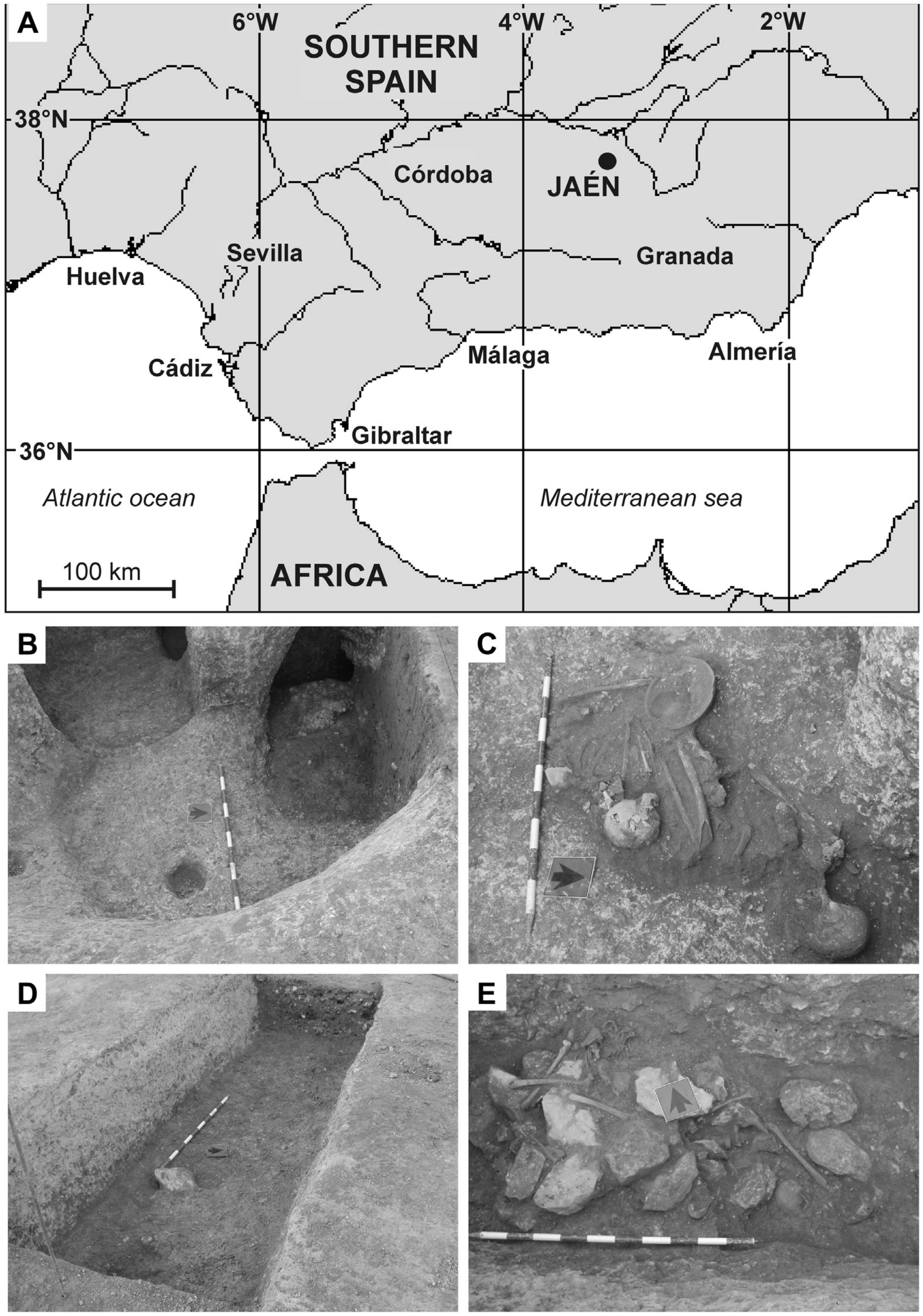

Fig. 1. Geographical location and photographs of the Marroquíes archeological site (Jaén, Andalucía, southern Spain). (A) Map of southern Spain indicating the situation of Jaén city. (B–E) Photographs of graves and detailed views of bone remains from Subestación (B–C) and Paseo Estación (D–E) areas of the Marroquíes archeological site. Scale bar = 1 m.

identified and consequently, ancient shell material used in this study is considered to have been deposited and preserved together at similar time and conditions, representing one single time-interval. Taphonomic and archeological evidence suggests shells were preserved in situ, without past or recent anthropogenic disturbance. A total of five fossil shells of the omnivorous *Rumina decollata* (Linnaeus, 1758) and ten ancient shells of the herbivorous *Cernuella virgata* (Da Costa, 1778) were collected for subsequent isotopic study. X-ray diffraction analyses carried out on three shells (one *Rumina* and two *Cernuella*) at the Instituto Andaluz de Ciencias de la Tierra (CSIC-Universidad de Granada) confirmed that the shell material recovered from the Marroquíes archeological site was 100% aragonitic and did not show evidence of recrystallization.

Modern specimens of *Rumina* ($n = 4$) and *Cernuella* ($n = 16$) from Jaén and Granada (Table 2) were also gathered and analyzed to establish a modern snail isotopic baseline against which fossil shells can be compared with. *C. virgata* (Gastropoda: Hygromiidae) is a generalized herbivorous air-breading snail. Its geographical range includes the Atlantic European coasts (from the Netherlands to Spain) and coastal regions of the Mediterranean region. This

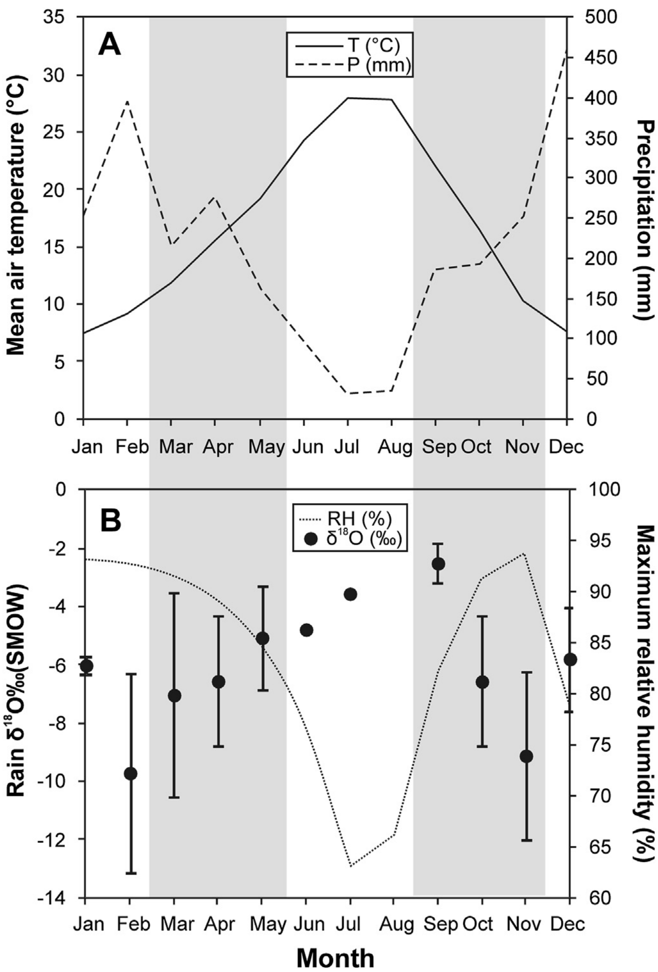

Fig. 2. Current climatic context of Jaén, southern Spain. Data taken from a meteorological station of Jaén regulated by the Junta de Andalucía (www.juntadeandalucia.es). Data represent climatic variations between 2008 and 2011 recording periods. (A) Monthly variation of air temperature (°C) and precipitation (mm). (B) Monthly variation of maximum relative humidity (%) and $\delta^{18}O$ values of the rain from Granada (Delgado-Huertas et al., 1991). Gray bands represent plausible active periods when snails are thought to be active and mostly precipitate shell material.

species generally resists dry habitats and prefers calcareous and anthropogenic substrates (e.g., Kerney and Cameron, 1979).

R. decollata (Gastropoda: Subulinidae) is an omnivorous snail natural from the Mediterranean but introduced in other continents, usually tolerant of dry and human-influenced habitats (e.g., Kerney and Cameron, 1979). *Rumina* feeds upon common garden snails and slugs and their eggs, annelids and plant matter. It has been used as a biological pest control to reduce populations of herbivorous snails that feed upon crops (e.g., Cowie, 2001).

3.2. Stable isotope analyses

Sample preparation and stable isotope analyses were carried out in the Instituto Andaluz de Ciencias de la Tierra (CSIC-Universidad de Granada). Each entire shell was cleaned ultrasonically in deionized water and crushed by hand with an agate mortar and pestle. Shell organic matrix was removed by placing the shell powder in 6% H_2O_2 overnight at room temperature. Thereafter, shells were rinsed three times with deionized water and oven-dried at 50 °C overnight.

Shell carbonate powder ($\sim$5 mg) was placed in a 12 ml exetainer™ vial and flushed with helium. The carbonate was converted to CO_2 by adding $\sim$0.2 ml of 100% phosphoric acid (H_3PO_4) at 50 °C during 24 h. The resulting CO_2 was analyzed isotopically using the Gas-Bench II and a Finnigan Delta PLUS^{XP} isotope ratio mass spectrometer (IRMS). All stable isotope results are reported in δ notation relative to the international standard 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 of the shell were calibrated against three in-house standards. The precision of the analyses was $\sim$0.1‰ (1σ standard deviation) for both isotopes based on the repeated measurement ($n = 30$) of standards. Replicate analyses of the same snail shell had an overall precision of $\sim$0.2‰ (1σ standard deviation) for $\delta^{18}O$ and $\delta^{13}C$.

3.3. Statistical analyses

Simple regression analyses were carried out to test the potential relationship between variables, and to identify the slope and intercept of the relationship. Mann–Whitney *U* test was used to test if samples differed in median values. Statistical analyses were performed using PAST 1.38b (Hammer et al., 2001), considering the significant level when $\alpha = 0.05$.

3.4. Snail evaporative steady-state flux balance mixing model for $\delta^{18}O$

Balakrishnan and Yapp (2004) developed a steady-state flux balance mixing model to better understand quantitatively the environmental controls on $\delta^{18}O$ values of land snail shells. The model portrays the relationship between the amount and isotopic composition of rain and the water in snail hemolymph, the diffusive flux of water from snail hemolymph by evaporation, and the temperature dependent oxygen isotope fractionation between snail hemolymph and aragonitic shell (Grossman and Ku, 1986). In this model, (1) air temperature, (2) $\delta^{18}O$ values of rain water (imbibed by the snail), (3) $\delta^{18}O$ values of water vapor, and (4) relative humidity are the most important environmental factors governing

Table 1
Radiocarbon results of bone collagen samples recovered from Subestación, Paseo Estación and García Triviño areas of the Marroquíes archeological site (Jaén, southern Spain).

Laboratory code	Sampling area	Sample type	^{14}C age (BP)	SD (±)	Calibrated age (cal BP)	SD (±)
Ua40052	García Triviño	Human rib	3830	30	4250	70
Ua40059	García Triviño	Dog	3980	40	4470	40
Ua40056	Paseo Estación	Human skull	3750	30	4090	60
Ua40057	Paseo Estación	Human skull	3790	30	4180	50
Ua40760	Paseo Estación	Ovicaprid?	3570	30	3880	40
Ua40763	Paseo Estación	Ovicaprid?	3590	30	3900	40
Ua40761	Subestación	Human tibiae?	3680	30	4020	50
Ua40762	Subestación	Human tibiae?	3720	30	4070	60

Table 2
Carbon and oxygen stale isotope values of modern and fossil land snail shells from the Marroquíes archeological site (Jaén, southern Spain).

Sample ID	Sampling locality	Geographical coordinates	Altitude	Species	Feeding habit	Age (cal BP)	δ^{18}O‰ (PDB)	δ^{13}C‰ (PDB)
CAZ-1-1	Cazorla, Jaén	37°54′N	826	*Cernuella virgata*	Herbivorous	Modern	−0.4	−9.6
CAZ-1-2	Cazorla, Jaén	37°54′N	826	*Cernuella virgata*	Herbivorous	Modern	−1.7	−10.4
CAZ-1-3	Cazorla, Jaén	37°54′N	826	*Cernuella virgata*	Herbivorous	Modern	−1.0	−9.0
CAZ-1-4	Cazorla, Jaén	37°54′N	826	*Cernuella virgata*	Herbivorous	Modern	−0.2	−10.2
CAZ-1-5	Cazorla, Jaén	37°54′N	826	*Cernuella virgata*	Herbivorous	Modern	−0.9	−10.7
CAZ-1-6	Cazorla, Jaén	37°54′N	826	*Cernuella virgata*	Herbivorous	Modern	−0.4	−9.3
CAZ-1-7	Cazorla, Jaén	37°54′N	826	*Cernuella virgata*	Herbivorous	Modern	−1.5	−10.8
CAZ-1-8	Cazorla, Jaén	37°54′N	826	*Cernuella virgata*	Herbivorous	Modern	0.4	−8.6
CAZ-1-9	Cazorla, Jaén	37°54′N	826	*Cernuella virgata*	Herbivorous	Modern	−1.9	−10.8
CAZ-1-10	Cazorla, Jaén	37°54′N	826	*Cernuella virgata*	Herbivorous	Modern	−2.6	−9.9
LEC-1-1	Lecrín, Granada	36°55′N	538	*Cernuella virgata*	Herbivorous	Modern	−0.4	−10.1
LEC-1-2	Lecrín, Granada	36°55′N	538	*Cernuella virgata*	Herbivorous	Modern	0.9	−10.1
LEC-1-3	Lecrín, Granada	36°55′N	538	*Cernuella virgata*	Herbivorous	Modern	−2.0	−10.5
LEC-1-4	Lecrín, Granada	36°55′N	538	*Cernuella virgata*	Herbivorous	Modern	0.9	−10.1
LEC-1-5	Lecrín, Granada	36°55′N	538	*Cernuella virgata*	Herbivorous	Modern	−0.1	−9.9
LEC-1-6	Lecrín, Granada	36°55′N	538	*Cernuella virgata*	Herbivorous	Modern	−2.8	−10.5
LEC-2-1	Lecrín, Granada	36°55′N	538	*Rumina decollata*	Omnivorous	Modern	−1.1	−9.2
LEC-2-2	Lecrín, Granada	36°55′N	538	*Rumina decollata*	Omnivorous	Modern	−1.6	−10.4
LEC-2-3	Lecrín, Granada	36°55′N	538	*Rumina decollata*	Omnivorous	Modern	−1.8	−9.6
LEC-2-4	Lecrín, Granada	36°55′N	538	*Rumina decollata*	Omnivorous	Modern	−1.5	−10.0
MQ-1-1	City center, Jaén	37°46′N	573	*Cernuella virgata*	Herbivorous	4470–3880	−4.9	−9.0
MQ-1-2	City center, Jaén	37°46′N	573	*Cernuella virgata*	Herbivorous	4470–3880	−2.6	−10.9
MQ-1-3	City center, Jaén	37°46′N	573	*Cernuella virgata*	Herbivorous	4470–3880	−1.2	−10.0
MQ-1-4	City center, Jaén	37°46′N	573	*Cernuella virgata*	Herbivorous	4470–3880	−3.4	−13.8
MQ-1-5	City center, Jaén	37°46′N	573	*Cernuella virgata*	Herbivorous	4470–3880	−1.2	−10.9
MQ-2-1	City center, Jaén	37°46′N	573	*Rumina decollata*	Omnivorous	4470–3880	−3.1	−10.9
MQ-2-2	City center, Jaén	37°46′N	573	*Rumina decollata*	Omnivorous	4470–3880	−3.3	−10.6
MQ-2-3	City center, Jaén	37°46′N	573	*Rumina decollata*	Omnivorous	4470–3880	−4.3	−9.4
MQ-2-4	City center, Jaén	37°46′N	573	*Rumina decollata*	Omnivorous	4470–3880	−3.8	−9.5
MQ-2-5	City center, Jaén	37°46′N	573	*Rumina decollata*	Omnivorous	4470–3880	−4.0	−11.4
MQ-2-6	City center, Jaén	37°46′N	573	*Rumina decollata*	Omnivorous	4470–3880	−2.5	−10.6
MQ-2-7	City center, Jaén	37°46′N	573	*Rumina decollata*	Omnivorous	4470–3880	−2.3	−10.8
MQ-2-8	City center, Jaén	37°46′N	573	*Rumina decollata*	Omnivorous	4470–3880	−4.0	−11.2
MQ-2-9	City center, Jaén	37°46′N	573	*Rumina decollata*	Omnivorous	4470–3880	−2.0	−8.1
MQ-2-10	City center, Jaén	37°46′N	573	*Rumina decollata*	Omnivorous	4470–3880	−2.2	−11.5

δ^{18}O values of the snail hemolymph and in turn, the shell (Balakrishnan and Yapp, 2004). Land snail body water is considered to have been lost only by evaporation ($\theta = 0$). Ambient water vapor is assumed to be in isotopic equilibrium with rain water (Balakrishnan and Yapp, 2004). If air temperature and local rain water δ^{18}O values are known or can be indirectly assumed (e.g., various commonly used paleoclimatic proxies reconstruct such variables), relative humidity during calcification at the soil–air interface may be predicted using this model (e.g. Balakrishnan et al., 2005a,b).

4. Results

Radiocarbon results indicate that Subestación, Paseo Estación and García Triviño areas within the Marroquíes archeological site ranged in age from ~4470 to ~3880 cal BP (Table 1), with an average value of 4110 ± 190 cal BP ($n = 8$). These new dates are consistent with values reported for other parts of the Marroquíes site (e.g., Zafra-de-la-Torre et al., 1999, 2003). Accordingly, archeological land snails recovered from these areas should have lived and grew their shells during the Copper Age interval. Radiocarbon dates reveal that snail shells from this study probably exhibit an age-mixing of ~590 y. This magnitude of time-averaging is expected for terrestrial shelly accumulations (Yanes et al., 2007).

Modern shells of *C. virgata* ($n = 16$) from the study area exhibit δ^{13}C values ranging from −10.8‰ to −8.6‰, with an average value of −10.0 ± 0.6‰ (Table 2; Fig. 3A). *R. decollata* shells ($n = 4$) ranged in δ^{13}C values from −10.4‰ to −9.2‰, averaging −9.8 ± 0.5‰ (Table 2; Fig. 3A). Thus, both species showed similar δ^{13}C values (Mann–Whitney *U* test, $U = 22$; $p = 0.37$). Fossil shells of *C. virgata* ($n = 5$) exhibited an average δ^{13}C value of −10.9 ± 1.8‰, ranging from −13.8‰ to −9.0‰ (Table 2; Fig. 3A). Ancient *R. decollata* shells ($n = 10$) showed an average δ^{13}C value of −10.4 ± 1.1‰, ranging from −11.5‰ to −8.1‰ (Table 2; Fig. 3A). Both species did not differ in shell δ^{13}C values (Mann–Whitney *U* test, $U = 23$; $p = 0.85$). Fossil shells (−10.6 ± 1.3‰; $n = 15$) were significantly lower in δ^{13}C values (Mann–Whitney *U* test, $U = 89$; $p = 0.043$) than modern specimens (−10.0 ± 0.6‰; $n = 20$). However, if the outlier of −13.8‰ is disregarded (Fig. 3A), no differences in shell δ^{13}C values are obtained between fossil and modern specimens. The dispersion of δ^{13}C values of both species was relatively comparable (Fig. 3A). Carbon and oxygen isotopic systematics of modern and fossil land snails from Jaén did not correlate with each other.

Modern *C. virgata* shells ($n = 16$) varied in δ^{18}O values between −2.8‰ and −0.9‰, with an average value of −0.9 ± 1.1‰ (Table 2; Fig. 3B). Modern shells of *R. decollata* ($n = 4$) exhibited an average δ^{18}O value of −1.5 ± 0.3‰, ranging from −1.8‰ to −1.1‰ (Table 2; Fig. 3B). The δ^{18}O values of the two snail species were statistically comparable (Mann–Whitney *U* test, $U = 20.5$; $p = 0.30$). However, *Rumina* exhibited narrower amplitude of δ^{18}O values than *Cernuella*, possibly due to a more selective active period during the wettest conditions and lower exposure to dryness (e.g., Yanes et al., 2009). Shells of fossil *C. virgata* ($n = 5$) displayed a range of δ^{18}O values from −4.9‰ to −1.2‰, averaging −2.7 ± 1.5‰ (Table 2; Fig. 3B), whereas fossil *R. decollata* ($n = 10$) varied in δ^{18}O values between −4.3‰ and −2.0‰, with an average value of −3.1 ± 0.9‰ (Table 2; Fig. 3B). The δ^{18}O values of both species were statistically equivalent (Mann–Whitney *U* test, $U = 20$; $p = 0.58$). Fossil specimens (−3.0 ± 1.0‰; $n = 15$) were significantly lower in δ^{18}O values (Mann–Whitney *U* test, $U = 28$; $p < 0.001$) than modern

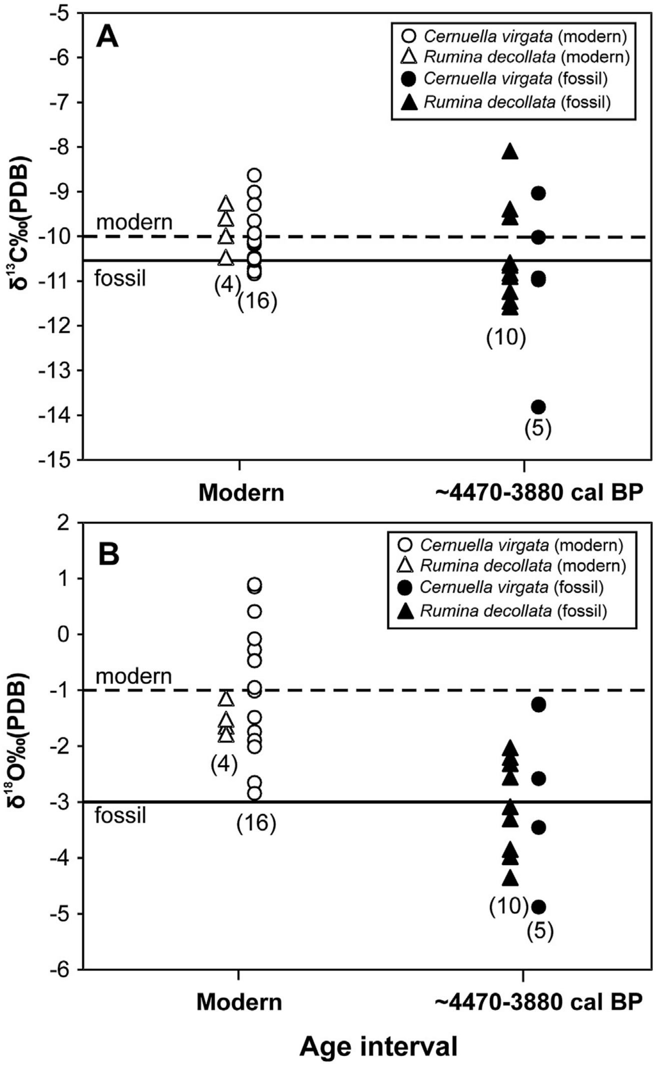

Fig. 3. Stable isotope composition of modern and fossil shells from the Marroquíes archeological site (Jaén, southern Spain). (A) Carbon stable isotope values of the shell. (B) Oxygen stable isotope value of the shell. Solid lines represent the average value of fossil shells whereas dashed lines represent the average value of modern shells. Numbers between brackets indicate the number of shells analyzed. Note that both species exhibit comparable carbon and oxygen stable isotope values.

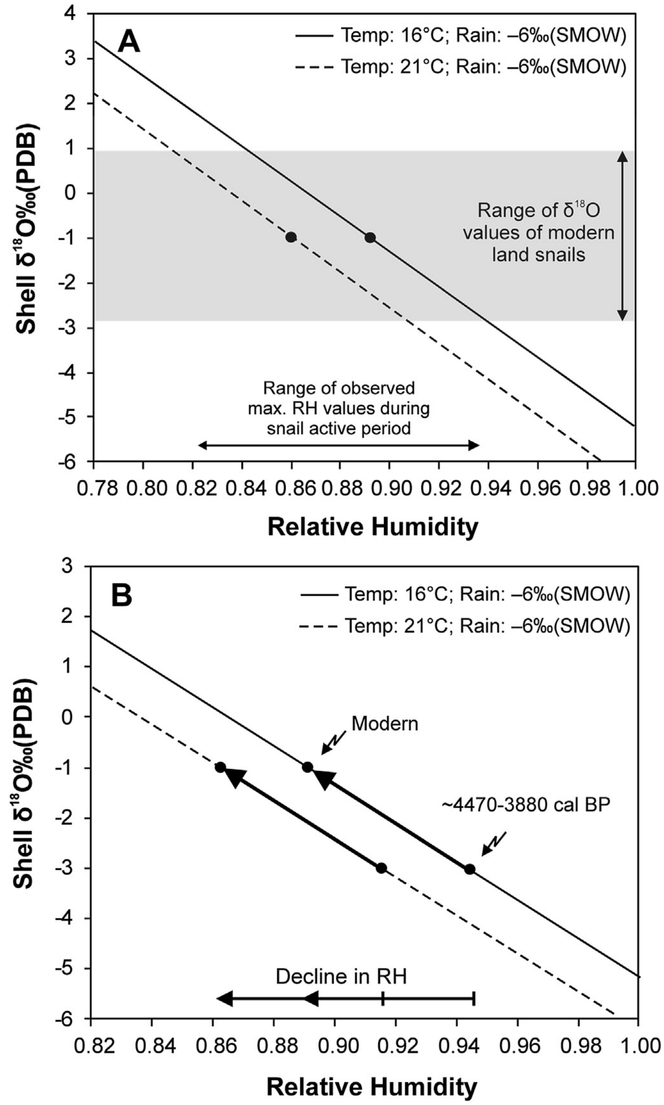

Fig. 4. Calculated shell $\delta^{18}O$ values using the snail evaporative steady-state flux balance mixing model by Balakrishnan and Yapp (2004). (A) Modern scenario. The shaded area depicts the range of measured shell $\delta^{18}O$ values of modern individuals from the study area whereas filled dots illustrate the average value. Note that empirical and hypothetical relative humidity values overlap. (B) Fossil scenario. Arrows show hypothetical trajectories of increasing average shell $\delta^{18}O$ values from the middle to late Holocene to the present as a function of decreasing relative humidity, assuming constant values for air temperature and rain water $\delta^{18}O$ values (see text). Solid line represents calculated shell $\delta^{18}O$ values if calcification occurred at ambient temperature during snail active period whereas dashed line portrays calculated shell $\delta^{18}O$ values if calcification occurred at temperatures ∼5 °C warmer than those from the environment (Zaarur et al., 2011).

individuals (-1.0 ± 1.1‰; $n = 20$). Snail shells of both species displayed similar amplitude of $\delta^{18}O$ values (Fig. 3B).

Calculations from the snail evaporative steady-state flux balance mixing model by Balakrishnan and Yapp (2004) suggest that modern individuals from Jaén and Granada having shell $\delta^{18}O$ values of -1.0 ± 1.0‰ precipitated carbonate during air temperatures of ∼16 °C, rain water values of −6‰(SMOW), and relative humidity of ∼89 ± 3%. These adopted temperature and rain $\delta^{18}O$ values for model calculations assume that snails mostly deposit shells during spring (from March to May) and fall (from September to November) seasons (Fig. 2). If calcification occurred at temperatures ∼5 °C higher than environmental temperatures (e.g., due to the sunlight-warm effect), as suggested by a recent clumped isotope study on modern land snails (see figure 3 in Zaarur et al., 2011), and the other variables remain constant, modern shells precipitated at relative humidity values of ∼86 ± 3% (Fig. 4A).

Model outputs indicate that fossil shells with an average measured $\delta^{18}O$ value of -3.0 ± 1.1‰ (Fig. 5A) deposited shell at times when ambient temperature and rain $\delta^{18}O$ values were likely similar than today (see discussion below), as suggested by several published regional paleoclimatic proxies (see Fig. 5B–C), but relative humidity was ∼95 ± 3% (Fig. 4B). If shell precipitated under temperatures ∼5 °C higher than observed environmental temperatures (Zaarur et al., 2011), fossil shells possibly grew under relative humidity values of ∼92 ± 3% (Fig. 4B).

5. Discussion

5.1. Paleovegetation inferences: shell $\delta^{13}C$ values

Both species of land snail exhibited statistically equivalent $\delta^{13}C$ values (Table 2; Fig. 3A) and therefore, results will be discussed using pooled data of both taxa. The $\delta^{13}C$ values of the shell

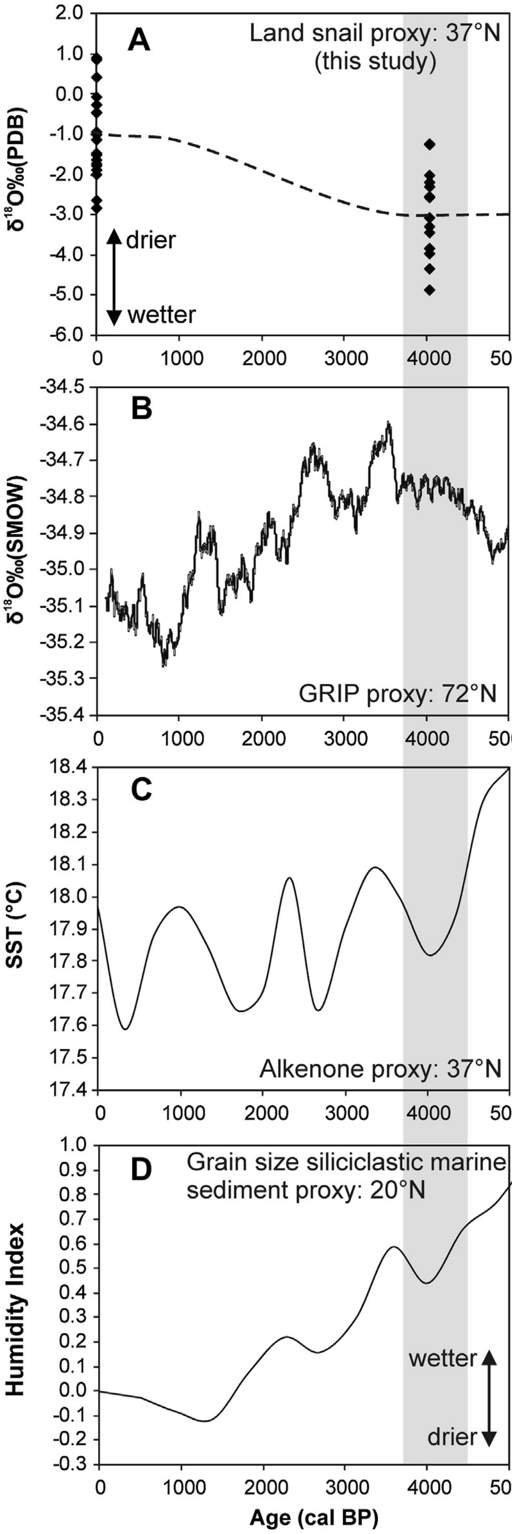

Fig. 5. Regional climatic proxies over the last 5000 cal BP. (A) Oxygen stable isotope values of land snails from Jaén, southern Spain. Gray band depicts the age range for fossil land snails analyzed in this study. (B) 100-point moving average of the oxygen stable isotope values from Greenland ice core (Dansgaard et al., 1989, 1993; GRIP Members, 1993; Grootes et al., 1993; Johnsen et al., 1997). (C) Sea Surface Temperature (SST) estimates based on the alkenones from a marine deep sea core off Iberian margin (Bard, 2002). (D) Humidity index estimates based on grain size siliciclastic marine sediment cores from northwest Africa (Tjallingii et al., 2008). Note that while average $\delta^{18}O$ values of rain (B) and temperatures (C) appear to be similar at ~4470–3880 cal BP (gray band) and today, humidity was somewhat higher during the middle to late Holocene than at present (A, D).

($-10.0 \pm 0.6‰$) suggests that modern specimens have consumed nearly exclusively C_3 plants with an average value of $\sim -26‰$, assuming an isotopic offset of $\sim 15‰$ between shell and body (Stott, 2002; Yanes et al., 2008), plus $\sim 1‰$ offset between body and plant (DeNiro and Epstein, 1978). This predicted value of C_3 plants is several per mil higher than measured values of non-lipid extracted plant litter from the study area, which vary between $-27.9‰$ and $-30.0‰$ (personal unpublished data). This suggests that other environmental factors besides $\delta^{13}C$ values of the surrounding vegetation should have affected the $\delta^{13}C$ values of modern snails. Controlling factors of shell $\delta^{13}C$ values, in addition to ingested foods, include variations in the contribution of limestone (e.g., Goodfriend and Hood, 1983; Goodfriend, 1987, 1999; Goodfriend and Ellis, 2002; Yanes et al., 2008). Contrary to small and minute gastropods (Pigati et al., 2004, 2010), medium to large size land snails (>10 mm), as the species studied here, are expected to consume variable amounts of limestone as a source of calcium to build their own shells. This unknown contribution of carbon derived from limestone may disguise to some degree the $\delta^{13}C$ values from the plant diet (e.g., Yanes et al., 2008). Other factors like variations in the atmospheric CO_2 seem to have a negligible direct effect on the $\delta^{13}C$ values of the shell (e.g., Stott, 2002; Metref et al., 2003; Balakrishnan and Yapp, 2004).

The carbon isotopic variability of $2.2‰$ among modern specimens ($n = 20$) may reflect daily/seasonal variations in metabolic rates, feeding habits and/or variable limestone contribution among individuals (e.g., Balakrishnan and Yapp, 2004). The amplitude of $\delta^{13}C$ values of fossil shells is comparable to that of modern individuals, suggesting similar degree of variations in the aforementioned factors during the middle to late Holocene and the present. In any case, the shell $\delta^{13}C$ values of modern (and fossil) specimens indicate a snail diet primarily based on C_3 plants, which is consistent with the documented dominance of C_3 species in the study area (e.g., Carrión et al., 2001, 2010; Anderson et al., 2011; Jiménez-Moreno and Anderson, 2012).

Despite the large overlap of $\delta^{13}C$ values between modern and fossil shells (Fig. 3A), ancient specimens were $\sim 0.6‰$ lower in $\delta^{13}C$ values than living individuals. Thus, ancient specimens consumed foods having an estimated value of $\sim -26.6‰$. This slightly lower $\delta^{13}C$ values may suggest somewhat lower water stress conditions at ~4470–3880 cal BP than today (Fig. 6A). If the extreme value of $-13.8‰$ is however overlooked, modern and fossil shells show similar $\delta^{13}C$ values, and therefore, comparable plant $\delta^{13}C$ values. Fossil snails recovered from 4180 ± 130 cal BP strata of the Los Castillejos archeological site (Yanes et al., 2011a) from Granada (Fig. 6A), exhibited significantly lower shell $\delta^{13}C$ values ($-9.2 \pm 0.7‰$; $n = 27$) than modern counterparts from the same locality ($-8.4 \pm 0.5‰$; $n = 15$). Hence, somewhat lower water stress conditions appear to have occurred at ~4180 ± 130 cal BP than in the present in southern Spain. This is consistent with other snail studies from the Mediterranean. Colonese et al. (2010b) suggested that middle Holocene shells were similar or slightly ^{13}C-depleted compared with living specimens. Other snail proxies from Italy (Bonadonna and Leone, 1995) and Spain (Yanes et al., 2011a) indicate that middle Holocene land snail shells of various species followed a diet primarily based on C_3 plants. This finding clearly reinforces the overall dominance of C_3 plants during the middle to late Holocene in southwestern Europe.

5.2. Paleoatmospheric inferences: shell $\delta^{18}O$ values

Modern specimens showed an average shell $\delta^{18}O$ value of $\sim -1‰$, up to $\sim 5‰$ higher than empirical rain $\delta^{18}O$ values. Such isotopic offset between shell and rain suggests that snail body water $\delta^{18}O$ values were modified by evaporation, the magnitude of which is largely linked to the local relative humidity (e.g.,

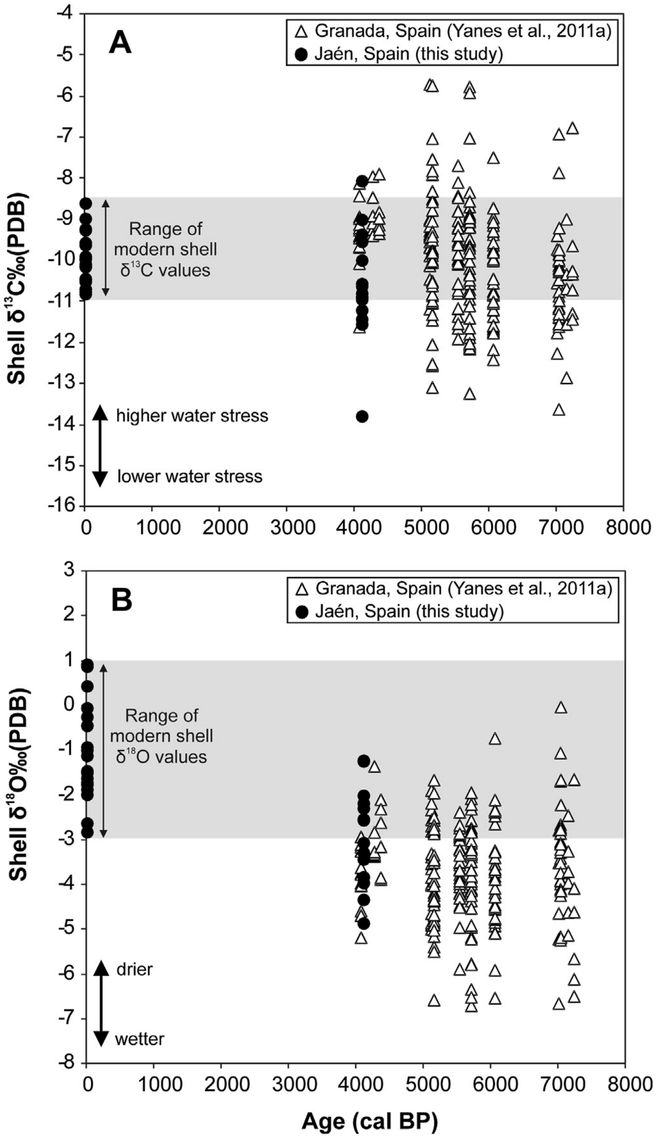

Fig. 6. Carbon (A) and oxygen (B) stable isotope values of middle to late Holocene land snails from southeastern Spain. Open triangles represent snail data from Granada, Spain (Yanes et al., 2011a). Filled circles correspond to data from Jaén, Spain (this study). Note that snails from the late Holocene remain to be surveyed in the region.

Balakrishnan and Yapp, 2004; Zaarur et al., 2011). Recent shells from Jaén may have precipitated at times when rain $\delta^{18}O$ was ~−6‰ (in isotopic equilibrium with water vapor) and air temperature was ~16 °C (Fig. 2A–B). The steady-state flux-balance mixing model by Balakrishnan and Yapp (2004) predicts that relative humidity during calcification should have been ~89%, on average (Fig. 4A). This predicted value by the model matches perfectly well with the measured mean maximum relative humidity value in Jaén during the snail active period (Fig. 2B). Accordingly, this model appears to calculate credible relative humidity values in the study area and therefore, may be used to estimate paleohumidity conditions.

The $\delta^{18}O$ values of fossil shells of both species were, on average, ~2‰ lower than modern specimens (Fig. 3B; Fig. 5A), suggesting that atmospheric conditions during calcification were probably different at ~4470–3880 cal BP than today. Several well-established paleoclimatic proxies from different localities can be used to deduce middle to late Holocene values for some environmental variables in southern Spain. For instance, the 100-point running mean of the $\delta^{18}O$ values from the Greenland Ice Core (GRIP) at the latitude of 72°N (Dansgaard et al., 1993, 1989; GRIP Members, 1993; Grootes et al., 1993; Johnsen et al., 1997) indicate that meteoric water between ~4500 and 3900 y was simply ~0.3‰ higher than present waters (Fig. 5B). Speleothem proxies from the eastern Mediterranean region documented basically the same $\delta^{18}O$ values over the last ~4500 y, with a subdued fluctuation of $\delta^{18}O$ values smaller than ~0.3‰ (see figure 2A in Bar-Matthews et al., 1999). In NW Iberian Peninsula, a newly studied Holocene speleothem also revealed minimal variation in the $\delta^{18}O$ values at ~4500–3900 y with respect to present-day (Railsback et al., 2011). These speleothem proxies suggest that the rain or water vapor $\delta^{18}O$ values, rainfall amount, the trajectory of rainfall or the temperature should have been comparable at ~4500–3900 y and the present in the Mediterranean. Alkenone data extracted from deep sea cores off SW Iberian Peninsula coast (Fig. 5C) indicate that temperatures at ~4500–3900 y were similar than today, with a difference lower than ~0.2 °C (Bard, 2002). This is consistent with alkenone and benthic foraminera proxies from several deep sea cores from the western Mediterranean (e.g., Cacho et al., 2001). According to these proxies, it seems logical to assume a possible paleoclimatic scenario for Jaén where the rain $\delta^{18}O$ values and the ambient temperatures at ~4470–3880 cal BP were relatively similar to those observed at present (Fig. 5). These paleoclimatic assumptions used together with the measured shell $\delta^{18}O$ values and the flux-balance mixing model by Balakrishnan and Yapp (2004) allow us to calculate relative humidity conditions at the time of shell growth. Model outputs predict that calcification during ~4470–3880 cal BP in Jaén occurred under average relative humidity values which were notably higher (~95%) than those from the present (~89%), whereas rain $\delta^{18}O$ values and air temperatures did not probably change severely (see continuous line in Fig. 4B). This pattern remains the same if shell deposition occurred at temperatures ~5 °C warmer than observed environmental temperatures during snail active period (Zaarur et al., 2011), that is, fossil shells precipitated at noticeable wetter conditions at ~4470–3880 cal BP than presently (~92% versus ~87%, respectively) (see dashed line in Fig. 4B). This second scenario is plausible if the shell was deposited principally during the daytime under substantial sunlight exposure, which would increase the body temperature during the biomineralization process with respect to the environmental temperature (Zaarur et al., 2011).

If alternatively, relative humidity (~89%) and ambient temperature (~16 °C) have remained constant from the mid-late Holocene to recent, the $\delta^{18}O$ values of fossil shells can only be explained in the context of the model if water $\delta^{18}O$ values imbibed by the snails were ~2% lower than today. However, based on published records (Fig. 5) such difference of values of environmental water at ~4470–3880 cal BP with respect to the present is unlikely. Other paleoclimatic scenarios were not explored because there is no convincing evidence from the published literature that document that rain $\delta^{18}O$ values and/or air temperatures in the western Mediterranean were appreciably different at ~4470–3880 cal BP and today.

The inferred wetter conditions during the Copper Age interval may have favored agricultural practices during that time interval in Jaén. Moisture conditions may have reduced the necessity of artificial irrigation of crops in this ancient civilization, as suggested for the early to middle Holocene human settlement of the Los Castillejos archeological site in Montefrío, Granada (Araus et al., 1997a, b; Aguilera et al., 2008).

The range of $\delta^{18}O$ values of ancient snails from Jaén largely overlap with a published Holocene snail proxy (Fig. 6B) from Granada, southern Spain (Yanes et al., 2011a). Both snail proxies suggest noticeable wetter conditions around 4470–3880 cal BP in southern Spain than at present (Fig. 6B). It is also worth mentioning that while late glacial and early-middle Holocene snail proxies from Europe are available, mainly from Italy (Bonadonna and Leone, 1995; Zanchetta et al., 1999; Yates et al., 2002; Colonese et al., 2007, 2010a,b, 2011; Kehrwald et al., 2010; Yanes et al., 2011a), European isotopic proxies of land snails from the mid-late Holocene interval are rare in the published literature.

5.3. Comparison with other paleoclimatic proxies

Middle Holocene climate in southern Spain has been studied by various continental proxies, mostly derived from lake sediments and pollen records. Anderson et al. (2011) studied the Laguna de Río Seco highland lake in Sierra Nevada (3020 m a.s.l., southern Spain), and observed that the early to middle Holocene was characterized by high abundance of deciduous *Quercus* and *Betula*, whereas dryness conditions overcame from the middle to late Holocene. A similar pattern was observed in alpine bog sediments of Borreguiles de la Virgen in Sierra Nevada at 2945 m a.s.l., southern Spain (Jiménez-Moreno and Anderson, 2012). Pollen proxies from Villaverde (870 m a.s.l.; south-central Spain) indicate that an increase of *Quercus* occurred between 7500 and 5900 cal BP as a response of increasing moisture and temperature, while xerophytes increased from ~5000 cal BP to the present due to increasing dryness (Carrión et al., 2001). A recent review by Carrión et al. (2010) stresses the fact that most published records indicate that the early to middle Holocene in southern Spain was portrayed by the dominance of tree species whereas xerophytes increased considerably thereafter. A significant drought and forest decline phase is identified starting at the Argaric period, 4300–3600 cal BP (Carrión et al., 2010 and references therein).

In a more regional scale, Roberts et al. (2011) combined model outputs and proxy data and concluded that the western Mediterranean was in general typified by (1) changes in the rainfall during the early Holocene, (2) increased rainfalls during the middle Holocene (see figure 5 in Roberts et al., 2011), and (3) a gradual aridification from the middle Holocene to the present (see also Pérez-Obiol et al., 2011). The oxygen isotopic data from a stalagmite in central Italy also revealed enhanced precipitation during the early-middle Holocene (Zanchetta et al., 2007). Downward off Mauritanian coast at ~20°N, DeMenocal et al. (2000) observed that the African Human Period, between 14800 and 5500 cal BP, was characterized by a considerable vegetation development in the Sahara zone. This was also recognized in the siliciclastic grain-size analysis off Mauritanian coast (Tjallingii et al., 2008), which showed a positive hydrological balance during the early-middle Holocene, while humidity fluctuated but overall declined from the middle Holocene to present-day (see Fig. 5D). These repeatedly documented wetter conditions during the early-middle Holocene at regional scale may have been caused by changes in the atmospheric circulation system like a southward shift in the north-westerlies and an increase in the North Atlantic winter precipitation (e.g., Mayewski et al., 2004; Moreno et al., 2005; Tjallingii et al., 2008).

According to the aforementioned proxies, at ~4500–3900 cal BP the wet phase already ended whereas the aridification process already started. Moreover, several proxies have actually identified a marked arid period around 4200 cal BP, especially in the eastern Mediterranean region (e.g., Migowski et al., 2006). The snail assemblage has been dated at ~4470–3880 cal BP, which is considered the beginning of the late Holocene. Consequently, these samples probably correspond to the initial phase of the well-documented aridification process in southern Spain. Nonetheless, the presented data here indicate that conditions were still wetter at ~4470–3880 cal BP than at present, as observed in a marine sediment proxy from northwestern Africa (Fig. 5D) and a land snail proxy from Granada (Fig. 6B). Furthermore, Faust et al. (2004) also observed that late Holocene climate-driven fluvial records from northern Tunisia (latitude: 36°N) often indicated enhanced fluvial dynamics and lower sedimentation rates around 4500–3000 cal BP, associated with an increase in groundwater level (see sediment series 3a in Fig. 3: Faust et al., 2004). Differing outcomes from all these proxies stress the fact that the Holocene in the Mediterranean is a complex period of marked dry/wet oscillations that vary in space and time (e.g., Mayewski et al., 2004; Jalut et al., 2009; Roberts et al., 2011; Magny et al., 2012). Thus, variations in moisture conditions may be in part affected by local and site-specific features. Factor affecting relative humidity at the soil–air interface (where snails live) may include temperature, presence of large bodies of water, soil moisture, and cloud and plant cover. If temperatures (and $\delta^{18}O$ values of rain and water vapor) have remained relatively similar during the studied time-intervals (Fig. 5), snails during ~4470–3880 cal BP deposited shell either near springs, on top of wetter soils, at shaded areas, and/or among denser vegetation (at an enclosed space), as opposed to snails living today in Jaén.

6. Conclusions

This study illustrates that land snail shelly accumulations from archeological sites are useful paleoenvironmental archives of ancient atmospheres in the western Mediterranean. These are the first results of carbon and oxygen stable isotope composition of land snail entire shells recovered from the Marroquíes archeological site (Jaén, southern Spain). New radiocarbon analyses carried out in bone collagen samples indicate snails lived during the Copper Age interval (~4470–3880 cal BP). The carbon stable isotope composition of fossil shells implies snails consumed primarily C_3 plants with comparable $\delta^{13}C$ values than plants living today in the area. The oxygen stable isotope composition of ancient shells was ~2‰ lower than that of modern counterparts, pointing to wetter conditions at ~4470–3880 cal BP than today. Calculations from a snail evaporative steady-state flux balance mixing model suggest that individuals deposited shell at times when relative humidity was noticeably higher (~92–95%) at ~4470–3880 cal BP and subsequently may have fluctuated but overall declined to present values (~86–89%). This study reinforces that the oxygen stable isotope composition of snail shells are sensitive to humidity fluctuations, even when rain $\delta^{18}O$ values and temperature seem to remain moderately similar. Also highlighted is the necessity of additional European land snail proxies, particularly from the middle to late Holocene interval.

Acknowledgments

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