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Paleo-environmental implication of clumped isotopes in land snail shells

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Abstract

Clumped isotopes analyses in modern land snail shells are reported and used to interpret shell oxygen isotopes within the context of terrestrial paleo-climatology. Carbonate clumped isotopes thermometry is a new technique for estimating the temperature of formation of carbonate minerals. It is most powerful as an indicator of environmental parameters in combination with $\delta^{18}O$, allowing the partitioning of the $\delta^{18}O$ signal into its temperature and water components. Results indicate that snail shell calcification temperatures are typically higher than either the mean annual or the snail activity season ambient temperatures. Small inter- and intra-snail variability suggests that shell aragonite forms at isotopic equilibrium so that the derived temperatures are an eco-physiological parameter reflecting snail body temperature at the time of calcification. We attribute these higher body temperatures to snail eco-physiological adaptations through shell color, morphology, and behavior. In combination with shell oxygen isotope composition, these temperatures allow us to calculate snail body water composition, which is in turn interpreted as a paleo-hydrological indicator, reflecting isotopic composition of local precipitation modified by local evaporation.

1. INTRODUCTION

Land snails have been used as an archive material for reconstructing past climatic and environmental conditions on land through eco-physiological analysis of snail species growth habitats, as well as through isotopic analysis of snail shell aragonite (Goodfriend, 1992). The occurrence of extant species in the Quaternary fossil record allows the use of modern analog approaches to estimate paleo-environmental conditions. In particular, the isotopic composition of snail shells together with snail faunal assemblages and shell morphology have been used to reconstruct environmental changes such as vegetation type coverage and temperature changes (Goodfriend, 1992). Oxygen isotope composition of carbonate minerals is the most popular proxy in paleo-climate research, used in both marine and terrestrial environments. It reflects the temperature dependent equilibrium isotopic fractionation between the mineral and the water in which it grows (e.g., Epstein et al., 1953). However, implementation of $\delta^{18}O$ for reconstruction of environmental temperatures requires an independent estimate of paleo-water composition, which is especially difficult in terrestrial environments, due to the complexity and variability of rain-water isotopic composition.

The aragonitic snail shell precipitates from bicarbonate dissolved in the snail body fluids, and is assumed to be in isotopic equilibrium with body water (Goodfriend, 1992). The main source for land snail body water is rain and dew that penetrate through the integument of the foot (Prior, 1985; Heller, 1993). $\delta^{18}O$ is therefore predominantly controlled by isotopic composition of the local rain (Balakrishnan and Yapp, 2004) and dew (Goodfriend et al., 1989) with potential effects of evaporation at the soil surface. The life span of land snails ranges from several

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months to 19 years (Barker, 2001) so that shell $\delta^{18}O$ may be associated with a specific season in small snails but in larger shells is likely to reflect precipitation over a number of years.

The first study of stable isotopes in land snail shells (Yapp, 1979) was followed by many more (Magaritz and Heller, 1980; Lecolle, 1985; Goodfriend et al., 1989; Goodfriend, 1990, 1992; Leng et al., 1998; Leone et al., 2000; Goodfriend and Ellis, 2002; Balakrishnan and Yapp, 2004; Balakrishnan et al., 2005; Zanchetta et al., 2005; Li et al., 2007; Yanes et al., 2008, 2009; Kehrwald et al., 2010) which investigated correlations between carbon and oxygen isotope compositions and environmental conditions. The approach was based on extrapolation from studies in aquatic snail shell carbonates whose $\delta^{18}O$ values, similarly to many marine biogenic carbonates, reflect the temperatures and isotopic composition of lake waters, and assuming a similar relationship in non-aquatic land snails. The pioneering work of Yapp (1979) and Magaritz and Heller (1980) examined $\delta^{18}O$ of land snail shells as a proxy for climatic and environmental conditions (mainly rainfall $\delta^{18}O$). Even though no simple correlation was found between the snail shells and the estimated meteoric water $\delta^{18}O$, they identified relative humidity as a key parameter in relating rainfall to snail body water composition.

In later studies, for example in France and Italy (Lecolle, 1985; Zanchetta et al., 2005), good correlations between shell $\delta^{18}O$ values and that of the environmental water were observed. These correlations did not hold, however, at high altitudes (1200 m above sea level) and in regions where the mean annual temperature was lower than 6 °C (Lecolle, 1985). Although several studies established a correlation between $\delta^{18}O$ of snail shell carbonate and local precipitation, the failure of these relationships at low temperature regions (Lecolle, 1985) remains a significant source of uncertainty in extrapolating snail shell $\delta^{18}O$ data in both space and time.

$\delta^{13}C$ signatures of shell carbonates and organic matter reflect the carbon sources of snail diet (Goodfriend, 1992), being indicative of vegetation types in the immediate snail environment (Goodfriend and Magaritz, 1987; Goodfriend and Ellis, 2002; Stott, 2002; Metref et al., 2003; Balakrishnan and Yapp, 2004). Carbon isotope records are thus interpreted to reflect variations in C_3 and C_4 plant distribution in response to changes in rainfall patterns (Goodfriend and Magaritz, 1987; Goodfriend, 1990). A complication, however, is potential carbon contribution to the shell from ingestion of carbonate rocks and atmospheric CO_2 (Goodfriend, 1992).

We examine clumped isotopes in modern land snail shells and use them to test the assumptions governing $\delta^{18}O$ interpretations. The carbonate clumped isotopes paleothermometry technique is based on the abundance of ^{13}C–^{18}O bonds in the carbonate lattice relative to that expected at a random distribution of isotopes among all isotopologues (quantified by the parameter Δ_{47}; Eiler, 2007). As such it measures the thermodynamically controlled preference of two heavy isotopes to create a bond with each other, and it does not depend on the absolute concentrations of ^{13}C and ^{18}O in the carbonate mineral. When the mineral precipitates under conditions of isotopic equilibrium this preference is temperature dependent and therefore records the temperature of mineral growth, independent of the oxygen isotope composition of the solution in which it grows (Wang et al., 2004; Ghosh et al., 2006; Eiler, 2007). The Δ_{47}–T relationship was first calibrated by Ghosh et al. (2006) through analysis of synthetic calcite and was then expanded to biogenic materials (summarized by Eiler (2007) and Tripati et al. (2010)). The temperatures derived from clumped isotopes can then be combined with carbonate $\delta^{18}O$, to obtain the oxygen isotopic compositions of the water in which the mineral formed.

Published data on aragonitic material of biogenic origin, both argonitic and calcitic, is in general agreement with the calcite calibration (Schauble et al., 2006; Eiler, 2007; Tripati et al., 2010). However, using the same approach in land snails we observed deviations from Δ_{47} values expected from ambient environmental temperatures, suggesting that snail calcification temperatures are higher than those in the snail growth habitat, with the temperature offset being larger at lower environmental temperatures. The calcification temperatures obtained are nevertheless useful for ecological and physiological studies of snails, and when considered together with shell $\delta^{18}O$, for determining snail body water isotopic compositions.

2. MATERIALS AND METHODS

Modern snail shells were collected in a variety of locations worldwide, covering a large range of environmental conditions, from hot and dry (e.g., Negev, Israel) to cold and humid (e.g., Davos, Switzerland) (Table 1). Specimens were either collected by us or obtained from the Yale Peabody Museum invertebrate collection. Additional sample information can be found in Supplementary Information. The samples were broken or empty shells collected in the vicinity of live active snails. Samples include a variety of species, with the same species available in several locations (*Helicidae Helix aspersa* from Palo Alto and San Simeon in California, and Haifa in the north of Israel). Different species growing in one location were compared as well (*Pleurodonte acuta* and *Orthalicus undatus* from Jamaica and *Sphincterochila zonata* and *Trochoidea simulate* from the Negev, in the south of Israel).

In preparation for isotopic analysis, shells were sliced vertically using a Buehler Isomet Low Speed Bone Saw, to allow removal of remaining organic tissue and soil particles. Shells were rinsed in DI water, and cleaned by sonication (Sonicor DSC-100TH). Shell material was dried in vacuum at room temperature and then ground in an agate mortar and pestle. All samples were identified as aragonitic by X-ray diffraction (using an automated Scintag PAD V diffractometer, in the XRD laboratory at Yale University).

Shell carbonate was digested overnight in 105% H_3PO_4 ($\rho = 1.93$ g/cm^3) at 25 °C. The CO_2 extracted was analyzed for $\delta^{18}O$, $\delta^{13}C$ and Δ_{47}. CO_2 was cryogenically extracted on a vacuum line and then cleaned by passing through a GC column (Supelco Q-Plot, 30 m long, 0.53 mm ID) at

Table 1a
Compiled data for all samples. $\delta^{18}O_{SBW}$ is the snail body water calculated using either Δ_{47} or the environmental temperature. $\delta^{18}O_P$ is the isotopic composition of local precipitation (Bowen and Revenaugh, 2003). All $\delta^{18}O$ values of water are referenced to VSMOW. Shell color codes are as follows: D = dark, M = Medium, B = brown and W = white.

Sample	Location	*n*	Species	$\delta^{13}C$ (‰) (VPDB)	$\delta^{18}O$ (‰) (VPDB)	Δ_{47} (‰)	Δ_{47} (‰) (temp.)	$\delta^{18}O_{SBW}$ (Δ_{47})	$\delta^{18}O_{SBW}$ (env. temp.)	$\delta^{18}O_P$	Shell color
sc-7	San Simeon, CA	3	*Helicidae Helix aspersa*	−10.91 ± 0.02	−0.66 ± 0.11	0.629 ± 0.007	29 ± 3	1.48	−1.31	−6.2	DB
sc-6	San Simeon, CA (O)	3	*Helicidae Helix aspersa*	−11.03 ± 0.21	−0.57 ± 0.16	0.656 ± 0.009	23 ± 2	0.12	−1.22	−6.2	DB
sc-5	San Simeon, CA (Y)	4	*Helicidae Helix aspersa*	−11.12 ± 0.28	−0.50 ± 0.03	0.663 ± 0.003	21 ± 1	−0.14	−1.15	−6.2	DB
sc-4	Palo Alto, CA	3	*Helicidae Helix aspersa*	−12.27 ± 0.05	1.16 ± 0.05	0.639 ± 0.015	27 ± 3	2.76	−0.30	−7.7	DB
sc-11	Palo Alto, CA	3	*Helicidae Helix aspersa*	−11.38 ± 0.06	0.40 ± 0.24	0.644 ± 0.010	26 ± 2	1.74	−1.06	−7.7	DB
sc-2	Negev, Israel (1)	4	*Sphincterochila zonata*	−0.96 ± 0.04	3.02 ± 0.03	0.646 ± 0.004	25 ± 1	4.26	2.83	−5.1	W
sc-1	Negev, Israel (4)	3	*Trochoidea simulate*	−6.44 ± 0.05	1.90 ± 0.01	0.651 ± 0.006	24 ± 1	2.88	1.70	−5.1	W + B
sc-8	Negev, Israel (6)	3	*Trochoidea simulate*	−7.24 ± 0.05	1.71 ± 0.02	0.640 ± 0.011	26 ± 2	3.23	1.52	−5.1	W + B
sc-3	Haifa, Israel	4	*Helicidae Helix aspersa*	−13.27 ± 0.05	0.72 ± 0.04	0.646 ± 0.010	25 ± 2	1.96	0.41	−1.5	DB
sc-pb-1	Pennsylvania	5	*Triodoesis notata*	−9.42 ± 0.05	−1.54 ± 0.11	0.653 ± 0.018	23 ± 4	−0.69	−3.81	−6.1	MB
sc-pb-2	East Saginaw, MI	6	*Triodoesis notata*	−9.69 ± 0.04	−3.64 ± 0.07	0.633 ± 0.016	28 ± 4	−1.76	−6.38	−8.9	MB
sc-pb-3	Washington, DC	3	*Triodoesis tridentata*	−13.61 ± 0.04	−2.93 ± 0.04	0.642 ± 0.009	26 ± 2	−1.49	−4.73	−4.5	MB
sc-pb-4	Florence, AL	4	*Triodoesis tridentata*	−11.30 ± 0.05	−1.08 ± 0.10	0.656 ± 0.012	23 ± 3	−0.34	−2.08	−6.5	MB
sc-pb-5	St. Louis County, MO	5	*Triodoesis notata*	−11.20 ± 0.05	−2.14 ± 0.06	0.623 ± 0.013	30 ± 3	0.31	−3.83	−6.8	MB
sc-pb-6	Jamaica	3	*Pleurodonte acuta*	−10.86 ± 0.01	−0.73 ± 0.04	0.607 ± 0.011	34 ± 3	2.58	0.85	−3	MB
sc-pb-7	Jamaica	3	*Orthalicus undatus*	−8.34 ± 0.05	0.10 ± 0.07	0.634 ± 0.005	28 ± 1	1.92	1.68	−3	W + B
sc-pb-8	Stock Island, FL	3	*Orthalicus reses (say)*	−11.14 ± 0.07	−1.64 ± 0.06	0.623 ± 0.003	30 ± 1	0.77	0.04	−4.3	W + B
sc-9	Davos, Switzerland (O)	3	*Helicidae Arianta arbustorum*	−10.57 ± 0.05	−3.13 ± 0.02	0.673 ± 0.017	19 ± 4	−3.25	−5.86	−10.4	DB
sc-10	Davos, Switzerland (Y)	3	*Helicidae Arianta arbustorum*	−10.49 ± 0.03	−2.48 ± 0.05	0.665 ± 0.006	21 ± 1	−2.21	−5.21	−10.4	DB

Table 1b
Climatologic data (seasonal temperature and amount of precipitation) of sample locations. Activity season was defined according to a combination of warm and wet conditions.

Location	MAT	Temperature		Precipitation		Activity season
		DJF	JJA	DJF	JJA	
San Simeon, CA	17	7	26	18	12	Winter
Palo Alto, CA	13	10	20	90	<3	Winter
Negev, Israel	19	11	24	<30	<3	Winter
Haifa, Israel	18	11	24	60	3	Winter
PA	10	−3	20	63	150	Summer
East Saginaw, MI	8	−5	20	45	105	Summer
Washington, DC	12	0	22	63	165	Summer
Florence, AL	15	1	23	84	225	Summer
St. Louis County, MO	12	−3	23	60	150	Summer
Jamaica	27	25	27	135	210	Year round
Stock Island, FL	25	22	27	60	210	Summer
Davos, Switzerland	8	−2	<15	<30	144	Summer

−20 °C (following Affek and Eiler (2006) and Huntington et al. (2009)). This is required to guarantee no interference of hydrocarbons with the mass 47 measurement (Eiler and Schauble, 2004). Measurements were performed using a Thermo-Finnigan MAT253 gas source isotope ratio mass spectrometer modified to simultaneously measure masses 44-49 in a dual inlet mode (in the Earth Systems Center for Stable Isotopic Studies at Yale University). The mass spectrometer cup configuration is identical to that used in Caltech for the original development of clumped isotopes thermometry (Eiler and Schauble, 2004). A notable modification in the Yale mass spectrometer is a larger aperture of the stainless steel capillary transferring CO_2 from the dual inlet bellows to the mass spectrometer source, resulting in a 16 V mass 44 signal for 40 mbar of CO_2 bellows pressure. An additional modification is the replacement of the original bellows potentiometer with a Bourns 3540s enabling a more efficient operative range of the bellows. These two modifications enabled the analysis of smaller samples (3–4 mg $CaCO_3$) than previously described (Huntington et al., 2009). Each measurement consisted of 90 cycles of a sample-standard comparison, with a signal integration time of 8 s. Isotopic measurements were performed in replicates of $n = 3$–6 per specimen, considering at least three replicates as a requirement for a reliable result. Reported errors are the statistical standard errors of these replicates, reflecting external analytical error, with the mass spectrometry data of all 90 cycles showing Gaussian distributions and standard error that is close to the shot noise limit (Merritt and Hayes, 1994). Both the $\delta^{18}O$ and $\delta^{13}C$ are referenced to the V-PDB scale as determined using a pre-calibrated Oztech CO_2 tank as a reference working gas, and verified using NBS-19. Δ_{47} is defined as the excess of CO_2 mass 47 signal over what is expected for random distribution of ^{13}C and ^{18}O among all isotopologues (Eiler et al., 2008; Huntington et al., 2009). Standardization is done using CO_2 that is heated at 1000 °C for 2 h, resulting in randomization (Wang et al., 2004). A series of such heated gases having a range of bulk compositions was used to correct for mass spectrometer effects such as non-linearity and scale compression that is due to dissociation–recombination reactions of CO_2 in the ion source (Huntington et al., 2009). The extent of scale compression varies among mass spectrometers as well as over time and source conditions (Affek et al., 2009; Dennis et al., in press). Scale compression in our system was accounted for by analyzing CO_2 at equilibrium at 25 °C, through isotope exchange with water, and verified through interlab comparison and measurements of carbonates having published Δ_{47} values, yielding Δ_{47} values that are within ~0.01‰ from previous estimates (Dennis et al., in press).

All climatological information is based on National Oceanic and Atmospheric Administration (NOAA) NCEP/NCAR Reanalysis data, taken as long-term mean for the years 1949–2011 (Kalnay et al., 1996).

3. RESULTS

Δ_{47} values observed in snail samples range between 0.607 ± 0.011‰ and 0.673 ± 0.017‰ (Table 1). Using the carbonate clumped isotopes thermometer calibration (Ghosh et al., 2006) these values correspond to a temperature range of 34 ± 3 and 19 ± 3.5 °C (Fig. 1). This range of temperatures is smaller than the range of mean annual temperatures (between 27 and 8 °C) or the estimated range of snail activity season temperatures (between 27 and 7 °C) in the sites from which the snail samples were collected. As snail activity requires both warmth and moisture, we define season of activity as the warm, rainy season; namely, year-round in tropical climates, winter in Mediterranean climates (in which summers are too dry), and summer in temperate climates (in which winters are too cold). The observed $\delta^{18}O$ and $\delta^{13}C$ values range between −0.96‰ and −13.61‰ and +3.02‰ and −3.64‰, respectively. No correlation was found between shells $\delta^{13}C$ and $\delta^{18}O$ (Fig. 2a), nor is there a correlation between either $\delta^{13}C$ or $\delta^{18}O$ and Δ_{47} values (Fig. 2b).

Intra-shell variations were examined by comparing segments growing at different stages of the snail life cycle – the upper part of the helix, reflecting material produced early in the snail's life vs. the lower part of the helix that

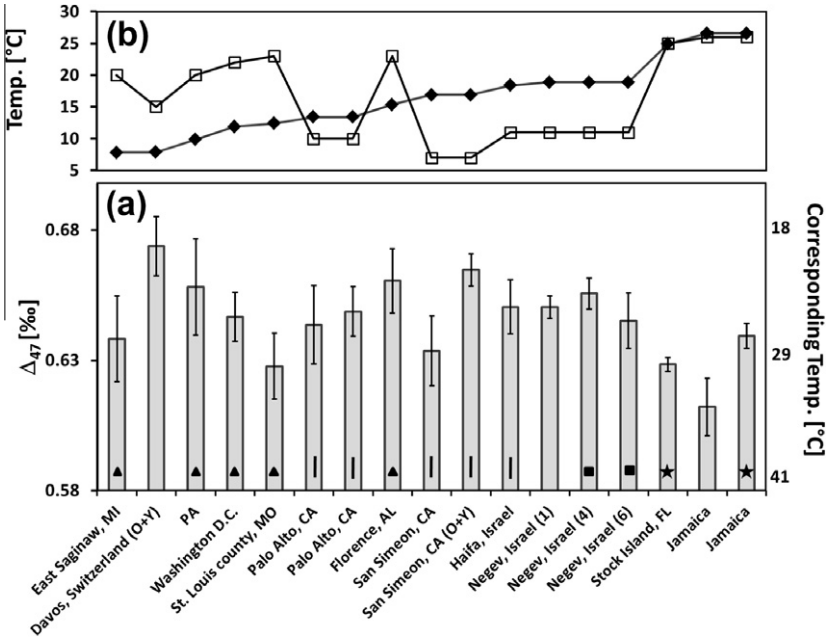

Fig. 1. (a) Clumped isotopes values in snail shells (left axis) and temperatures calculated (right axis) based on Ghosh et al. (2006). Snail locations are ordered according to increasing mean annual temperatures (from left to right). Snails of the same species are marked by the same symbol. (b) Mean annual (diamonds) and season activity (squares) temperatures of each location.

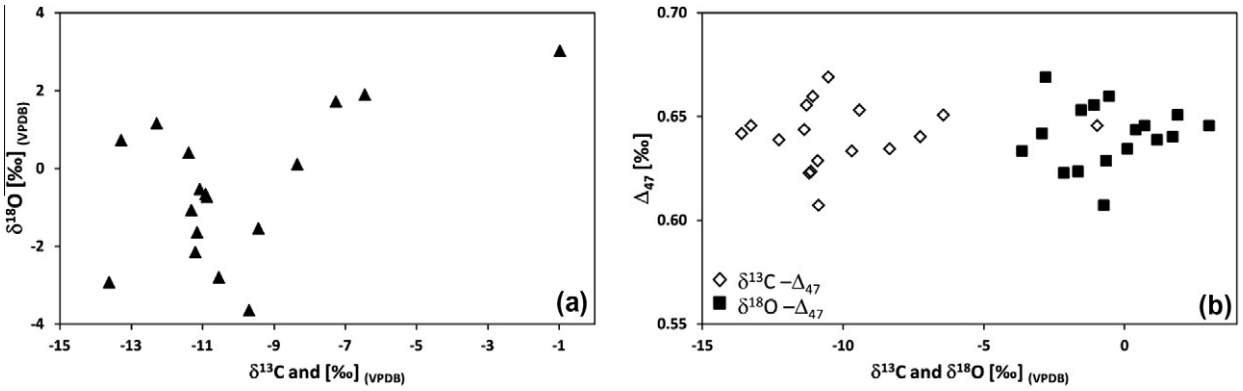

Fig. 2. $\delta^{18}O$ vs. $\delta^{13}C$ (a) and Δ_{47} vs. both $\delta^{18}O$ and $\delta^{13}C$ (b) observed in land snail shells. In all cases no correlations are observed (linear regression R^2 values of 0.333, 0.003 and 0.003 for $\delta^{18}O$ vs. $\delta^{13}C$ and Δ_{47} vs. either $\delta^{18}O$ or $\delta^{13}C$, respectively).

formed later (specimens from San Simeon, CA, and Davos, Switzerland; Table 2). No significant difference in either Δ_{47} or $\delta^{13}C$ were observed within a specimen, with Δ_{47} values of $0.656 \pm 0.009‰$ and $0.663 \pm 0.003‰$ (sc-5 and sc-6 from San Simeon, CA; Table 1) and $0.673 \pm 0.017‰$ and $0.665 \pm 0.006‰$ (sc-9 and sc-10 from Davos, Switzerland). Respective $\delta^{13}C$ values are $-11.03 \pm 0.21‰$ and $-11.12 \pm 0.28‰$ (sc-6 and sc-5), and $-10.57 \pm 0.05‰$ and $-10.49 \pm 0.03‰$ (sc-9 and sc-10). Variations in $\delta^{18}O$, when comparing the lower and upper helix, were not significant in the San Simeon samples: $-0.57 \pm 0.16‰$ and $-0.50 \pm 0.03‰$ (sc-6 and sc-5), whereas somewhat larger variations were observed in the Davos sample: $-3.13 \pm 0.02‰$ and $-2.48 \pm 0.05‰$ (sc-9 and sc-10). All other data is reported for whole shell analysis.

To examine inter- and intra-species variations, we compared Δ_{47} values of two individual snails of the same species (Table 3), as well as two different species collected in the same location (Table 4), for several locations. Detailed data are given in Table 1. In the Negev desert,

Table 2
Intra shell comparison of Δ_{47} in different growth stages of *Helicidae Helix aspersa* from San Simeon, CA and *Helicidae Arianta arbustorum* from Davos, Switzerland. Upper part of the helix represents an early growth stage of the snail (Y) and lower part of helix represents an older age (O).

Sample	Δ_{47} (‰)
San Simeon, CA (O)	0.656 ± 0.009
San Simeon, CA (Y)	0.663 ± 0.003
Davos, Switzerland (O)	0.673 ± 0.017
Davos, Switzerland (Y)	0.665 ± 0.006

Table 3
A comparison of the same species from the same location in the Negev, Israel, San Simeon and Palo Alto, CA.

Sample	Species	Δ_{47} (‰)
Negev, Israel (sc-1)	*Trochoidea simulate*	0.651 ± 0.006
Negev, Israel (sc-8)	*Trochoidea simulate*	0.640 ± 0.011
Palo Alto, CA (sc-4)	*Helicidae Helix aspersa*	0.639 ± 0.011
Palo Alto, CA (sc-11)	*Helicidae Helix aspersa*	0.644 ± 0.015
San Simeon, CA (sc-5 + 6)	*Helicidae Helix aspersa*	0.660 ± 0.006
San Simeon, CA (sc-7)	*Helicidae Helix aspersa*	0.629 ± 0.013

Table 4
Comparing the Δ_{47} of different snail species from the same location.

Sample	Species	Δ_{47} (‰)
Negev, Israel (sc-2)	*Sphincterochila zonata*	0.646 ± 0.004
Negev, Israel (sc-1)	*Trochoidea simulate*	0.651 ± 0.006
Negev, Israel (sc-8)	*Trochoidea simulate*	0.640 ± 0.011
Jamaica (sc-pb-6)	*Pleurodonte acuta*	0.607 ± 0.011
Jamaica (sc-pb-7)	*Orthalicus undatus*	0.634 ± 0.007

Israel, Δ_{47} values of *Sphincterochila zonata* (sc-2) and two specimens of *Trochoidea simulate* (sc-1 and sc-8) did not vary significantly with Δ_{47} values of 0.646 ± 0.004‰, 0.651 ± 0.006‰, and 0.640 ± 0.011‰, respectively. Their $\delta^{13}C$ values, on the other hand, were highly variable, especially between species, with values of -0.96 ± 0.04‰ (sc-2), -6.44 ± 0.05‰ and -7.24 ± 0.05‰ (sc-1 and sc-8). $\delta^{18}O$ values differed somewhat among species but only slightly among individuals within the same species, with values of $+3.02 \pm 0.03$‰ (sc-2), $+1.90 \pm 0.01$‰ and $+1.71 \pm 0.02$‰ (sc-1 and sc-8). The comparison of *Pleurodonte acuta* and *Orthalicus undatus* from Jamaica showed significant differences in Δ_{47}, with values of 0.607 ± 0.011‰ and 0.634 ± 0.005‰, (sc-pb-6 and sc-pb-7), as well as in $\delta^{13}C$ with values of -10.86 ± 0.01‰ and -8.34 ± 0.05‰ and in $\delta^{18}O$, with values of -0.73 ± 0.04‰ and $+0.10 \pm 0.07$‰, respectively. Two *Helicidae Helix aspersa* (sc-4 and sc-14) individuals from Palo Alto, CA, showed no significant difference in Δ_{47} (0.639 ± 0.015‰ and 0.644 ± 0.010‰), and a similar range of $\delta^{13}C$ and $\delta^{18}O$ intra-species variability (-12.27 ± 0.05‰ and -11.38 ± 0.06‰ for $\delta^{13}C$, $+1.16 \pm 0.05$‰ and $+0.40 \pm 0.24$‰ for $\delta^{18}O$). In San Simeon, CA, however, two individuals of *Helicidae Helix aspersa* (sc-7 and sc-5-6), showed significant variability in Δ_{47} with values of 0.629 ± 0.013‰ and 0.660 ± 0.006‰, but only small variability in $\delta^{13}C$ (-10.91 ± 0.02‰ and -11.07 ± 0.25‰) and $\delta^{18}O$ (-0.66 ± 0.11‰ and -0.53 ± 0.10‰), for samples sc-7 and sc-5-6, respectively.

4. DISCUSSION

4.1. Carbon and oxygen isotopes

Carbon isotopes in snail shells are typically interpreted to reflect snail diet (Stott, 2002), in particular the relative contribution of C_4 vs. C_3 vegetation, thus providing information about paleo-vegetation and related hydrological conditions. We can therefore infer that, with the exception of one specimen from the Negev, Israel, all the analyzed snails fed almost exclusively on C_3 plants, in agreement with the C_3 dominance in their environment (Woodward and Lomas, 2004). Sample sc-1 from The Negev, Israel, had an anomalously high isotopic $\delta^{13}C$ value of -0.96‰, and most likely fed on C_4 or CAM plants.

In general, trends in oxygen isotope composition of a shell are related to local environmental conditions, namely, more ^{18}O enriched values are characteristic of hot and dry regions and more depleted values are associated with colder and more humid environments. However, as noted previously (Lecolle, 1985; Zanchetta et al., 2005; Yanes et al., 2009), this correlation is not straightforward. The expected correlation with environmental conditions such as ambient temperature and rainfall $\delta^{18}O$ yielded reasonable estimates in some cases, but this approach did not hold in cases of low ambient temperatures (Lecolle, 1985). Yet, in spite of this complication, most geochemical studies assume that calcification temperature is identical to ambient temperature (either mean annual or snail growing season). Our clumped isotopes data indicate that calcification temperatures are higher than ambient temperatures, and in cold environments the difference tends to be larger, providing a likely explanation for these oxygen isotope discrepancies.

4.2. Clumped isotopes

4.2.1. Calcification temperatures

The range of calcification temperatures recorded by the snails is small (19–34 °C) relative to the range of the temperatures associated with snail activity season in the different locations (7–27 °C). Seasonal and diurnal variations could have provided an explanation to the temperature discrepancy; however, we found no correlation between either mean annual ambient or snail activity season temperatures and temperatures obtained from clumped isotopes (Figs. 1 and 3a). In some locations (Israel and California) Δ_{47} reflects temperatures that are close to the local annual mean even though snails in these regions are active almost only during the winter (Heller, 1993). In most other locations, the temperature recorded by the snail shells is higher than ambient, even when accounting for a possible bias towards shell growth in summer.

Comparing the clumped isotopes temperatures to the local ambient temperatures (either mean annual or snail activity season; Fig. 3a) reveals a general trend in which

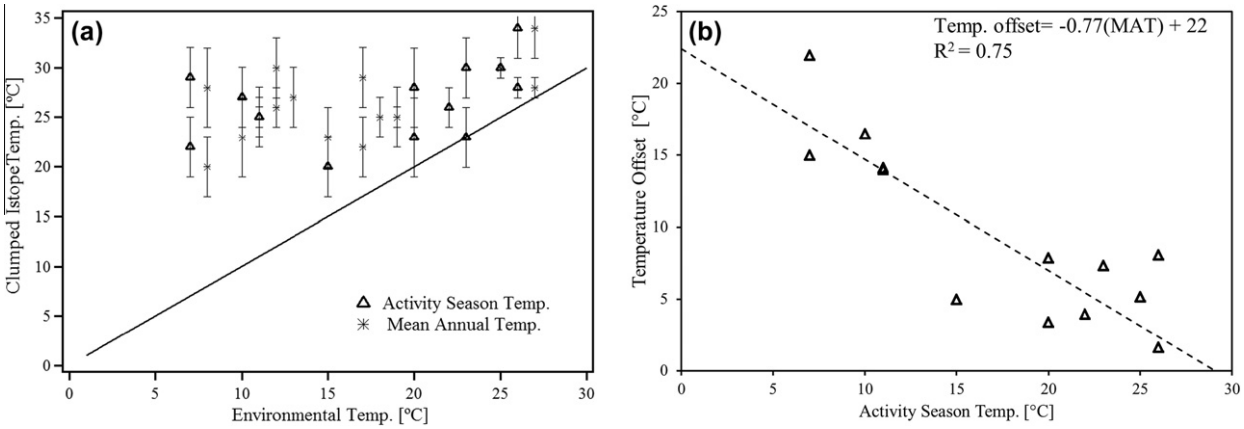

Fig. 3. (a) A comparison of measured clumped isotopes temperatures in land snail shells to local mean annual and snail activity season temperatures. (b) The offset of clumped isotopes derived snail calcification temperatures from activity season temperature as compared to the local season activity temperature. Note that the linear fit is a convenient approximation but is not a physiology-based function and is therefore valid for interpolation but not extrapolation.

higher environmental temperatures correlate with higher growth temperatures. However, the absolute calcification temperatures in our samples are always higher than local temperatures, with calcification temperatures being close to uniform across most locations. The observed temperature offset between clumped isotopes temperatures and local snail activity season temperatures is higher where environmental temperatures are lower (Fig. 3b). This trend is consistent with past observations in which environmental temperatures could plausibly explain the observed shell $\delta^{18}O$ in warm regions, but not in colder, high elevation sites (Lecolle, 1985; Yanes et al., 2009).

Although generally ignored in geochemical studies, it has been shown that coloration and morphology of the shell, as well as behavioral lifestyle adaptation may significantly affect snail body temperature (Heath, 1975; Dittbrenner et al., 2009), leading to snail body temperatures, and hence calcification temperatures, that are typically warmer than ambient. Dark colored shells may increase snail body temperature by up to 12 °C above environmental temperature by exposure to direct sunlight, as the snail basks in the sun (Heath, 1975). At the same time, thick white shells and a lifestyle that involves being away from the warm soil surface (by climbing on vegetation) may moderate snail body temperature in regions where soil and daytime air temperatures exceed a critical value (Heller, 1993). Shell color, morphology, and behavior may thus provide a specific adaptation that allows the snail some control of its body temperature. Indeed, in our measurements, the largest offset between clumped isotopes temperatures and ambient temperatures was observed in the dark brown colored shells. The smallest difference was observed in the lighter, medium-gray shells. In view of this, it might be surprising that white shells of snails from the Negev, Israel, show a larger temperature difference. This, however, can result from other morphological or behavioral controls on body temperature, such as longer hours of exposure to the sun during the winter activity period. Though not observed in our dataset, we expect that in especially warm regions snail calcification temperatures may be lower than ambient. The observed Δ_{47} values thus reflect the temperature at the site of calcification, which is likely to be close to snail body temperature during the time of snail activity.

The observation that calcification and ambient temperatures differ from each other has implications beyond snail eco-physiological aspects, and addresses specifically the use of snail shells for paleo-environmental reconstruction. This calcification temperature reflects the conditions recorded by shell $\delta^{18}O$ and is therefore the temperature relevant for estimating $\delta^{18}O$ of the associated water, typically interpreted as a paleo-hydrology indicator. Clumped isotopes in land snails therefore serve as a way to correctly resolve the temperature and water signatures in shell $\delta^{18}O$, required to reconstruct snail body water $\delta^{18}O$ as a proxy for hydrological parameters such as rainfall and relative humidity.

4.2.2. Shell growth under equilibrium conditions

Whereas the notion of calcification temperature that is higher than ambient temperature is consistent with biological evidence for elevated snail body temperature, an alternative potential explanation to the Δ_{47} observations may be aragonite formation under non-equilibrium conditions. Isotopic dis-equilibrium in Δ_{47} has been observed in speleothem carbonate (Affek et al., 2008; Daëron et al., 2011) and is associated with CO_2 degassing out of solution (Guo, 2008).

Precipitation under conditions of isotopic equilibrium is a key assumption in interpretation of carbonate isotopic composition based on an empirical $\delta^{18}O$–T calibration relationship (Epstein et al., 1953; Grossman and Ku, 1986; Kim and O'Neil, 1997; Kim et al., 2007), although potential deviation from equilibrium in these $\delta^{18}O$–T calibrations is often argued (e.g., Coplen, 2007; Dietzel et al., 2009). For clumped isotopes, a Δ_{47}–T relationship developed by Ghosh et al. (2006) followed calcite precipitation procedures that were based on the Kim and O'Neil (1997) approach and is therefore assumed to reflect a nominal

equilibrium relationship. This assumption is based primarily on the observed conformity of a large variety of biogenic carbonates to that calibration (summarized by Eiler, 2007; Tripati et al., 2010), including mostly marine organisms but also some terrestrial carbonates such as teeth (Eagle et al., 2010). The conformity of biogenic carbonates that are either calcite, aragonite, or apatite to the same Δ_{47}–T relationship suggests that there is no clumped isotopes fractionation associated with the mineral formation, so that isotopic equilibrium distribution of ^{13}C–^{18}O bonds is determined in the solution (Guo, 2008). A notable exception from the nominal equilibrium conformity are speleothem carbonates that significantly deviate from that relationship (Affek et al., 2008; Daëron et al., 2011; Wainer et al., 2011) as a result of kinetic isotope effects associated with CO_2 degassing and hydration and hydroxylation reactions of CO_2 (Guo, 2008). *A priori* we expect that clumped isotopes in land snails would follow the general biogenic Δ_{47}–T relationship, assuming that snail body solution is at isotopic equilibrium. Deviation from equilibrium, however, may be a potential alternative explanation to the observed lower-than-expected Δ_{47} values. We therefore conducted a number of tests, as described below, to help us assess precipitation conditions.

Kinetic isotope effects are expected to be associated with variations in mineral growth rates and are likely to be reflected as heterogeneity in Δ_{47} values within a shell. In principle, this could be tested in snails that grow year round with growth rates that vary seasonally. Unfortunately, the relatively large sample size required for clumped isotopes analysis (~15 mg for 3–4 replicates) does not allow such high resolution sampling within one shell. Furthermore, seasonal variations in $\delta^{13}C$ and $\delta^{18}O$ may merely reflect variations in diet and body water as opposed to dis-equilibrium. We therefore focus on low resolution phenological comparison of shell produced early in the snail life cycle (young) vs. shell produced by a mature snail, irrespective of seasonality. No significant young vs. old difference was observed in two specimens growing under different conditions (*Helicidae Helix aspersa* from San Simeon, CA and *Helicidae Arianta arbustorum* from Davos, Switzerland; Tables 1 and 2), in spite of observed variations in $\delta^{13}C$ and $\delta^{18}O$ values, consistent with shell aragonite forming under isotopic equilibrium.

Deviation from equilibrium will affect not only Δ_{47} but also $\delta^{18}O$ and $\delta^{13}C$ so that under kinetically controlled conditions they are expected to be correlated with each other. No such correlation was observed either within individual shells or among all snail samples (Fig. 2). Although in itself this is not a proof for isotopic equilibrium, as $\delta^{13}C$ and $\delta^{18}O$ may vary due to uncharacterized external parameters, such as snail diet, the absence of correlation does not support kinetic isotope effects as the main mechanism for Δ_{47} offset from ambient temperatures.

There is no *a priori* reason to expect that different species at one location would show the same Δ_{47} value. Nor is it expected that individuals of the same species from different climatic regimes would show the same body warming above ambient temperatures. Instead, the offset from the Δ_{47} expected for growing season ambient temperatures would depend on a combination of biological and environmental conditions (Heath, 1975; Heller, 1993). For example, a snail growing in warm, dry conditions, such as the Negev in Israel, is likely not to be active during peak warmth and to prefer activity in winter. An additional adaptation would be the thick white shell of *Sphincterochita zonata* that prevents excessive heating of snail body (Heller, 1993). In that view, it is not surprising to see a difference in Δ_{47} values between the two differently colored snails from warm, humid conditions in Jamaica, with the species of a darker color (*Pleurodonte acuta*) showing higher calcification temperature (Table 4). Though not direct evidence for equilibrium carbonate formation, these observations are in support of a biological adaptation of calcification temperature, rather than a non-equilibrium isotope effect as the main control of Δ_{47} values. It is encouraging, however, to note that snails of similar color and shell thickness that grow under similar climatic conditions, such as Israel and California (except for one individual in San Simeon, CA, discussed below) show similar Δ_{47} values, consistent with isotopic equilibrium (Table 3).

Contrary to these two samples, the two specimens of *Helicidae Helix aspersa* from San Simeon, CA, show Δ_{47} values that are significantly different (0.660 ± 0.006 and 0.629 ± 0.013). It is possible that the specimen that recorded lower calcification temperatures lived in a shady, more humid micro-environment (such as a shady garden or lawn) while the other lived in a sunnier micro-environment or that one of them happened to capture anomalous cool or warm climatic years. Even though these samples were collected in the same location, the difference in their actual living micro-environments could affect their bodies and hence calcification temperatures. It seems very likely that these snails, collected as shell fragments of snails living in a garden, did not grow in a natural environment and hence would not necessarily reflect regional conditions.

4.3. Rain water reconstruction

Early studies of snail shell carbonates (e.g., Yapp, 1979) attempted to correlate shell and rain-water $\delta^{18}O$ values using mean annual temperatures. As no direct relationship was found, later studies used the temperatures and rainfall $\delta^{18}O$ associated with times of snail activity (Leng et al., 1998; Balakrishnan et al., 2005). We combine clumped isotopes temperatures with shell $\delta^{18}O$ to calculate $\delta^{18}O$ of snail body water (using the Grossman and Ku (1986) calibration as corrected by Kobashi and Grossman (2003)). This is then compared to estimated $\delta^{18}O$ of local precipitation (based on the mapping model of Bowen and Revenaugh (2003) as given in wateriso.eas.purdue.edu/waterisotopes/; Fig. 4). Since snail growth temperatures are generally higher than mean annual temperatures, the calculated isotopic compositions of snail body water when using the clumped isotopes temperatures are more enriched than those obtained using environmental temperatures (by 0.2–4.1‰). These water values are slightly better correlated with rainfall $\delta^{18}O$ (R^2 of 0.784 vs. 0.735), highlighting the link between rain and body water that is modified, however, by enhanced evaporative enrichment in dry regions (Fig. 4).

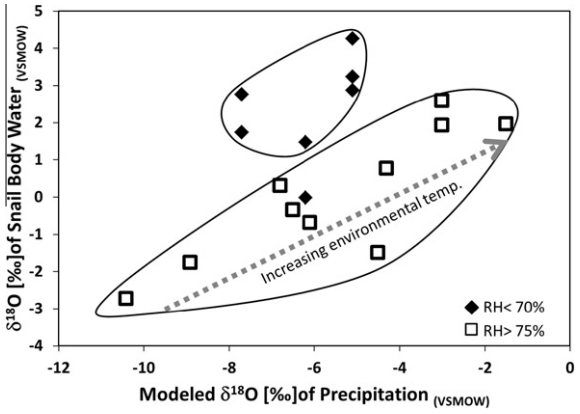

Fig. 4. Oxygen isotope composition of the snail body water, calculated from clumped isotopes temperatures and shell $\delta^{18}O$, compared to $\delta^{18}O$ of annual local precipitation. Regions are divided to humid (RH > 70%) and dry (RH < 70%). Precipitation $\delta^{18}O$ is based on Bowen and Revenaugh (2003). RH data from NCEP/NCAR reanalysis (Kalnay et al., 1996).

Snails obtain water through the integument of the foot (Prior, 1985; Heller, 1993), so that the source of body water is mostly rain, dew, and soil surface water, and a possible small contribution from digested vegetation, with both rain and dew reflecting condensed local vapor (Welp et al., 2008). Snail body water would therefore reflect the local rainfall, with $\delta^{18}O$ modified by soil surface evaporation, the extent of which depends mostly on the local relative humidity (RH). Snail shells reflect the rainfall occurring during the snails' growing season, with most snails being dormant during the dry or cold seasons.

The effect of evaporation may be characterized by separately examining body water in snails that grow in humid vs. dry conditions, with low RH resulting in more enriched body water (Fig. 4). Our data clearly divides into two groups following the local RH (high RH is >75%, low RH is <70%; Fig. 4). In both groups, snail body water shows clear ^{18}O enrichment relative to local rainfall, with enrichment being more pronounced at low RH locations. Within the high RH locations there is a weak but noticeable trend with temperature. Precipitation $\delta^{18}O$ values in warmer regions are typically higher, but show a lower effect of evaporative enrichment in snail body water; instead, the $\delta^{18}O$ difference between precipitation and snail body water reflects the temperature dependence of equilibrium fractionation factor in evaporation.

The two San Simeon snails discussed above as recording calcification temperatures that are significantly different from each other are split between the two RH groups. The higher temperature one falls within the low RH group, close to the samples from adjacent Palo Alto, whereas the other specimen is an outlier, and plots within the high RH group, consistent with our assumption that this individual represents anomalous conditions, either a human-modified (shaded and irrigated) micro-environment or an unusual year in terms of both temperature and moisture availability.

Body water $\delta^{18}O$ can thus be used as an indicator of variations in rainfall $\delta^{18}O$ and local evaporation. Our data indicates that knowing the actual calcification temperature is necessary in order to estimate water composition and that this might vary among regions and climatic conditions, and be affected by species-specific parameters such as color and shell morphology. Using land snails for paleo-hydrology reconstruction would further benefit from an understanding of the link between rainfall and body water $\delta^{18}O$. This may be facilitated by focusing snail-based paleo-hydrological analysis on specific regions and combining it with a thorough regional-scale characterization of the relationship between modern-day rain and snail $\delta^{18}O$.

Although it is possible that the offset between calcification temperature and ambient temperatures is in itself climate-related (and therefore region-specific), it is more likely that it reflects species-specific biological and behavioral adaptations. These biological adaptations are likely to vary in response to prolonged exposure to different environmental conditions associated with climate states that are radically different from the Holocene, such as those prevalent over the glacial–interglacial cycles. In other words, although in a colder climate a snail might not be able to warm its body to the same temperature possible under modern-day conditions, it does not necessarily imply a constant ambient-to-body temperature offset, as the species is able to adapt over time to the colder climate. As such, a regional analysis of modern snails may not be sufficient to develop a calcification-to-ambient temperature transfer function and the independent information provided by clumped isotopes would be required to determine paleo-calcification temperature. The potential climatic information stored in snail shells is therefore related to hydrological parameters that can be derived from $\delta^{18}O$, using the calcification temperature derived from Δ_{47}.

4.4. Implications for previous studies – revisiting Yapp (1979)

To illustrate the potential use of snail isotopic analysis as a paleo-hydrological tool, we used our clumped-isotopes based calcification temperatures to re-examine one of the early published datasets. Yapp (1979) performed a regional $\delta^{18}O$ study, comparing different modern species from climatically diverse regions (Utah, Wisconsin, South Carolina, Washington, Ohio, Louisiana, Mexico, and Norway) with estimated environmental temperatures ranging 13–22 °C and RH ranging 50–80%. Given that no Δ_{47} data is available for that dataset, we estimate calcification temperature based on the offset observed in our data from ambient temperature (both MAT given in the original study and snail activity season temperatures average based on NCAR reanalysis data; Fig. 3b). It is important to note that these calcification temperatures are only a first estimate and should not replace direct measurements, but may still serve as an illustrative example. These temperatures are then combined with shell $\delta^{18}O$ values to reconstruct the respective snail body water $\delta^{18}O$ (see SI for the complete dataset).

Since calcification temperatures are always higher than environmental temperatures, the resulting snail body water is more enriched than the original estimates, thus revealing the evaporative enrichment of surface water. Also here, dry regions (Sevier County, Utah; Lower Garnd Coulee,

Washington; and Taos County, New Mexico) reflect large water enrichment. The limited temperature range (20–22 °C) of the dataset, however, does not allow testing for enrichment trend with temperature. Using mean annual environmental temperatures, Yapp (1979) showed that the degree of isotopic enrichment in the snail is negatively correlated with local RH. Our re-analysis of his data does not change this basic conclusion, but emphasizes the role of local evaporation.

4.5. Paleo-climate implications

Whereas our observations have interesting implications for the eco-physiological behavior of land snails, they also suggest that the use of snail shells for paleo-climatic reconstruction on land is not as straightforward as may have been hoped. Clumped isotopes temperatures in land snail shells cannot be used to directly reconstruct air temperatures, but can be useful in a paleo-hydrological context as they provide the calcification temperatures required to extract rainwater isotopic signals out of shell oxygen isotopic composition. Snail shell isotopic composition records climatic conditions, but these are modified by species-specific calcification temperatures and region-specific evaporative enrichment at the soil surface. Hence, land snail-based paleo-hydrology requires a comparison with modern oxygen and clumped isotopes analysis, preferably of similar snail species, to provide a baseline for regional evaporation and its effect on snail body water.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gca.2011.08.044.

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