Oxygen stable isotopic disparities among sympatric small land snail species from northwest Minnesota, USA

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The oxygen isotopic composition (δ18O) of land snail shells can be a valuable paleoenvironmental archive if the climatic parameters that influence the isotopic system are fully understood. Previous calibration studies have examined a limited number of species or individuals, and most have focused on larger (>10 mm) taxa, which do not represent the dominant shell material in the Quaternary fossil record. In this study, we evaluate the δ18O values of small land snails (<10 mm), which are common in modern settings and are often preserved in a wide array of Quaternary geologic and archeologic deposits. Our primary goal was to determine if coexisting species record equivalent isotopic information in their shells, regardless of differences in their ecology, dietary habits, behavior, and/or body size. We collected and analyzed 265 individuals of 11 species from 12 sites in northwest Minnesota (USA), which exhibits extremely abundant and diverse terrestrial malacofauna in North America. We did not observe significant correlations between shell δ18O values and the type of ecosystem (forest/grassland) or hydrologic setting (upland/lowland). However, the majority of species differed significantly in shell δ18O values. Larger taxa (Catineilla, Succinea, Discus) consistently yielded higher δ18O values than smaller taxa (Euconulus, Gastrocopta, Hawaii, Vallonia), by up to ~3‰. These isotopic offsets among sympatric taxa could be attributed to a number of physical, behavioral, and/or evolutionary traits, including the ability of larger species to tolerate drier conditions better than their smaller counterparts, differences in their preferred microhabitats or phylogenetic non-independence. Regardless of the reason, our results imply that researchers should not combine isotopic data from different types of land snails without first investigating modern specimens to determine if it is appropriate. Moreover, our data suggest that combining instrumental climate data, a snail flux-balance model, and shell δ18O values can help us to better understand the ecology of land snails.

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1. Introduction

More than 1200 species of land snails have been identified and described in North America (see recent review by Nekola, 2014a). Over 40% of these species have shells that are categorized as small (<5–10 mm), minute (<2 mm), or micro (<2 mm) (Nekola, 2005; hereafter we refer to them collectively as “small”). Small land snails are present in a wide variety of ecosystems, ranging from the tropics to the high Arctic (Nekola and Coles, 2010; Nekola, 2014a,b), and their shells are frequently preserved in archeological, lacustrine, fluvial, wetland, loess, alluvial, and colluvial deposits (Yapp, 1979; Goodfriend and Ellis, 2000; Balakrishnan et al., 2005a; Paul and Mauldin, 2013). Even though small snail shells are abundant—often accounting for 90% or more of regional faunas (Nekola, 2014b), accessible, and are a potential source of paleoenvironmental and paleoecological information, their scientific study in North America has been mostly limited to radiocarbon dating (Pigati et al., 2004, 2010, 2013, 2015; Rakovan et al., 2010; Rech et al., 2012). Thus far, relatively few studies have used them to evaluate or reconstruct past environmental conditions in North America (Yapp, 1979; Goodfriend and Ellis, 2000; Balakrishnan et al., 2005a; Paul and Mauldin, 2013).

Fossilized shells of small land snails are excellent candidates for use in paleoenvironmental studies because (1) they exhibit a broad latitudinal/spatial coverage within the continental realm, spanning a wide variety of habitats ranging from rainforests to deserts, (2) they are highly sensitive to climate/environmental change, (3) they are plentiful and well preserved throughout the Quaternary geologic and archeologic records, (4) they are composed entirely of aragonite, so the presence of contaminants in fossil shells (typically in the form of calcite) can be detected using standard x-ray diffraction techniques, and (5) most Quaternary species are extant, so direct comparisons and calibrations using living counterparts are usually possible.
The oxygen isotopic composition (δ18O) of small fossil shells holds particular promise as a paleoenvironmental proxy. Yapp’s pioneering study (1979) was the first to exploit the relation between climate and oxygen isotopes in land snail shells, and inspired several dozen studies aimed at reconstructing various climate parameters. However, shell δ18O values are affected by multiple atmospheric variables that are often difficult to discriminate, including rainwater δ18O, water vapor δ18O, relative humidity, and temperature (Balakrishnan and Yapp, 2004). Thus, proper interpretation of shell δ18O values of land snail shells remains challenging.

Calibration studies using modern specimens from areas with well-constrained climate data can improve the interpretation of fossil shell δ18O data. In North America, only a handful of field studies have carried out calibration assessments using modern land snails (Yapp, 1979; Sharpe et al., 1994; Goodfriend and Ellis, 2002; Balakrishnan et al., 2005b; Yanes, 2015). In general, these studies have examined a limited number of species or individuals, and most have focused on larger (> 10 mm) taxa, which do not represent the most abundant shell material found in the Quaternary fossil record.

Another uncertainty when evaluating land snail isotopic data is whether or not different coexisting (sympatric) species record equivalent environmental information in their shells, regardless of potential differences in their ecology, dietary habits, behavior, and/or body size. Many land snail isotope studies have focused on a single species (e.g., Yanes et al., 2008; Colosene et al., 2010, 2011; Prendergast et al., 2015, 2016), which minimizes problems associated with potential variations resulting from differing shell ecologies or species-specific biological fractionations (i.e., vital effects). In other studies, however, researchers have combined isotopic data derived from multiple species (e.g., Yapp, 1979; Balakrishnan et al., 2005a,b; Zanchetta et al., 2005). In some instances, species that overlap in space and time appear to track equivalent isotopic information (e.g., Zanchetta et al., 2005; Yanes et al., 2009, 2011, 2013; Yanes and Romanek, 2013; Yanes, 2015), but this is not always the case (Balakrishnan et al., 2005b; Yanes et al., 2013). This issue is particularly relevant for studies with a broad spatio-temporal scope because land snail assemblages can vary tremendously over both space and time, making it difficult or impossible to use a single taxon for all analyses.

In this study, we evaluate the oxygen isotopic composition of multiple key species of small land snails collected from forest and grassland ecosystems in northwest Minnesota. The study sites are located in a mid-latitude region (46-48°N) at the transition between taiga, deciduous forest, and grassland biomes (Fig. 1), and contains a rich and diverse assemblage of land snails (Nekola, 2003). Moreover, relevant climate data from this sampling region is readily accessible for comparison with snail shell δ18O values. This study tests the hypothesis that multiple species of small land snails collected from the same region record equivalent δ18O values in their shells, regardless of differences in the local ecosystem (forest or grassland), hydrologic setting (upland or lowland), and physical properties (shell size, morphology, etc.). The δ18O data are then compared to climate data from the region and examined using an evaporative steady-state flux balance–mixing model for land snails developed by Balakrishnan and Yapp (2004). The results from this work will serve as a much-needed methodological reference for investigators working on fossil land snails in the geologic and archeologic records of North America and elsewhere.

2. Material and methods

2.1. Geographic and climatic setting

We collected live snails from a total of twelve localities in northwest Minnesota, including (from north to south) Beaches WMA, Halma Roadside, Halma Swamp, Strathcona, Old Mill SP, Oak Ridge, Hoot Forest, Eastlund, Waubun SE, Callaway N, Bluestem Prairie, and Barnesville WMA (Fig. 1; Table 1; Supplementary Table S1). These localities were chosen because they have similar climates, are positioned at approximately the same elevation (~330–567 m a.s.l.), exhibit comparable and relatively pristine woodland and grassland areas, and contain a diverse and abundant assemblage of small land snails (Nekola, 2003). In addition, air temperature and other climate data for the area are readily accessible through the National Oceanic and Atmospheric Administration’s National Centers for Environmental Information (https://www.ncdc.noaa.gov). We selected time-series climate data for the recording period between 2010 and 2017 from the northwest Minnesota region. Monthly average air temperatures in northwest Minnesota range from −13.7 °C to +20.9 °C, with an average annual temperature of −4.8 °C. The total annual precipitation amount is ~596 mm, with summer months being the wettest. In addition, the weighted average annual δ18O value of precipitation is −12 ± 1‰ (relative to Standard Mean Ocean Water; SMOW), ranging from −27‰ to −20.5‰ (SMOW) (Henderson et al., 2010).

2.2. Land snail field collection strategy

Documentation of terrestrial gastropods from each site was accomplished by hand collection of larger shells and litter sampling for smaller taxa from representative 100–1000 m² areas (Nekola, 2003). Soil litter sampling was primarily used as it provides the most complete assessment of site faunas (Oggier et al., 1998). As suggested by Emberton et al. (1996), collections were made at places of high micromollusc density, with a constant volume of soil litter (~4 l) being gathered at each site. For woodland sites, sampling was concentrated along the base of rocks or trees, on soil-covered bedrock ledges, and/or at places found to have an abundance of shells. For grassland sites, samples consisted of small blocks (~125 cm³) of turf, loose soil and leaf litter accumulations under or adjacent to shrubs, cobbles, boulders, and/or hummocks, and other locations observed to have an abundance of shells.

2.3. Land snail species

The malacofauna of northwest Minnesota include at least 41 different extant species (Hubricht, 1985; Nekola, 2003). Eleven species were selected for the present study including: Catinella avara (Say, 1824), C. exilis (Leigh and Jean, 1972), Discus catkiliensis (Pilsbry, 1896), Discus cronkhiti (Newcomb, 1865) [aka D. whitei (Newcomb, 1865)], Euconulus cf. alderi (Gray, 1840), E. fulvus (Müller, 1774), Gastrorota contracta (Say, 1822), G. tappaniana (Adams, 1842), Hawaiiia minuscula (Binney, 1841), Succinea (Novisuccinea) ovalis (Say, 1817), and Vallonia gracilicosta (Reinhart, 1883). Species were identified to species level based on shell features, geographic distribution, published descriptions, and anatomic comparisons with the mollusk collection at the University of New Mexico curated by one of us (JCN). We selected these species because they have high abundance in both modern settings and Quaternary sites across North America. We note that although the Succineidae (e.g., Catinella and Succinea) are usually medium to large in shell size (>10 mm), the results of previous work by our research group indicate they are similar to many smaller shells in terms of isotopic systematics (Pigati et al., 2010). Succineidae shells are also among the most common Pleistocene to Holocene land snail fossils, and thus we have included them in the present analysis.

Land snail physiology, ecology, and behavior suggest that survival and environmental factors depend on their ability to maintain adequate levels of internal body water across a wide variation of air temperatures and relative humidity (Wilbur and Yonge, 1964; Cook, 2001; Pearce and Ørstan, 2006). During periods of activity, snails secrete mucus, which is mostly made of water, and high levels of relative humidity reduce the risk of desiccation via evaporation. To accommodate these needs,
snails are most active when temperatures are above freezing and relative humidity is high (RH > 75%), mostly at night and immediately following precipitation events. Considering these requirements, we assume that the small land snails studied here build their shells between April and October (7 months of the year), when temperatures in northwest Minnesota are warmest (exceeding 10 °C) and precipitation is highest. Accordingly, the oxygen isotope composition of the shell should primarily record the environmental conditions of the part of the year when snails are active.

2.4. Laboratory analyses

Shells of 265 individual snails were rinsed in deionized water and subjected to ultrasonication to remove organic and detrital contaminants. Entire cleaned shells were ground manually in an agate mortar and pestle, and then analyzed at the Center for Stable Isotopes at the University of New Mexico using the method described by Spötl and Vennemann (2003). Samples were loaded in 12 ml borosilicate exetainers, which were flushed with He and reacted for 12 h with H₃PO₄ at 50 °C. The resulting CO₂ was measured in a Gasbench device coupled to the continuous flow Isotope Ratio Mass Spectrometer (CF-IRMS) from Thermo Fisher Scientific Delta V Plus. The isotope ratio measurements were calibrated using repeated measurements of the international standards NBS-19 and NBS-18. Isotopic results are reported in the standard δ notation relative to the international standard Pee Dee Belemnite (PDB). Overall precision was ±0.1‰ for δ¹⁸O and ±0.08‰ for δ¹³C (1σ).

2.5. Statistical analyses

All statistical analyses were performed using PAST 3.12 software (Hammer et al., 2001) considering statistical significance at α = 0.05. Kruskal-Wallis and Mann the Whitney-U tests were used to determine whether groups of median oxygen isotope values differ significantly from one another.

3. Results

The shell δ¹⁸O values of all analyzed modern small land snails (n = 265) from northwest Minnesota (Fig. 1) range between −9.1‰ and −2.5‰, with an overall average of −5.9 ± 1.1‰ (1σ; Table 1; Table S1). These values fall within the range of published shell δ¹⁸O values from North America, which currently varies between −11.9‰ and +1.0‰ (Yapp, 1979; Sharpe et al., 1994; Goodfriend and Ellis, 2002; Balakrishnan et al., 2005a; Yanes, 2015). Average δ¹⁸O values of snails collected from grassland settings (−5.9 ± 1.2‰) were essentially the same as those collected from forested areas (−6.0 ± 0.9‰) (Fig. 2A), and average δ¹⁸O values of snails collected from upland areas (−5.7
± 1.2%) were similar to those from lowland areas (−6.2 ± 0.9‰) (Fig. 2B). In addition, when all species and localities are pooled together, shell δ¹⁸O values do not correlate with δ¹³C values (r = −0.09; p = 0.109; n = 265), which themselves range from −12.8 to −2.6‰, with an overall average of −9.1 ± 2.1‰. Although δ¹³C values are not the focus of this paper, we note that snails from grasslands exhibited δ¹³C values that were several per mil higher than those from forests (Fig. 2A), suggesting that small snails may record a significantly different diet/vegetation depending on the local ecosystem that likely reflects the isotopic signature of consumed and assimilated C₃ plants with different water-stress levels (Farquhar et al., 1989).

Interestingly, when examining the oxygen isotope data as a whole, there are significant differences (Kruskal-Wallis, p < 0.001) in the δ¹⁸O values between individual taxa (Fig. 3A). Isotopic differences are significant for both median and mean values. For example, species of the genera Catinella and Succinea, both of which are in the Succineidae family, and species of the genus Discus consistently yielded the highest shell δ¹⁸O values, whereas species of the genera Euconulus and Hawaiia yielded the lowest shell δ¹⁸O values (Fig. 3A). This pattern held true at individual sites as well, with the δ¹⁸O values of Catinella, Succinea, and Discus always exhibiting the highest δ¹⁸O values and Euconulus and Hawaiia consistently yielding the lowest δ¹⁸O values (Fig. 3B–D). The isotopic dispersion, or range of isotopic values, between sites and species within a site remained relatively comparable (Table 1; Figs. 2, 3A–D).

Some individuals yielded extreme δ¹⁸O values that can be statistically considered outliers because they are notably distant from the values obtained from the remaining individuals within the population (see dots and asterisks in Fig. 3). This reinforces the idea that numerous specimens rather than one or two should be analyzed per sampling site to capture the full range of isotopic variability within a snail population.

### 4. Discussion

#### 4.1. Influence of snail body size on shell δ¹⁸O values

Of the 11 taxa studied here, Succinea exhibits the largest adult body size (≥ 20 mm in adult shell length) followed by Catinella (6–9 mm), Discus (average = 5 mm), and Euconulus, Gastrocopta, Hawaiia, and Vallonia averaging only 2–3 mm (Nekola, 2014a). Interestingly, the average shell size for these species showed a positive relationship with the average shell δ¹⁸O values, with shells of the larger taxa (Succinea, Catinella, Discus) consistently yielding δ¹⁸O values that are higher than the smaller taxa (Fig. 4). This correlation between snail size and shell δ¹⁸O values has been documented in studies of larger snails from Europe, including Cepaea nemoralis (Yanes et al., 2012) and Spihincteroida candissimina (Yanes and Fernández-López-de-Pablo, 2016), and the small Caracollina lentilica from the Canary Islands (Bullard et al., 2017), but has not been documented previously for small land snails in North America.

Some studies have suggested that larger snails are better adapted to dryness because they are less prone to water loss via evaporation as a consequence of a lower surface-to-volume ratio and/or aperture size relative to overall shell size (Nevo et al., 1983; Yanes et al., 2012). The actual reasons behind the observed link between snail size and shell δ¹⁸O are still unknown. We, however, can speculate that this pattern could perhaps result from differences in physical and/or behavioral characteristics between the different types of small land snails studied here. For example, the larger taxa may be active during the day when humidity is relatively low, whereas smaller taxa are active at night when humidity is higher. Similarly, larger taxa may be active during different parts of the year and be able to tolerate drier months better, therefore experiencing different seasonal conditions than smaller taxa. Alternatively, they may all be active during the same time periods, but
larger taxa could be more mobile, thereby encountering more open-air conditions than smaller taxa living deep within leaf litter microenvironments. It is also possible that these small taxa prefer to live within very specific microhabitats within the leaf litter itself. For example, small land snail taxa may be stratified vertically in relation to litter depth, with different species preferring particular depths (Boag, 1985; personal field observations, 2010–2017). In such cases, snails living at greater depths would be more protected from diurnal, and perhaps even seasonal, climate fluctuations than those living at shallower depths. Lastly, it is possible that shell δ18O systematics is a function of evolutionary relationships with some phylogenetic groups exhibiting lower shell δ18O values than others for reasons that are independent of shell size.

An additional consideration is the potential for species-specific biological fractionation, or “vital effects”, which could affect the shell δ18O values of some or all of the taxa studied here. Vital effects are entirely unknown for both small and large land snails and can only be differentiated from the potential microenvironmental effects described above by raising snails in the laboratory under controlled conditions, which is beyond the scope of this study. Thus, for now, we assume that vital effects in small land snails are negligible, although we acknowledge that further testing is required to evaluate if this is truly the case.

4.2. Influence of interspecies differences on relative humidity calculations

We used the snail evaporative steady state flux balance-mixing model developed by Balakrishnan and Yapp (2004) to determine if calculated relative humidity values are significantly different for the coexisting snail species in northwestern Minnesota assuming that other variables are held constant. The model establishes a relationship between the δ18O of water vapor, the flux and δ18O of water imbibed from local precipitation, the amount and isotopic composition of the snail body fluid, the diffusive flux of water from the snail body by evaporation, and the temperature-dependent oxygen isotope fractionation between the snail body fluid and the aragonitic shell (Grossman and Ku, 1986). The model also considers the flux of water output from the body fluid (fH2O) relative to the flux of precipitation ingested by the snail (fH2O). The ratio is called θ and is defined as θ = fH2O / fH2O. Balakrishnan and Yapp (2004) demonstrated that it is appropriate to assume that water vapor is in isotopic equilibrium with the precipitation. Finally, these authors also demonstrated that it is appropriate to assume that snail body water is lost by evaporation from the snail body plus some liquid water expelled by the snail (i.e., θ = 0), which introduces minimal error in calculations. Accordingly, θ = 0 is adopted here for the calculations.

Model calculations were constrained using the measured shell δ18O values, the air temperature when snails likely deposited shell (−14–17 °C), and the δ18O values of precipitation in the studied area (−10‰ to −12‰ [SMOW]). Two scenarios were considered. The first, snails were active and grew shell for a period of 7 months, between April and October, when average air temperature was −14 °C and average rain δ18O was −12‰ (SMOW) (solid line in Fig. 5). The second, snails were active only for 5 months, between May and September, when average air temperature was −17 °C and average rain δ18O was −10‰ (SMOW) (dashed line in Fig. 5).

If two of the species with extreme δ18O values (e.g., Euconulus and Discus) from a specific microhabitat (e.g., Halma Swamp) are examined in the context of this model, the model results suggest that Euconulus was active at times or in areas when RH was at least 5% higher than when Discus was active (Fig. 5). The calculated RH values from the model suggest that land snails are active and grow shell at times when RH is higher than the average RH for Minnesota (−60%). Although we acknowledge that there is large uncertainty associated with RH calculations, the model can provide credible climate scenarios during snail activity.

An alternative scenario is that both snails were active under similar RH conditions, but the larger Discus was active when temperature was warmer (−17 °C) and rainwater δ18O value was higher (−10‰ vs SMOW) while the smaller Euconulus was active at cooler temperatures (−14 °C) and lower rain δ18O values (−12‰ vs SMOW). This alternative situation suggest that the larger species was active during a shorter (warmer/drier) time of the year (5-months) than the smaller one, tracking therefore a higher rain δ18O value (−10‰ vs SMOW).

In any case, our results imply that for investigations that use small snail δ18O values as a paleoclimatic proxy, it is necessary to analyze and compare data from the same or closely similar taxa (perhaps also similar in shell size), rather than combining data extracted from different species.

If the differences in the median and mean δ18O values of small snails from North America indeed reflect differences in snail active periods or microhabitat preferences, then the shell δ18O values could be used to complement ecological studies that attempt to assess snail behavior in different environments/ecosystems. This is particularly relevant for small snails because little is known about their ecology and behavior at the species and microhabitat level, which is not surprising considering the small size of the snails and how difficult it is to observe them in the field.

5. Conclusions

In contrast to some published studies, our results show that sympatric small land snails from northwest Minnesota exhibit significantly different oxygen isotopic compositions. Species within a given genus yielded similar values, but δ18O values diverged at the genus and family levels. Specifically, shells of larger taxa (Catineilla, Succinea, Discus) consistently yielded higher δ18O values, whereas smaller taxa (Euconulus,
Gastrocopta, Hawaiia, Vallonia) yielded lower values. This relation holds true when examining the entire dataset as a whole, which encompassed 265 individual specimens from 12 localities, as well as for taxa coexisting at any given site.

The differences in δ¹⁸O values between various genera are likely due to some combination of (1) differences in the ability of some taxa to better tolerate drier conditions on a daily or seasonal basis, (2) differences in the mobility between large and small taxa which could potentially expose more mobile taxa to drier conditions compared to those seques-tered deep under leaf litter, and (3) potential habitat preferences within a given microsite leading to vertical stratification of taxa and differential exposure to the elements. Regardless of the exact reason, our results clearly demonstrate that it is not always appropriate to combine the oxygen isotopic data of different genera or families and that calibration

![Fig. 3. A. Oxygen isotope values of modern land snails from all localities from northwest Minnesota combined, separated by species. B. Oxygen isotope values of snails from Halma Swamp only. C. Oxygen isotope values of snails from Waubun SE only. D. Oxygen isotope values of snails from Oak Ridge only. Data are presented as box-plots. Line inside the box represents median value. Box extremes represent quartile distribution. Whiskers depict range of values. Dots and asterisks depict outliers. Numbers between parentheses represent the number of shells analyzed.](image1)

![Fig. 4. Relationship between land snail shell maximum length (mm) and mean oxygen isotope composition of the shell. Dots depict mean values whereas whiskers represent the standard deviation of the data. Body size data was adopted from Nekola (2014a) and the species descriptions provided by the Carnegie Museum of Natural History webpage (http://www.carnegiemnh.org/science/mollusks/index.html). Note how larger body size snails (e.g., Catinella, Succinea, and Discus) exhibit higher oxygen isotope values, despite occupying the same habitat and microhabitat together with smaller contemporaneous species.](image2)
using modern specimens from the same genus/species is critical in order to properly evaluate isotopic data derived from fossil shells.

Finally, $\delta^{18}O$ values of the shells of small land snails can be evaluated using a published snail flux balance-mixing model constrained with observed climatic data to reconstruct climatic variables, such as relative humidity, that are otherwise extremely difficult to constrain. Our results suggest that, in addition to use in paleoenvironmental studies, interesting ecological or behavioral characteristics may be investigated using stable isotopes of small snail shells. This approach may be particularly valuable for small land snails, considering that observing their behavioral characteristics through field observations is nearly impossible, and little to nothing is known about their ecology.

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