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Stable isotope ecology of land snails from a high-latitude site near Fairbanks, interior Alaska, USA

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ABSTRACT

Land snails have been investigated isotopically in tropical islands and mid-latitude continental settings, while high-latitude locales, where snails grow only during the summer, have been overlooked. This study presents the first isotopic baseline of live snails from Fairbanks, Alaska (64°51′N), a proxy calibration necessary prior to paleoenvironmental inferences using fossils. δ^{13}C values of the shell (-10.4 ± 0.4‰) and the body (-25.5 ± 1.0‰) indicate that snails consumed fresh and decayed C_3-plants and fungi. A flux-balance mixing model suggests that specimens differed in metabolic rates, which may complicate paleovegetation inferences. Shell δ^{18}O values (-10.8 ± 0.4‰) were ~4‰ higher than local summer rain δ^{18}O. If calcification occurred during summer, a flux-balance mixing model suggests that snails grew at temperatures of ~13°C, rainwater δ^{18}O values of ~−15‰ and relative humidity of ~93%. Results from Fairbanks were compared to shells from San Salvador (Bahamas), at 24°51′N. Average (annual) δ^{18}O values of shells and rainwater samples from The Bahamas were both ~10‰ ^{18}O-enriched with respect to seasonal (summer) Alaskan samples. At a coarse latitudinal scale, shell δ^{18}O values overwhelmingly record the signature of the rainfall during snail active periods. While tropical snails record annual average environmental information, high-latitude specimens only trace summer season climatic data.

Introduction

Land snails contain an aragonitic shell that is fairly durable in archeological and paleontological sites and, accordingly, is commonly preserved in Quaternary outcrops (Goodfriend, 1992, 1999). Commonly, land snails are the only biological material that is both abundant and well-preserved in terrestrial sedimentary records, so they may be the only biotic source of paleoclimatic information. In particular, the carbon (δ^{13}C) and oxygen (δ^{18}O) isotopic composition of land snail shelly records are valuable terrestrial proxies generally used to reconstruct the paleovegetation (e.g., relative abundance of photosynthetic pathways) and the paleoatmospheric conditions (e.g., rainfall and humidity), respectively (Balakrishnan and Yapp, 2004). However, the calibration and validation of land snails as a credible environmental proxy are not as straightforward because both the δ^{13}C and the δ^{18}O values are influenced by multiple variables simultaneously (Balakrishnan and Yapp, 2004), and the magnitude at which various physical parameters affect the isotopic signature of the shell may vary between species, localities and even the temporal and spatial scale considered. Thus, studies that quantify the environmental significance of the isotopic signature of land snails using present-day samples are timely and highly valuable for paleoclimatologists and paleontologists.

Laboratory experiments by Stott (2002) and Metref et al. (2003) indicated that the δ^{13}C values of the shell are a function of the δ^{13}C values of the consumed and assimilated plant matter, whereas atmospheric CO_2 and carbonate ingestion had a minor effect in the shell δ^{13}C values. However, this does not seem to be the case in every setting and for all taxa because snails occupying carbonate-rich areas may incorporate carbon from limestone ingestion into their shells (Goodfriend and Hood, 1983; Goodfriend, 1987; Yanes et al., 2008; Pigati et al., 2010, 2013). Moreover, a recently published laboratory experiment by Zang et al. (2014) proposed that although the snail shell is predominantly influenced by diet, both carbonate ingestion and atmospheric CO_2 could also play a role. Balakrishnan and Yapp (2004) pointed out that different individuals of the same and different taxa might vary in metabolic rates, which could be reflected in the snail carbon isotope pool and may further complicate paleovegetation inferences.

While no published laboratory experiment has attempted to monitor the controlling factors of shell δ^{18}O values to date, Balakrishnan and Yapp (2004) used empirical and theoretical data and concluded that the dominant variables controlling the δ^{18}O values of the shell include rainwater δ^{18}O values, water vapor δ^{18}O values, relative humidity and temperature. It is generally assumed that O_2 from the atmosphere and the δ^{18}O values of water derived from ingested plants both have a negligible effect in the snail oxygen isotope pool (Balakrishnan and Yapp, 2004). Despite the large number of environmental variables

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controlling snail shell $\delta^{18}O$ values, the majority of published field studies suggest that snails seem to be principally influenced by rain $\delta^{18}O$ values (Lécolle, 1985; Zanchetta et al., 2005; Yanes et al., 2008, 2009) or by both rain $\delta^{18}O$ values and relative humidity (Balakrishnan et al., 2005a,b; Yanes et al., 2011a). Conclusively, the environmental meaning of the isotopic codes of land snail shells can be complex to understand but potentially useful to deduce informative aspects of past continental climates.

Apart from the relative temporal continuity of fossil shells in Quaternary sedimentary records, land snails exhibit a large spatial presence along latitude, ranging from the tropics to the high-arctic tundra (Pearce and Örstan, 2006). However, this proxy has been principally calibrated and used in tropical-subtropical oceanic islands (Baldini et al., 2007; Yanes et al., 2008, 2009, 2011a, 2013a; Yanes and Romanek, 2013) and mid-latitude coastal and inland continental sites (e.g., Yapp, 1979; Lécolle, 1985; Goodfriend and Ellis, 2000, 2002; Zanchetta et al., 2005; Balakrishnan et al., 2005a,b; Kehrwald et al., 2010; Colonese et al., 2007, 2010a,b, 2011, 2013a,b; Yanes et al., 2011b, 2012, 2013b; Yanes et al., 2014).

The present work investigates the isotopic composition of land snails from a woodland ecosystem in Fairbanks (Alaska), at the latitude of 64°51′N, longitude of 147°49′W, and elevation of 189 m (a.s.l.). This study presents land snail stable isotope systematics from the highest-latitude continental interior locality reported in the published literature after Yapp (1979), who analyzed four land snail shells from the coastal site of Sandnessjoen (Norway), at a latitude of ~66°N. Quaternary permafrost soils and eolian deposits from Alaska are rich in fossil land snails (Pigati et al., 2013) and these materials would potentially provide valuable insights into past climates at high latitudes. But before ancient shells from Alaska can be used to deduce paleoenvironments, proxy calibration and validation are necessary using present-day samples. This work discusses the environmental significance of carbon and oxygen stable isotope values of modern land snail tissues (body and shell) and potential dietary sources (vascular and non-vascular plants, soil organics and fungi) from a boreal forest of Fairbanks and explores the utility of land snails as an environmental archive at high latitudes. Isotopic data from Alaska (64°N) are explored using published flux-balance mixing models by Balakrishnan and Yapp (2004). Finally, modern land snail samples from the tropical island of San Salvador, Bahamas (at the latitude of ~24°N) were analyzed and discussed for comparison with Alaskan counterparts.

Methods

Present climate in Fairbanks

Fairbanks, interior Alaska (Fig. 1A), exhibits a continental climate, with extreme seasonal variability in solar radiation. The Alaskan Climate Research Center (http://climate.gi.alaska.edu/) indicates that for the recording period between 1981 and 2010, annual precipitation averaged 274.6 mm, ranging from 54.9 mm in July to 6.4 in March (Fig. 2A). The mean annual air temperature is −2.5°C, varying from 16.9°C in July to −22.2°C in January (Fig. 2A). Snow falls year-round except for the summer months (Fig. 2B). Average annual relative humidity is 61.8%, and fluctuates from 72.5% in December to 48.5% in May (Fig. 2B). Maximum relative humidity values reach up to ~99% in August. The climate in Fairbanks during the summer (from May to September), when snails are active and grow their shell, is characterized by mean air temperatures of ~13°C, total precipitation of ~181 mm, and average relative humidity of ~57% (Figs. 2A–B). The stable isotope composition of the summer precipitation in Fairbanks is available from the Bonanza Creek ELTER webpage (http://www.lter.uaf.edu/data_detail.cfm?datafile_pkey=66). For the recording period between June 2009 and August 2010 (snail samples in this study were live collected in August 2010), the summer rainwater exhibited an average $\delta^{18}O$ value of $-15 \pm 3‰$ ($n = 204$), ranging from −6.5‰ to −23.6‰ (Figs. 2C–D).

Hibernation of land snails

Land snails are ectothermic, i.e., they do not regulate their internal body temperature. Consequently, snails that inhabit high-latitude regions, which receive substantial amounts of snow during part of the year and below zero air temperatures during many months, hibernate for a large number of months (Gomot de Vaufleury, 2001). Snails can be either freezing-tolerant (those who survive freezing body fluids) or freezing-intolerant (those who cannot stand freezing body fluids and survive by extending their supercooling ability) (Ansart et al., 2002). Snail physiological and ethological mechanisms to survive cold hardiness include (Nicolai et al., 2010, 2012): (1) reducing the volume of freezable water, (2) accumulating cryoprotectans (e.g., polyhydric alcohols, saccharides, free amino acids and lipids) by body water loss and increasing of body fluid osmolarity, which lowers the freezing point of the snail body fluid, (3) borrowing into the soil and (4) forming

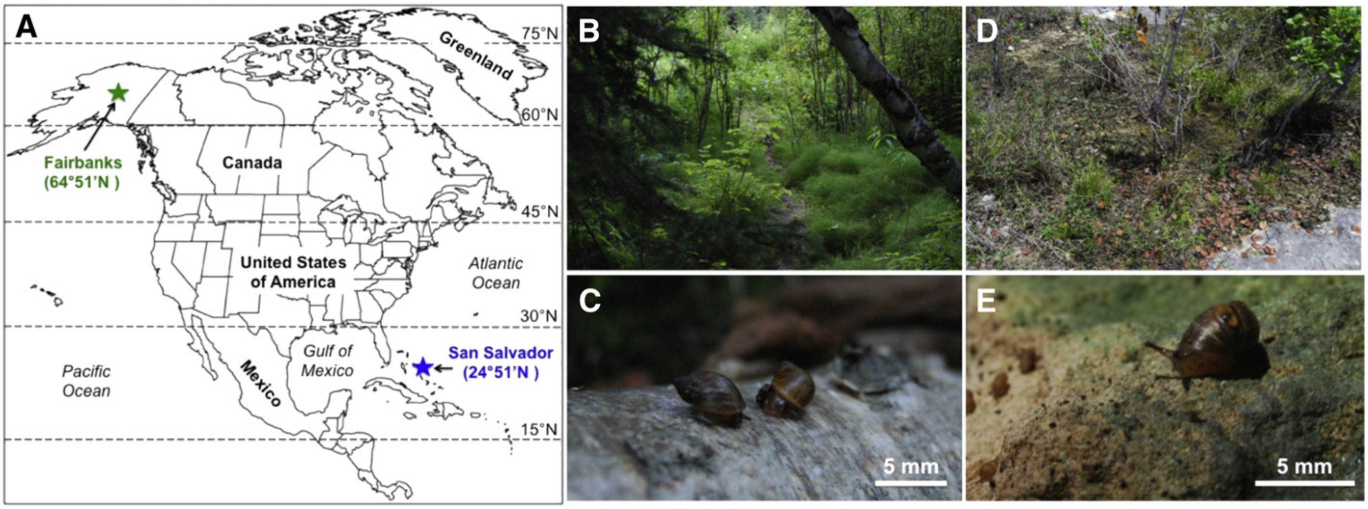

Figure 1. Geographical location of the two sampling sites (Fairbanks and San Salvador Island) in this study (A). Photographs of the interior boreal forest sampled (B) and the snail species (*Succinea strigata*) collected (C) in Fairbanks. Photographs of the coastal environment sampled (D) and the snail species (*Succinea barbadensis*) gathered (E) in San Salvador, Bahamas.

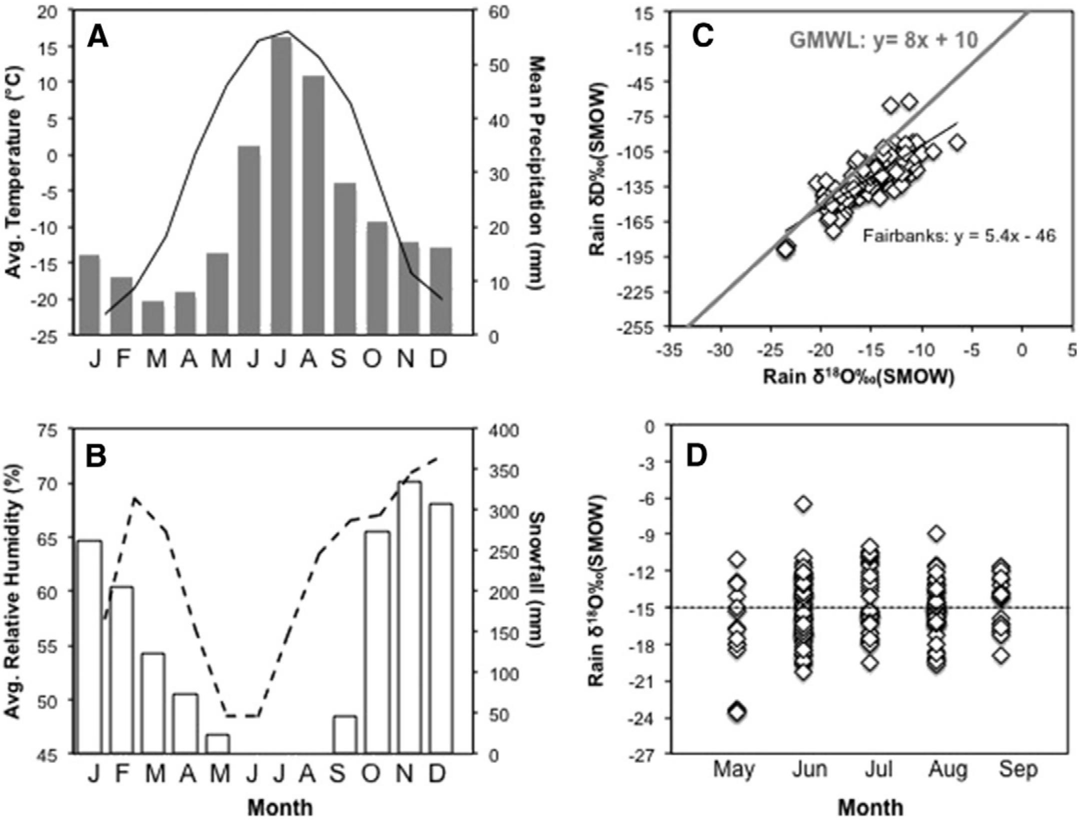

Figure 2. Present climate in Fairbanks. (A) Mean monthly temperature and precipitation. (B) Average monthly relative humidity and snowfall. (C) Deuterium and oxygen stable isotope values of summer rainfall in Fairbanks. Plotted data come from June, July, August and September 2009 and May 2010. (D) Oxygen stable isotope values of the summer rainfall in Fairbanks (from June 2009 to May 2010) by month.

an epiphragm. Many land snail species from high-latitudes are, in part, freezing tolerant, i.e., they can survive some ice formation within their body for a limited time, with variable supercooling abilities (Ansart et al., 2002). Snail mortality during hibernation can be high and may control community dynamics, however, despite relatively high mortality rates, high-latitude snails may live from several months to more than 2 yr (Nicolai et al., 2012).

Target land snail species and sampling protocol

Fairbanks

All snail samples ($n = 35$ total) were live-collected in the woodlands of Fairbanks, interior Alaska, about 1 km north of the University of Alaska, Fairbanks, during August 2010 (Table 1). After visual searching for snails, two species of small (<5 mm) land snails were found: *Succinea aff. strigata* ($n = 25$) and *Euconulus aff. fulvus* ($n = 10$). Several specimens of each species were deposited in the Carnegie Museum of Natural History at Pittsburg, with catalog numbers of CM139308 for *Euconulus* and CM139309 for *Succinea*. The family Succineidae exhibits a cosmopolitan geographical distribution, being present in almost every continent, and exhibits an extensive fossil record, especially in Quaternary loess deposits of North America (Pigati et al., 2010, 2013) and Europe (Moine et al., 2005, 2008). Succineid species often live from 1 to 2 yr and are associated with swamp and periodically flooded areas. Generally, succineids have a semelparous life cycle, reproducing in the summer and hibernating during winter (Örstan, 2010). The family Euconulidae also exhibits a widespread geographical distribution. In particular, *Euconulus fulvus* is broadly present in North America, especially in cool and humid soils containing dead wood, and seems to tolerate non-calcareous soils.

San Salvador, Bahamas

For comparative purposes, a total of n = 15 *Succinea barbadensis* specimens were collected in the tropical carbonate-rich island of San Salvador (Bahamas) during July 2010 (Table 2). Fresh dead shells of *Succinea* were gathered from a coastal site near Cockburn Town. The sampling locality was characterized by open-vegetation dominated by shrubs and grasses. The mean annual temperature in this island is ~24°C and the amount-weighted rain $\delta^{18}O$ value is −4.5‰ (SMOW), on average. Organic matter samples from this locality were not available. For additional details about the geology and environmental setting of San Salvador see Yanes and Romanek (2013).

Organic matter sample collection and preparation

Several potential carbon food resources of snails, including fresh tree leaves ($n = 1$), mosses ($n = 1$), fungi ($n = 1$) and soil organic matter ($n = 1$) were collected next to the sampled snail assemblage in Fairbanks (Table 3;Fig. 1B). In addition, snail body tissues of some *Succinea* specimens ($n = 20$ out of 25 *Succinea* individuals) and homogenized snail feces from several *Succinea* specimens ($n = 1$) were selected for isotopic analysis. About 1.5 mg of snail tissue and ~5 mg of plant and fungi tissue were weighed in a tin capsule, crimped and combusted in an Elemental Analyzer (EA). The CO_2 and N_2 produced after combustion were analyzed using the IRMS. Analytical uncertainty was ±0.1‰.

Carbonate sample preparation

Shells were cleaned in distilled water and ultrasonication, and dried at 40°C overnight. Entire shells ($n = 35$ total) were finely ground manually using an agate mortar and pestle. Entire shell was preferred over

Table 1
Stable carbon and oxygen isotope results of live-collected land snails from Fairbanks (Alaska) collected during August 2010.

Snail ID	Species	Locality	Latitude	Body $\delta^{13}C$ (PDB)	Shell $\delta^{13}C$ (PDB)	$\Delta^{13}C$ (Shell-Body)	Shell $\delta^{18}O$ (PDB)
FAI-snail-1	*Succinea strigata*	Fairbanks, Alaska	64°51′N		−10.9		−10.3
FAI-snail-2	*Succinea strigata*	Fairbanks, Alaska	64°51′N		−10.3		−10.4
FAI-snail-3	*Succinea strigata*	Fairbanks, Alaska	64°51′N		−10.5		−11.3
FAI-snail-4	*Succinea strigata*	Fairbanks, Alaska	64°51′N		−10.4		−10.8
FAI-snail-5	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−24.9	−10.2	14.7	−10.9
FAI-snail-6	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−25.3	−10.4	14.9	−10.7
FAI-snail-7	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−26.1	−10.8	15.3	−10.7
FAI-snail-8	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−24.2	−10.2	14.0	−10.8
FAI-snail-9	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−26.0	−9.9	16.1	−10.8
FAI-snail-10	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−26.0	−10.8	15.3	−10.4
FAI-snail-11	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−24.9	−10.8	14.1	−10.9
FAI-snail-12	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−27.6	−11.0	16.7	−11.1
FAI-snail-13	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−24.7	−10.2	14.6	−10.8
FAI-snail-14	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−25.9	−10.7	15.1	−10.8
FAI-snail-15	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−26.2	−10.0	16.2	−10.3
FAI-snail-16	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−26.3	−10.1	16.2	−11.0
FAI-snail-17	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−23.0	−9.8	13.2	−11.3
FAI-snail-18	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−25.9	−10.4	15.4	−10.6
FAI-snail-19	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−25.3	−10.3	15.0	−11.0
FAI-snail-20	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−25.9	−11.1	14.9	−10.5
FAI-snail-21	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−24.3	−9.7	14.6	−11.3
FAI-snail-22	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−25.3	−10.8	14.5	−10.9
FAI-snail-23	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−26.5	−10.4	16.2	−10.8
FAI-snail-24	*Succinea strigata*	Fairbanks, Alaska	64°51′N	−25.4	−10.6	14.8	−10.8
FAI-snail-25	*Succinea strigata*	Fairbanks, Alaska	64°51′N		−10.4		−10.8
FAI-snail-26	*Euconulus fulvus*	Fairbanks, Alaska	64°51′N		−11.2		−10.9
FAI-snail-27	*Euconulus fulvus*	Fairbanks, Alaska	64°51′N		−10.4		−10.3
FAI-snail-28	*Euconulus fulvus*	Fairbanks, Alaska	64°51′N		−10.8		−10.7
FAI-snail-29	*Euconulus fulvus*	Fairbanks, Alaska	64°51′N		−10.8		−11.1
FAI-snail-30	*Euconulus fulvus*	Fairbanks, Alaska	64°51′N		−10.3		−11.3
FAI-snail-31	*Euconulus fulvus*	Fairbanks, Alaska	64°51′N		−10.6		−10.9
FAI-snail-32	*Euconulus fulvus*	Fairbanks, Alaska	64°51′N		−10.8		−10.5
FAI-snail-33	*Euconulus fulvus*	Fairbanks, Alaska	64°51′N		−10.5		−11.8
FAI-snail-34	*Euconulus fulvus*	Fairbanks, Alaska	64°51′N		−10.5		−11.9
FAI-snail-35	*Euconulus fulvus*	Fairbanks, Alaska	64°51′N		−11.0		−9.4

intrashell analyses because the goal of this work was to evaluate the dominant climatic controlling factors of snails over their annual-biannual lifespan. In addition, because analyzed species were considerably small (<5 mm maximum length) with quite thin shells, intrashell analyses were not possible. Samples were treated with 3% H_2O_2 overnight to remove potential organic contaminants. About 150 μg of carbonate was weighted in a 6 ml Exetainer^{TM} vial that was subsequently flushed with helium. The carbonate was then converted to CO_2 gas by adding 0.1 ml of 100% H_3PO_4 at 25°C. The resulting CO_2 was analyzed after 24 h using the GasBench II connected to an IRMS. Analytical uncertainty was ±0.1‰ for both carbon and oxygen isotopes.

Stable isotope analysis

Samples were measured in the stable isotope facility of the Department of Earth and Environmental Sciences, University of Kentucky. Organic matter samples (snail body tissue, snail feces, plants, fungi and soil organic matter) were analyzed in a Costech Elemental Analyzer

Table 2
Stable carbon and oxygen isotope results of modern land snail shells from San Salvador Island (Bahamas) collected during July 2010.

Snail ID	Species	Locality	Latitude	Shell $\delta^{13}C$ (PDB)	Shell $\delta^{18}O$ (PDB)
CT-snail-1	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−6.5	−0.2
CT-snail-2	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−5.8	−0.4
CT-snail-3	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−6.3	−0.7
CT-snail-4	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−4.6	+0.1
CT-snail-5	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−5.7	−0.7
CT-snail-6	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−5.5	−0.2
CT-snail-7	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−7.1	+0.8
CT-snail-8	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−6.4	−0.7
CT-snail-9	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−9.0	−1.3
CT-snail-10	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−7.5	−0.7
CT-snail-11	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−7.5	−0.6
CT-snail-12	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−6.1	−0.7
CT-snail-13	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−7.1	−0.5
CT-snail-14	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−4.6	+0.1
CT-snail-15	*Succinea barbadensis*	Cockburn Town, San Salvador	24°60′N	−6.9	−0.9

Table 3
Stable carbon isotope values of succineid snail feces and potential snail dietary sources in the boreal forest of Fairbanks, Alaska. Each bulk sample represents a mixture of multiple individuals that were homogenized together.

Sample ID	Sample type	Locality	Latitude	δ^{13}C (PDB)
FAI-feces-1	Snail feces	Fairbanks, Alaska	64°51′N	−29.3
FAI-diet-1	Tree leaves	Fairbanks, Alaska	64°51′N	−28.1
FAI-diet-2	Fungi	Fairbanks, Alaska	64°51′N	−19.7
FAI-diet-3	Moss	Fairbanks, Alaska	64°51′N	−34.4
FAI-diet-4	Bulk soil organics	Fairbanks, Alaska	64°51′N	−27.1

(ESC 4010) connected to a continuous flow isotope ratio mass spectrometer (IRMS) Finnigan Delta$^{\mathrm{PLUS}}$ XP. Aragonitic shells were measured in a GasBench II connected to the same IRMS. All stable isotope results are reported in δ notation relative to the international standard Pee Dee Belemnite (PDB) for both organic matter and carbonate samples. The δ values are defined as:

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

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

Results

Fairbanks

Two species of land snails were found during a field survey in August 2010 in a boreal forest of Fairbanks (Table 1): (1) *Succinea strigata* ($n = 25$), with a maximum shell length of ~5 mm (Fig. 1C), and (2) *E. fulvus* ($n = 10$), a microsnail with maximum shell length of ~2 mm. Specimens were found alive attached to fallen logs on the forest floor. Despite their difference is size, both species showed statistically equivalent (*t*-test, $p > 0.05$) carbon and oxygen isotope values in their shells, and therefore, they are treated collectively. Shell δ^{13}C values ranged from −11.2‰ to −9.7‰ (Figs. 3A–B), with an average value of −10.4 ± 0.4‰ ($n = 35$). Bulk body tissue was measured for 20 *Succinea* specimens. Body δ^{13}C values ranged from −27.6‰ to −23.0‰ (Fig. 3B), averaging −25.5 ± 1.0‰ ($n = 20$). Shell and body δ^{13}C values correlated weakly (-Fig. 3B). While shells showed a reduced range in δ^{13}C values (Δ^{13}C = 1.4‰), body tissues exhibited significant variations among specimens (Δ^{13}C = 4.6‰). Shells were, on average, 15‰ ^{13}C-enriched with respect to body tissue (Fig. 4A). Potential food resources for land snails in the studied habitat included C_3 vascular plants, moss, fungi and decayed soil organic matter. One sample of each potential carbon source was measured and displayed respective δ^{13}C values of −28.1‰, −34.4‰, −19.7‰ and −27.1‰ (Table 3; Fig. 4A). Finally, a homogenized sample of snail feces from multiple *Succinea* specimens showed a δ^{13}C value of −29.3‰ (Fig. 4A). Snail body tissue was ~3.8‰ higher in ^{13}C than snail feces (Table 3).

The δ^{18}O values of the snail shells from Fairbanks ranged from −11.9‰ to −9.4‰ (Figs. 3A–B), averaging −10.8 ± 0.4‰ ($n = 35$). On average, shell δ^{18}O values are ~4.2‰ ^{18}O-enriched with respect to summer rainwater from the region (Fig. 4B).

San Salvador, Bahamas

For comparison, several specimens of *S. barbadensis* collected in July of 2010 in Cockburn Town, San Salvador (Bahamas), located at the latitude of 24°N (Figs. 1A, D–E), were analyzed isotopically (Table 2). The shell δ^{13}C showed an average value of −6.4 ± 1.0‰ ($n = 10$), ranging from −9.0‰ to −4.6‰. Thus, shells from Bahamas were ~4‰ higher in ^{13}C than counterparts from Fairbanks.

Shell δ^{18}O values ranged from +0.8‰ to −1.3‰ (Fig. 3C), with an average value of −0.4 ± 0.5‰ ($n = 10$). Bahamian shells were ~4.9‰ higher in δ^{18}O than local rainfall (Fig. 4B). Snail shell δ^{18}O values from the Bahamas were, on average, ~10‰ higher (in PDB scale) than shells from Fairbanks (Fig. 4B). Similarly, annual rainfall δ^{18}O values from the Bahamas were, on average, ~10‰ higher (in SMOW scale) than summer rain from Fairbanks (Fig. 4B).

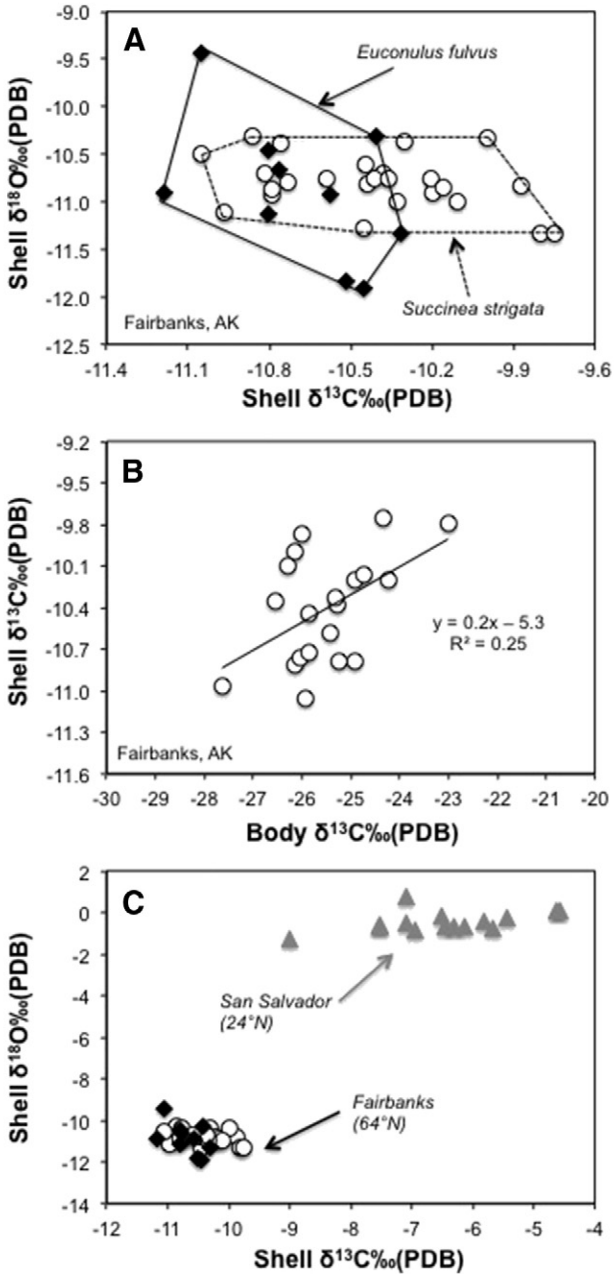

Figure 3. Stable isotope results of land snails from Fairbanks. (A) Oxygen and carbon stable isotope values of modern land snail shells. (B) Relationship between carbon stable isotope values of succineid land snail shell and body tissue from Fairbanks. (C) Carbon and oxygen stable isotope values of land snail shells from Fairbanks and San Salvador Island.

Discussion

Carbon stable isotopes

Stott (2002) and Metref et al. (2003) showed that snails primarily incorporate carbon isotopes from the plant diet in their tissues, whereas other carbon sources such as atmospheric CO_2 and carbonate ingestion had a negligible influence. Thus, the carbon isotope composition of snail tissues has been used as a proxy for paleovegetation at low-to-mid

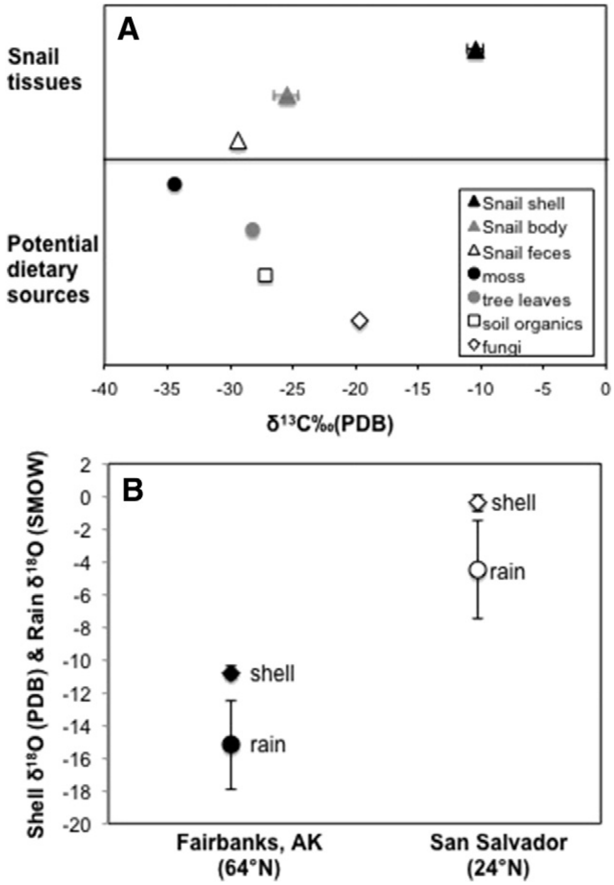

Figure 4. (A) Carbon stable isotope values of land snail tissues and potential snail food resources from Fairbanks, Alaska. Triangles depict results from snail tissues, including feces (open symbol), body tissue (gray symbol) and shell (black symbol). Circles depict plant samples (moss = black symbol; tree leaves = gray symbol). Open "quadrat depicts soil organic matter and open diamond depicts fungi. Note that only samples of snail shell and body included multiple individuals whereas the remaining samples depict a single analysis. (B) Comparison of the oxygen stable isotope values of land snail shells (diamonds) and rainfall (circles) between Fairbanks (filled symbols) and San Salvador Island (open symbols).

latitude sites. While studies in carbonate-rich areas suggest that snails may incorporate carbon isotopes derived from limestone ingestion, which complicates plant ecology inferences (Goodfriend and Hood, 1983; Goodfriend, 1987; Yanes et al., 2008, 2012, 2013a), snails that occupy carbonate-poor areas (e.g., forests with acidic soils), may offer more accurate information about local vegetation cover. In the case of the studied boreal forest here, soil samples did not contain calcium carbonate, so the influence of carbonate ingestion in snail shells is assumed to be negligible. However, snails may utilize other carbon sources in addition to vascular plants. In fact, in woodland areas, snails consume, apart from C_3 plants, other food resources including moss, fungi, algae, tree sap, and decayed organic matter (Speiser, 2001), which, in turn, may differ significantly in carbon isotope values (Fig. 4A). Snail body tissue is enriched in ^{13}C with respect to diet by ~1‰ (DeNiro and Epstein, 1978; Stott, 2002; Metref et al., 2003). Subtraction of 1‰ from the average $\delta^{13}C$ of the body tissue of the Fairbanks snails yields a value of −26.5 ± 1.0‰. In Fig. 4A it is illustrated that the average snail body $\delta^{13}C$ value plots close to the value of fresh tree leaves (−28.1‰) and the soil organic matter sample (−27.1‰), suggesting that the measured *Succinea* specimens from Fairbanks included significant proportions of living and decayed C_3 plant matter in their diet, probably with some assimilation of fungi (−19.7‰). It seems less likely that *Succinea* included moss (−34.4‰) in its diet.

An additional complication in interpreting the carbon isotope composition of land snail shells is the potential variations in metabolic rates among sympatric specimens. The snail evaporative steady-state flux balance model developed by Balakrishnan and Yapp (2004) was used to evaluate whether or not snails from Fairbanks experienced noticeably different metabolic rates. The model relates the amount and isotopic composition of food resources, bicarbonate in the snail hemolymph, and the diffusive flux of respired CO_2. The flux of dissolved bicarbonate output from the hemolymph (f_o) relative to the flux of diet (f_{in}) is called ϕ ($=f_o / f_{in}$) and varies with metabolic rate (Balakrishnan and Yapp, 2004). Model calculations were constrained by the measured $\delta^{13}C$ values of shell and body tissue and the mean air temperature during snail active period, assumed to be ~13°C. The model, in combination with the $\delta^{13}C$ values of snails from Fairbanks, indicates that specimens varied in metabolic rate, with ϕ ranging from 0.00 to 0.20 (Fig. 5). This suggests that variations in shell $\delta^{13}C$ values may be in part explained by differing metabolic rates among specimens. This finding differs from some published results, which did not observe significant variations in model-derived metabolic rates among *Sphincterochila candidissima* specimens (Yanes et al., 2013a) and *Theba geminata* individuals (Yanes et al., 2013b) both from carbonate-rich areas from mid- and low-latitude sites, respectively. Accordingly, potential variations in snail metabolic rates appear to be species-dependent and require additional examination).

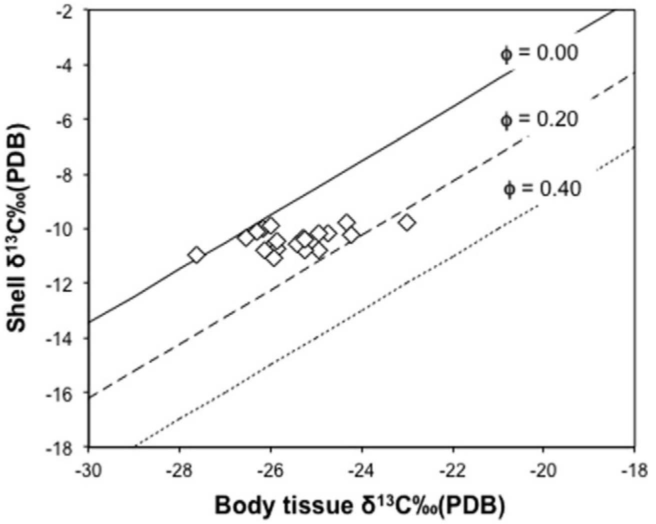

Figure 5. Calculated curves of land snail shell $\delta^{13}C$ values as a function of body $\delta^{13}C$ values, which depict snail's diet, based on the steady-state flux balance model by Balakrishnan and Yapp (2004). Calculations were made assuming that calcification occurred at average air temperatures of 13°C.

Succinea specimens from the tropical island of San Salvador (Bahamas) showed a wider range of shell $\delta^{13}C$ values, and were, on average, ~4‰ higher than those from Fairbanks (Fig. 3C). The higher variability in shell $\delta^{13}C$ values of specimens from San Salvador is explained by the higher variation in $\delta^{13}C$ values of potential carbon sources in the island, which includes the presence of both C_3 and C_4 plants, and carbonate-rich marine sediments (Baldini et al., 2007; Yanes and Romanek, 2013). The results from the present work suggest that even when individuals follow variable metabolic rates (like those from Fairbanks), higher dispersion in $\delta^{13}C$ values is expected at carbonate-rich localities where multiple photosynthetic pathways coexist. This study also suggests that snails from boreal forests, which grow and reproduce in a narrow temporal window during the warmest (ice-free) months, may experience higher variability of metabolic rates than snails from mid-to-low latitudes, with longer active periods throughout the year.

Oxygen stable isotopes

Snail shells from Fairbanks exhibited a rather narrow range of $\delta^{18}O$ values, averaging −10.8 ± 0.4‰ (Figs. 3A–B). This is probably one of the most negative oxygen isotope values for land snail shells reported in the published literature. Even though specimens of *Succinea* and *Euconulus* exhibited statistically comparable δ^{18}O values, the microsnail *Euconulus* displayed a larger dispersion than *Succinea* (Fig. 3A). This larger dispersion suggests that the microsnail may have experienced higher evaporation rates than *Succinea* specimens. The shell δ^{18}O values of snails from Fairbanks were examined using the evaporative steady-state flux balance-mixing model published by Balakrishnan and Yapp (2004). The model links the amount and isotopic composition of external liquid water, liquid water from the snail hemolymph, the diffusive flux of water from the hemolymph by evaporation, and the temperature dependent oxygen isotope fractionation between water and aragonite (Grossman and Ku, 1986). This model considers that δ^{18}O values of water and water vapor, relative humidity and temperature are the most important variables that control shell δ^{18}O values (Balakrishnan and Yapp, 2004). The model considers the flux of liquid water output from the hemolymph (f_o) relative to the flux of liquid water imbibed (f_{in}), a ratio called θ ($=f_o / f_{in}$). Model calculations assume that ambient water vapor is in isotope equilibrium with the imbibed liquid water and that water is lost by evaporation (Balakrishnan and Yapp, 2004). Model outputs are constrained by the measured shell carbonate δ^{18}O values, the summer temperature in Fairbanks (~13°C), and the δ^{18}O values of the summer rainfall (−15‰ vs. SMOW). If land snails from Fairbanks, with a measured shell δ^{18}O value of −10.8‰, grew their shells at times when temperatures were about 13°C and rainwater δ^{18}O values were around −15‰, the model predicts that relative humidity conditions during calcification were near 93% (Fig. 6). This relatively high value for RH is indeed reached during the summer months in Fairbanks, indicating that *Succinea* and *Euconulus* specimens from the study area grow at notably moist conditions during the summer months.

Succinea shells from the tropical island of San Salvador (Bahamas) displayed an average δ^{18}O value of −0.4‰, that is, 10.4‰ higher than snails from Fairbanks (Fig. 3C). The average annual rain δ^{18}O value in San Salvador is −4.5‰(SMOW) and the annual mean temperature is 24°C (Baldini et al., 2007; Yanes and Romanek, 2013). Annual rainwater δ^{18}O values from San Salvador are ~10‰ higher than summer rainwater values from Fairbanks (Fig. 3C). Thus, the isotopic offset between shells from Fairbanks and San Salvador is equivalent to the observed offset between respective rainfall isotopic values. Using the model by Balakrishnan and Yapp (2004) and assuming that snails grew their shells year-round in San Salvador, shells should have been deposited at times when relative humidity was ~86%, on average (Fig. 6). This calculated value of relative humidity during calcification overlaps with predicted values by Yanes and Romanek (2013) for modern Cerionidae and Chondropomidae snails collected at different localities within the same island. Hence, different snail species from the Bahamas seem to have grown under comparable RH values. The results reported here suggest that succineid snail shells from the Bahamas were deposited at drier conditions than specimens from Fairbanks.

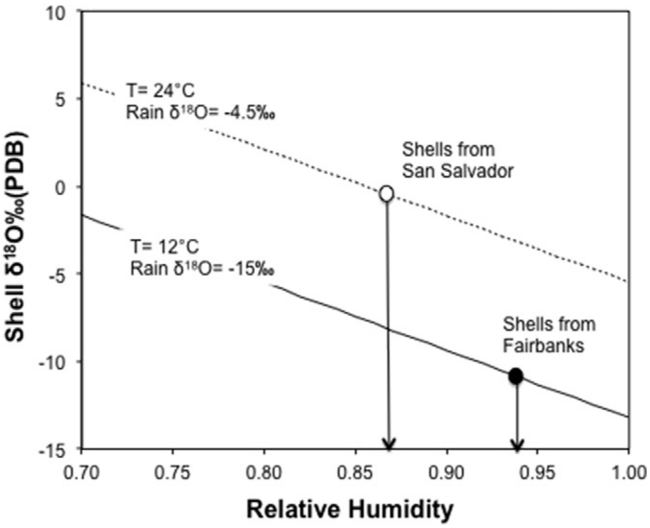

Figure 6. Calculated curves of land snail shell δ^{18}O values as a function of relative humidity (RH) using the evaporative steady-state flux balance-mixing model by Balakrishnan and Yapp (2004). Curves were calculated for two temperatures (24°C and 13°C) and rainfall δ^{18}O values (−4.5‰ and −15.0‰). Arrows illustrate that, on average, succineid shells from San Salvador grew their shells at RH of ~86% while succineid shells from Fairbanks were deposited at times when RH was ~93%.

Despite the complexity associated with multiple (rather than one) climatic variables controlling snail shell δ^{18}O values, meaningful paleoclimatic inferences can be deduced from fossil snails, especially at coarse temporal/spatial scales. This study shows that at a coarse latitudinal scale, shell δ^{18}O values seem to be predominantly a function of input rainfall δ^{18}O values. Snail shells recovered from Quaternary outcrops in Alaska will provide valuable summer season paleoclimatic data, especially paleorainfall δ^{18}O values and paleohumidity.

Conclusions

Land snail shells from high latitudes are useful proxies for summer climatic conditions. Land snails from Fairbanks, interior Alaska, primarily recorded the carbon isotope values of the snail's diet in their shell and body tissue, which included living and decayed C_3 plant matter and some assimilation of fungi. A flux-balance mixing model suggested that measured individuals exhibited significantly different metabolic rates, in contrast to some published data from mid to low latitudes. Thus, variations in shell δ^{13}C values among individuals of the same species may result from variable metabolic rates rather than from variable diets. The oxygen isotope composition of snails from Fairbanks (−10.8 ± 0.5‰ vs PDB) is likely the most negative value reported for land snails. A flux-balance mixing model indicates that analyzed snails from Fairbanks primarily deposited their shell during the summer season, when relative humidity was ~93%. A comparison between succineid specimens from Alaska (64°N) and the tropical island of San Salvador (24°N) suggests that the local δ^{18}O values of the rainfall during snail active periods are probably the dominant control of shell δ^{18}O values at a coarse latitudinal scale. While at the microhabitat scale, the shell δ^{18}O values might be complex to understand due to the high number of environmental factors operating collectively, the shell δ^{18}O values at rough latitudinal scales seem to be a meaningful proxy for rainfall δ^{18}O.

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