RESEARCH ARTICLE

Key Points:
- Clumped isotopes of snail shells are a season-specific geothermometer in monsoonal region
- *Cathaica* sp. snails had calcification temperatures 3–5°C higher than *Bradybaena* sp. snails
- Climatic seasonality determined the faunal assemblages of land snails in north China

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Stable and clumped isotopes in shell carbonates of land snails *Cathaica* sp. and *Bradybaena* sp. in north China and implications for ecophysiological characteristics and paleoclimate studies

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Abstract Knowledge of ecophysiological characteristics of different land snail species is crucial for defining climatic significance of snail faunal assemblages. However, little work has been done in this aspect, hindering our obtaining unambiguous paleoclimatic information using these proxy indicators. Here we document for the first time the different ecophysiological characteristics of *Cathaica* sp. and *Bradybaena* sp. land snails using the stable isotopes and clumped isotope (Δ_{47}) of the shell carbonates. The Δ_{47}-derived temperatures for both species revealed a robust correlation with environmental temperatures. Moreover, the temperatures for *Cathaica* sp. are 3–5°C higher than those for *Bradybaena* sp. land snails, indicating different ecophysiological adaptations or growing seasons of the two species. Specifically, *Cathaica* sp. snails prefer living in a warm-humid summer, whereas *Bradybaena* sp. snails are active in the relatively cool-arid spring and/or autumn. The result testifies to the Δ_{47} in snail shell carbonates as a promising paleothermometer in monsoonal region and presents new insight into paleoclimatic explanation of these land snail species. This finding highlights the importance of climatic seasonality in the changes of the faunal assemblages of land snails.

1. Introduction

Land snails have been recognized as a useful archive material for reconstructing past climatic and environmental conditions because they are sensitive to seasonal climatic and ecological changes [*Goodfriend*, 1992; *Wu et al.*, 1996]. To date, most of the important findings in the paleoclimatic field achieved using land snails are derived from analyzing their faunal assemblages, although stable isotopes of snail shell carbonates have been used recently [*Goodfriend*, 1999; *Balakrishnan et al.*, 2005a, 2005b; *Yanes et al.*, 2009; *Gu et al.*, 2009]. For example, land snail fossils from Chinese loess deposits have been categorized into two groups: cold-arid and thermo-humid species, which represent, respectively, the East Asia winter and summer monsoon conditions [*Wu and Li*, 2008; *Li et al.*, 2008; *Huang et al.*, 2012; *Rousseau and Wu*, 1997]. The division of these snail fossil assemblages is based on the type of soil horizon (i.e., loess, paleosol, or weakly developed paleosol) that certain species abundantly occur in combined with experimental observations of the ecological behavior of living snails. However, despite that some work has been done to link snail ecophysiology to environment [*Baldini et al.*, 2007; *Chiba and Davison*, 2009; *Colonese et al.*, 2014], there is still a lack of systematic ecophysiological study to definitely characterize each land snail species especially in China [*Wu and Li*, 2008], which leaves at least three open questions. First, there may be large uncertainty in paleoclimate reconstruction when using snail faunal assemblages because of the ambiguous division between different ecotypes for certain snail species. Second, the quantitative linkage of snail faunal assemblages to climatic factors (e.g., temperature and rainfall) is not robust, which hampers the development of a reliable mollusc-climate factor transfer function [*Wu et al.*, 1996]. Third, there has been no study of the changes in the ecophysiological habits of land snails in the adaptation to changing environments.

More recently, carbonate clumped isotope (expressed as Δ_{47}, quantifying the excess abundance of CO_2 of mass 47 ($^{13}C^{18}O^{16}O$) in carbonate relative to the theoretical random distribution) have been developed as a new paleothermometer [*Ghosh et al.*, 2006] for reconstruction of paleoenvironments [*Came et al.*, 2007;

Passey et al., 2010; *Henkes et al.*, 2013; *Hren et al.*, 2013] and paleoelevation [*Quade et al.*, 2013; *Fan et al.*, 2014] and to constrain diagenetic alteration [*Dennis and Schrag*, 2010]. The clumped isotope in land snail shells have been used to elucidate the temperatures at which the shell carbonates precipitated, thus reflecting the environmental temperature [*Eagle et al.*, 2013] or indicating paleohydrological changes in combination with the shell oxygen isotope composition [*Zaarur et al.*, 2011]. However, there are some controversial results with use of the snail shell clumped isotope to reconstruct environmental temperatures. For example, *Zaarur et al.* [2011] did not find a universal relationship between the Δ_{47}-derived land snail calcification temperature and the environmental temperature when studying different land snail taxa from a variety of locations, including tropical, desert, and temperate environments [*Zaarur et al.*, 2011]. By contrast, *Eagle et al.* [2013] argued that land snail calcification temperatures were correlated with environmental temperatures if they only adopted the data from the environments most similar to monsoonal China [*Eagle et al.*, 2013]. In this context, the above conclusion deserves further examination for land snails of the same species from different locations in typical monsoonal climate regions.

To address these major gaps, we collected modern snail shells of two dominant species in north China (i.e., *Cathaica* sp. and *Bradybaena* sp.) along a southwest-northeast transect covering a temperature gradient for warm month mean temperature (April–October) from 17.5°C to 21.8°C with little rainfall difference (i.e., maximum difference in mean annual precipitation < 70 mm) (Figure 1a). Clumped isotope and stable isotopes were then measured in the shell carbonates of the two species, with the objective of justifying the use of snail shell clumped isotope as a proxy indicator of environmental temperatures and distinguishing differences in the ecophysiological habits of the two snail species.

2. Materials and Methods

2.1. Samples

A total of 24 live adult land snails were collected from eight study sites in north China in July to September 2013 (Figure 1a). The live snails of both *Cathaica* sp. and *Bradybaena* sp. were collected at each site. The two species collected from each site occupied the same or adjacent habitats, which are mostly semiarid shrubs. This ensures that they grew at the same time and in comparable conditions. For an individual snail, its shell and body were separated in field. Aragonite of the shell samples were analyzed for stable and clumped isotopes. Individual snail shells were first cleaned with a toothbrush to remove large fragments of soil and organic material stuck on the shell surfaces. The shells were then gently crushed to large fragments and were sonicated and washed 6 times in deionized water. This procedure produced clean shell material free from any macroscopic contaminants. The shell material was then dried at 50°C overnight and ground to a fine powder with a pestle and mortar. To ensure the representative of every aliquot of the samples, each shell powder was homogenized and adequately mixed before analysis. One individual shell usually weighs about 100 mg, which is sufficient for both the δ^{13}C/δ^{18}O and the Δ_{47} analyses.

2.2. Lifestyles of *Cathaica* sp. and *Bradybaena* sp. Land Snails

At present, there are several studies describing the lifestyles and habits of *Bradybaena* sp. land snails (i.e., *Bradybaena similaris* and *Bradybaena ravida*) mainly based on field observation and culture experiments with objective to prevent the damage on vegetables and fruit plants by these animals [*Zhang et al.*, 1986; *Xu et al.*, 2002; *Liu et al.*, 2007; *Wang*, 2008]. We summarize it as below. In general, the *Bradybaena* sp. land snails can usually live for one or one and half years [*Zhang et al.*, 1986]. At the beginning of March, some individual *Bradybaena* sp. land snails, which hibernated in the environments facing to sun and with relatively humid condition, start to emerge from hibernation. Since then, more and more *Bradybaena* sp. land snails come out to look for food along with a rise in air temperature (>10°C). These land snails are most active in the months from April to June. Subsequently, they go into hibernation in July and August because the air temperature becomes too high. When the temperature drops to below 30°C at the end of August, they start to be active again and the following autumn (September and October) is their second growing season. The *Bradybaena* sp. land snails grow very slowly after October and go into hibernation when air temperature is below 10°C. According to culture experiment, the favorable temperature is 17–28°C for *Bradybaena ravida* land snails, which react quickly and eat a lot of food under such a condition [*Wang*, 2008]. Moreover, when the temperature increased above 30°C or decreased below 12°C, the activity and food intake of the *Bradybaena ravida* land snails were largely reduced.
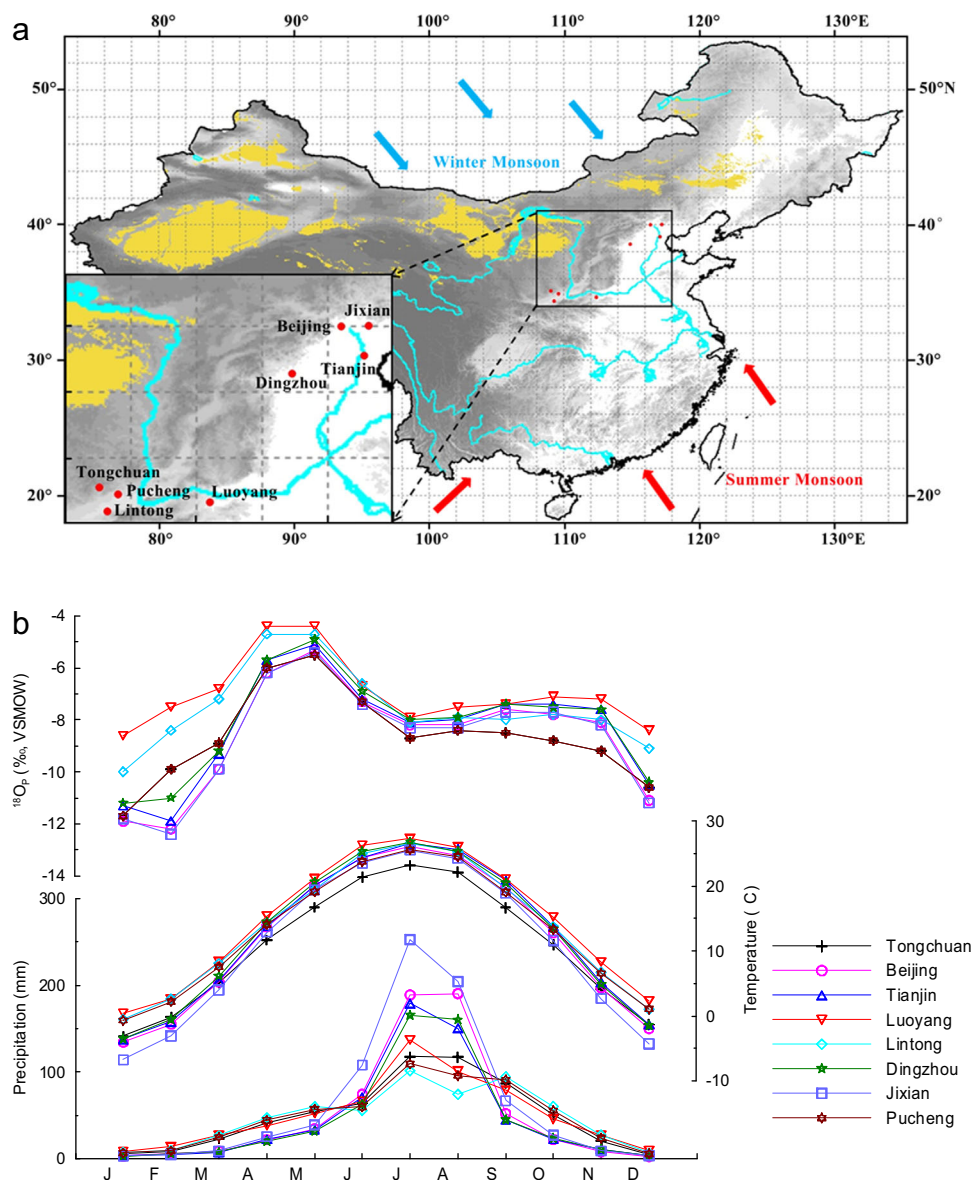
Figure 1. (a) Map showing the positions of the study sites and the settings. Atmospheric circulation: winter monsoon (blue arrows) and summer monsoon (red arrows). (b) Monthly mean data for the weighted $\delta^{18}O$ of precipitation ($\delta^{18}O_P$), temperature, and precipitation for the study sites. The precipitation and temperature data are averaged values over 51 years (1951–2001) and come from the China Meteorological Data Sharing Service System. The mean weighted $\delta^{18}O_P$ values were computed using the online isotopes in precipitation calculator provided by *Bowen* [2008] and provided at wateriso.utah.edu/waterisotopes/.

Another study on *Bradybaena similaris* land snails reveals that the temperature of 20–25°C and the relative humidity of ∼90% are the most optimal condition for this species [*Xu et al.*, 2002]. As similar as *Bradybaena ravida*, *Bradybaena similaris* would eat little and be inactive when the temperature was higher than 30°C or lower than 15°C and the relative humidity was lower than 66%. By contrast, studies about lifestyle of *Cathaica* sp. land snails are rather scarce. A recent study has reported an appropriate temperature of 10–30°C for *Cathaica* (*Cathaica*) *orestias* to live [*Yang and Ren*, 2012]. The *Cathaica* (*Cathaica*) *orestias* land snails usually go into hibernation during winter time (from the middle of October to the beginning of March). However, hibernation in summer is seldom witnessed. For example, the damages on fruit trees caused by the *Cathaica* (*Cathaica*) *orestias* land snails occurred continuously from May to October in the year of 2007 and 2009 in Tianshui city, Gansu Province [*Yang and Ren*, 2012]. Moreover, the *Cathaica* (*Cathaica*) *orestias* has a habit of living in groups in the trees and crouching on trunk or twigs for food [*Zhang et al.*, 2014].

2.3. Stable and Clumped Isotope Measurement of Shell Carbonates

We extracted CO_2 from carbonates by reaction with anhydrous phosphoric acid, following the method of *Ghosh et al.* [2006]. Briefly, approximately 15 mg of sample was reacted with ∼103% phosphoric acid (density 1.90 g/mL) under vacuum in a sealed glass vessel at a temperature of 25°C for approximately 16 h (overnight). The produced CO_2 was cryogenically purified on a vacuum line by passing through three traps in the following sequence, namely, a water trap immersed in liquid nitrogen + acetone slurry (approximately −85°C), an absorbent trap (filled with Porapak Q^{TM}, a divinyl benzene polymer) immersed in liquid nitrogen + ethylene glycol slurry (about −15°C) and another water trap immersed in liquid nitrogen + acetone slurry (approximately −85°C). This cleaning procedure is required to guarantee no interference of hydrocarbons and halocarbons with the mass 47 measurement [*Eiler and Schauble*, 2004]. The purified CO_2 was then collected into a small glass sample vessel using liquid nitrogen for determination. The $\delta^{13}C$, $\delta^{18}O$, and Δ_{47} in the CO_2 derived from the phosphoric acid digestion of carbonates were determined using a Thermo Scientific MAT 253 isotope ratio mass spectrometer, which was modified to simultaneously measure mass 44–49 in a dual-inlet mode, utilizing the published configuration and methods [*Cui and Wang*, 2014]. The measurement was performed at a signal level of 16 V for mass 44. Each measurement consisted of 64 cycles of a sample-standard comparison, with a signal integration time of 26 s. It takes a total of two and half hours to analyze one sample. Both the $\delta^{13}C$ and $\delta^{18}O$ are reported relative to the VPDB scale as determined using a precalibrated CO_2 tank gas as a reference working gas, and verified using NBS-19. An absolute reference frame for the Δ_{47} measurements was defined by analyzing the CO_2 heated at 1060°C for 3 h and the CO_2 equilibrated with water at 25°C for approximately 72 h, following the method developed by *Dennis et al.* [2011]. The Δ_{47} values of our samples were calibrated using the absolute reference frame. NBS-19 was analyzed along with the samples every day to constrain daily shift of mass spectrometer. The Δ_{47} values measured on NBS-19 relative to the absolute reference frame ranged from 0.379 to 0.388 (mean value: 0.383), which are close to the published mean value of 0.392 by *Dennis et al.* [2011]. The Δ_{47} values of samples were finally normalized by adjusting the measured Δ_{47} values of NBS19 to the published value (0.392) to obtain an internally consistent data set and compare it with previously published results. To convert Δ_{47} values into calcification temperatures, we use the equation: $\Delta_{47} = 0.0636 (10^6 \cdot T^{-2}) - 0.0047$ [*Dennis et al.*, 2011].

$\delta^{13}C$ and $\delta^{18}O$ of shell segments sampled by microdrill on shells along growth band were measured using GasBench II linked to MAT253 isotope ratio mass spectrometer (Thermo Fisher). The sample powers of about 100 μg were reacted with 100% H_3PO_4 at 72°C for 1 h. The resultant CO_2 was purified by passing through two $NAFION^{TM}$ water traps and a PoraPlot Q chromatograph column (maintained at 45°C) and then introduced to mass spectrometer for isotopic measurement. Stable isotope results are reported relative to the Vienna PeeDee Belemnite (VPDB) standard with an external analytical precision of 0.06‰ and 0.10‰ for $\delta^{13}C$ and $\delta^{18}O$, respectively.

3. Results

3.1. Variations of the Δ_{47} Values in Shell Carbonate Along the Transect

The Δ_{47} values observed in the shell carbonate range from 0.677 to 0.699 for *Bradybaena* sp. snails and from 0.659 to 0.682 for *Cathaica* sp. snails (Table 1). Using the clumped isotope thermometer calibration [*Dennis et al.*, 2011], these values correspond to a temperature range of 32–28°C and 37–31°C for *Bradybaena* sp. snails and *Cathaica* sp. snails, respectively. Comparison of the clumped isotope temperatures to the local ambient temperatures (warm month mean temperature (April–October); Figure 2) reveals a robust correlation of snail growth temperatures with environmental temperatures for both species (i.e., r = 0.893, $p < 0.01$ for *Cathaica* sp. and r = 0.864, $p < 0.01$ for *Bradybaena* sp. using Pearson correlation test). Furthermore, the observed calcification temperatures of *Cathaica* sp. are 3–5°C higher than those of *Bradybaena* sp. land snails (Figure 2).

3.2. Changes in the Stable Carbon and Oxygen Isotopes of Shell Carbonate

The $\delta^{13}C$ and $\delta^{18}O$ values measured on the snail shell carbonates are shown along with the sampling sites (Figure 3 and Table 1). The $\delta^{13}C$ and $\delta^{18}O$ values for the shell carbonate of *Bradybaena* sp. range from −12.34‰ to −10.36‰ and from −6.97‰ to −1.51‰, respectively. The values observed in the shell carbonate of *Cathaica* sp. have relatively large variations, i.e., −13.61‰ to −8.56‰ for $\delta^{13}C$ and −8.33‰ to −1.74‰ for $\delta^{18}O$ (Figure 3). However, there is no systematic difference in either the $\delta^{13}C$ or $\delta^{18}O$ values between the

Table 1. Compiled Data of Stable and Clumped Isotopes for All Samples

Sample	Location	Species	$\delta^{13}C$ (‰) (VPDB)	$\delta^{18}O$ (‰) (VPDB)	Δ_{47} (‰)	Δ_{47} Temp. (°C)
BJ-B-1	Beijing	*Bradybaena* sp.	−11.35 ± 0.007	−2.54 ± 0.016	0.688 ± 0.009	30 ± 2
BJ-B-2	Beijing	*Bradybaena* sp.	−11.05 ± 0.001	−3.91 ± 0.003	0.692 ± 0.009	29 ± 2
BJ-C-1	Beijing	*Cathaica* sp.	−11.16 ± 0.001	−4.69 ± 0.002	0.659 ± 0.007	37 ± 2
BJ-C-2	Beijing	*Cathaica* sp.	−12.17 ± 0.001	−3.85 ± 0.002	0.675 ± 0.009	33 ± 2
TJ-B-1	Tianjin	*Bradybaena* sp.	−11.90 ± 0.007	−5.90 ± 0.015	0.691 ± 0.008	29 ± 2
TJ-B-2	Tianjin	*Bradybaena* sp.	−10.74 ± 0.007	−4.17 ± 0.018	0.683 ± 0.008	31 ± 2
TJ-C-1	Tianjin	*Cathaica* sp.	−9.69 ± 0.007	−8.33 ± 0.012	0.670 ± 0.007	34 ± 2
TJ-C-2	Tianjin	*Cathaica* sp.	−11.39 ± 0.007	−3.66 ± 0.018	0.662 ± 0.010	36 ± 3
TJ-C-3	Tianjin	*Cathaica* sp.	−11.25 ± 0.007	−4.60 ± 0.016	0.669 ± 0.008	34 ± 2
JX-B-1	Jixian	*Bradybaena* sp.	−12.01 ± 0.009	−4.06 ± 0.018	0.692 ± 0.008	29 ± 2
JX-C-1	Jixian	*Cathaica* sp.	−12.38 ± 0.001	−5.47 ± 0.002	0.677 ± 0.009	32 ± 2
JX-C-2	Jixian	*Cathaica* sp.	−10.83 ± 0.005	−4.91 ± 0.014	0.663 ± 0.007	36 ± 2
DZ-B-1	Dingzhou	*Bradybaena* sp.	−10.36 ± 0.007	−6.54 ± 0.012	0.686 ± 0.008	30 ± 2
DZ-C-1	Dingzhou	*Cathaica* sp.	−13.61 ± 0.007	−7.00 ± 0.017	0.664 ± 0.008	35 ± 2
TC-B-1	Tongchuan	*Bradybaena* sp.	−11.98 ± 0.007	−4.35 ± 0.015	0.699 ± 0.008	28 ± 2
TC-C-1	Tongchuan	*Cathaica* sp.	−8.56 ± 0.001	−1.74 ± 0.002	0.682 ± 0.007	31 ± 2
PC-B-1	Pucheng	*Bradybaena* sp.	−11.99 ± 0.007	−6.61 ± 0.012	0.699 ± 0.007	28 ± 2
PC-B-2	Pucheng	*Bradybaena* sp.	−11.23 ± 0.001	−1.51 ± 0.002	0.688 ± 0.009	30 ± 2
PC-B-3	Pucheng	*Bradybaena* sp.	−11.07 ± 0.001	−2.25 ± 0.002	0.695 ± 0.009	28 ± 2
PC-C-1	Pucheng	*Cathaica* sp.	−11.58 ± 0.005	−8.04 ± 0.012	0.677 ± 0.007	32 ± 2
LT-B-1	Lintong	*Bradybaena* sp.	−10.92 ± 0.001	−6.97 ± 0.002	0.681 ± 0.008	32 ± 2
LT-C-1	Lintong	*Cathaica* sp.	−9.50 ± 0.001	−1.98 ± 0.002	0.669 ± 0.007	34 ± 2
LY-B-1	Luoyang	*Bradybaena* sp.	−12.34 ± 0.007	−4.70 ± 0.013	0.677 ± 0.008	32 ± 2
LY-C-1	Luoyang	*Cathaica* sp.	−13.27 ± 0.005	−3.08 ± 0.014	0.666 ± 0.008	35 ± 2

Bradybaena sp. land snails and the *Cathaica* sp. land snails. Moreover, no clear trend is observed in the $\delta^{13}C$ and $\delta^{18}O$ values for the two species geographically northward or westward. When plotting $\delta^{13}C$ against $\delta^{18}O$ of the shell carbonates, there is a slightly positive correlation between the two isotope compositions for *Cathaica* sp. land snails and a slightly negative correlation for *Bradybaena* sp. land snails (Figure 4).

4. Discussion

4.1. Clumped Isotope of Land Snail Shells as Paleothermometer in Monsoonal Regions

The robust correlation of shell carbonate Δ_{47}-derived temperatures with environmental temperatures observed for the two species (Figure 2) suggests a potential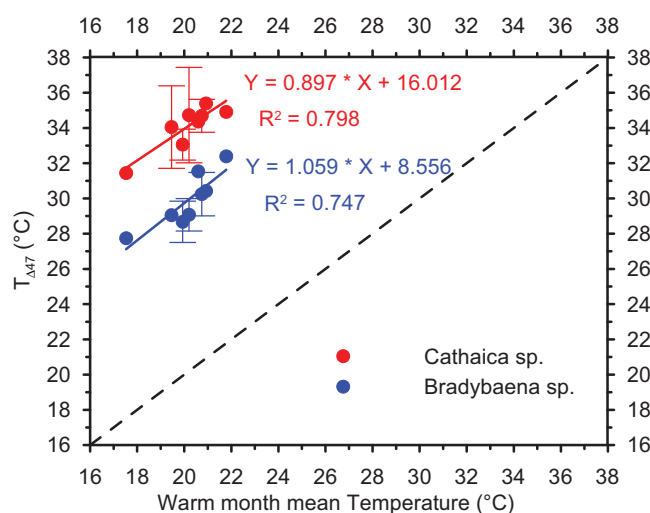 application of snail shell clumped isotope in reconstructing paleotemperatures in monsoonal climate regions. Moreover, the Δ_{47}-derived temperatures for *Cathaica* sp. are 3–5°C higher than those for *Bradybaena* sp. land snails, which may reflect the different eco-physiological adaptations of the two species. Previous studies have shown that coloration and morphology of the shell, as well as behavioral adaptation, may significantly affect snail body temperature [*Heath*, 1975; *Dittbrenner et al.*, 2009]. For example, dark colored shells may increase the snail body temperature by up to 12°C above the environmental temperature by exposure to direct sunlight [*Dittbrenner et al.*, 2009]. *Cathaica* sp. land snails have thick, milky-white shells with brown growth bands (Figure 5), which protect the snail body from direct sunlight during its activity in the sun. As
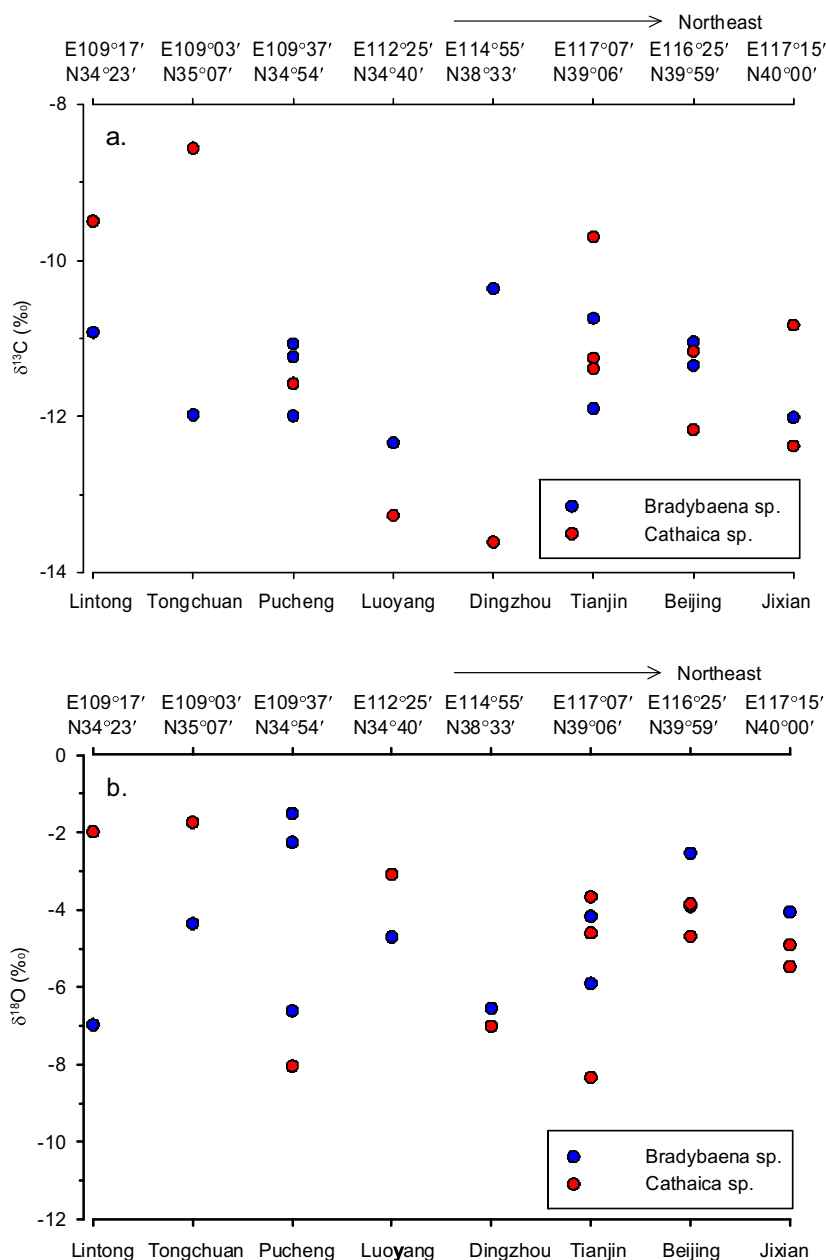

Figure 2. Comparison of the clumped isotope temperatures ($T_{\Delta47}$) of snail shell carbonates with the local warm month mean temperatures (April–October). Error bars denote standard deviation (SD) for the mean $T_{\Delta47}$ values of multiple individual shells. Note that the $T_{\Delta47}$ values of *Cathaica* sp. are 3–5°C higher than those of *Bradybaena* sp. land snails.

Figure 3. Changes in the (a) $\delta^{13}C$ and (b) $\delta^{18}O$ values of the snail shell carbonates at the sampling sites along a southwest-northeast transect.

aforementioned, the *Cathaica* (*Cathaica*) *orestias* like living in groups in the trees [*Zhang et al.*, 2014], which would increase the time of their exposure to the sunlight. *Bradybaena* sp. land snails have very thin, yellow-ish, and transparent shells, which do not provide protection from sunlight. Therefore, the differences in the colors and appearances of shells may partially explain the different ecophysiological adaptations of the two species and thus the different calcification temperatures. However, the different Δ_{47} temperatures observed for the two species may represent the different seasons that they live in. This could be examined further by the stable carbon and oxygen isotopes of shell carbonates of the two species, which is discussed in detail below.

Some recent studies have shown that the $\delta^{13}C$ value of shell carbonate primarily reflects the carbon isotope composition of the snail diet [*Francey*, 1983; *Goodfriend and Ellis*, 2002; *Liu et al.*, 2007]. However, consumption of carbonate may be also a source of carbon intake by land snails especially in some regions abundant
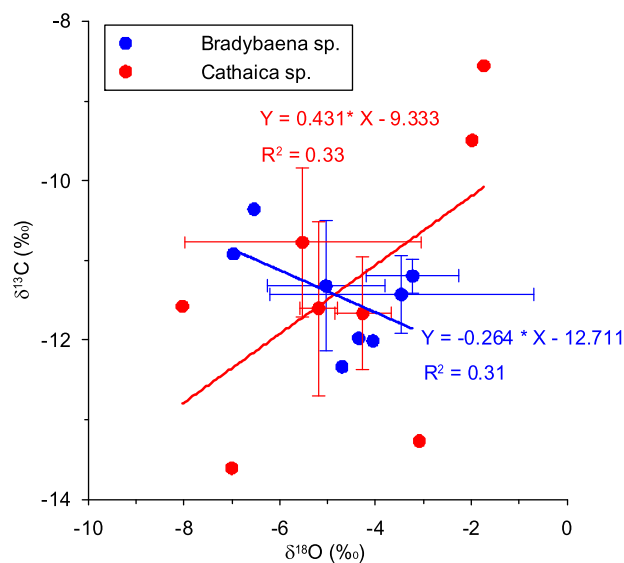
Figure 4. Plots showing the correlation of $\delta^{13}C$ with $\delta^{18}O$ for snail shell carbonates. Error bars denote standard deviation (SD) for the mean $\delta^{13}C$ and $\delta^{18}O$ values of multiple individual shells. Note the positive $\delta^{13}C$-$\delta^{18}O$ relationship for the *Cathaica* sp. snails in contrast to the negative $\delta^{13}C$-$\delta^{18}O$ relationship for the *Bradybaena* sp. snails.

in deposits of limestone or other carbonate rocks [*Goodfriend and Hood*, 1983; *Goodfriend and Stipp*, 1983; *Pigati et al.*, 2004, 2010]. By applying a ^{13}C enrichment factor of 14.2‰ in the shell relative to the snail body in north China [*Liu et al.*, 2007], we find that the $\delta^{13}C$ of snail organic tissues is $-26.54‰$ to $-24.56‰$ for *Bradybaena* sp. snails and $-27.81‰$ to $-22.76‰$ for *Cathaica* sp. snails. Because the snail body has the same carbon isotope composition as the snail's food [*Goodfriend and Ellis*, 2002; *Liu et al.*, 2007], the calculated $\delta^{13}C$ of snail organic tissues for the two species indicate the main food source as being C_3 plants, which is consistent with the vegetation grown at the study sites. Since there is no old carbonate deposits at our study sites, soil carbonate may be a potentially extra source of carbon to our land snail shells. Ingestion of soil carbonate would make the $\delta^{13}C$ value of shell carbonate rela-

tively higher than that for land snail only ingesting plant detritus because $\delta^{13}C$ of soil carbonate is more positive than that of soil organic matter [*Cerling and Quade*, 1993]. In this case, estimated $\delta^{13}C$ of plant food should be lower than the above calculated $\delta^{13}C$ of snail organic tissues. This further confirms a main food source of C_3 plants. A plot of $\delta^{13}C$ against $\delta^{18}O$ of the shell carbonates shows a slightly positive correlation for *Cathaica* sp. land snails and a slightly negative correlation for *Bradybaena* sp. land snails (Figure 4). The different $\delta^{13}C$–$\delta^{18}O$ relationships for the two species suggest different growth seasons. In previous studies, the $\delta^{18}O$ values of land snail shell carbonate have been related to the $\delta^{18}O$ of the ambient rainwater ($\delta^{18}O_P$) [*Yanes et al.*, 2009; *Gu et al.*, 2009; *Zaarur et al.*, 2011]. In monsoonal climate regions, low $\delta^{18}O_P$ values occur in summer due to the amount effect, overshadowing the dependence of $\delta^{18}O_P$ on temperature [*Johnson and Ingram*, 2004; *Vuille et al.*, 2005; *Yang et al.*, 2012]. This effect is visible as a distinct dip in the $\delta^{18}O_P$ values during July to September at our study sites (Figure 1b). Moreover, the $\delta^{13}C$ values of C_3 plants in north China usually decrease with rainfall increase [*Wang et al.*, 2003; *Liu et al.*, 2005]. This negative response is more apparent in summer because most of the annual precipitation occurs in the summer at our study sites. As a result, the $\delta^{13}C$ values of C_3 plant tissues grown in summer are more negative than those in spring and autumn. In this case, the positive $\delta^{13}C$–$\delta^{18}O$ relationship for *Cathaica* sp. snails suggests that *Cathaica* sp. land snails start to grow in spring but experience the bulk of their growth in summer, when the carbon isotopes in the snail diet and the oxygen isotopes in rainwater are lower. In contrast, the negative $\delta^{13}C$–$\delta^{18}O$ relationship for *Bradybaena* sp. land snails indicates that they are active in spring and autumn, when snail diets have relatively positive $\delta^{13}C$ values, whereas the $\delta^{18}O$ value of rain water is more positive in spring and low in autumn (Figure 1b). As we summarized above, modern observations confirm the seasonal activity for *Bradybaena* sp. land snails [*Zhang et al.*, 1986; *Xu et al.*, 2002; *Liu et al.*, 2007; *Wang*, 2008]. Meanwhile, a study of the oxygen isotopes of living land snails (*Bradybaena ravida redfieldi*) in Zhenjiang in southern China shows that the shells of *Bradybaena ravida redfieldi* were mostly grown during spring [*Sun et al.*, 2009]. The above conclusion was made based on the fact that $\delta^{18}O$ values of the whole shell samples display little difference from those of the shell lip samples collected in April–June. This at least excludes the possibility that *Bradybaena* sp. would grow their shells in summer. Based on the seasonal difference in the activity of the two species, both the $\delta^{13}C$ and $\delta^{18}O$ of the shell of *Bradybaena* sp. snails should be higher than those of *Cathaica* sp. snails. However, no consistent difference was observed for the $\delta^{13}C$ and $\delta^{18}O$ values (Figure 3), which may be attributed to the different niche or microenvironments where the snails lived. At the same time, the *Cathaica* sp. snails could consume some C_4 plants, which are much more abundant in summer and have higher $\delta^{13}C$ values than C_3 plants, which also influences the expected relationship of the
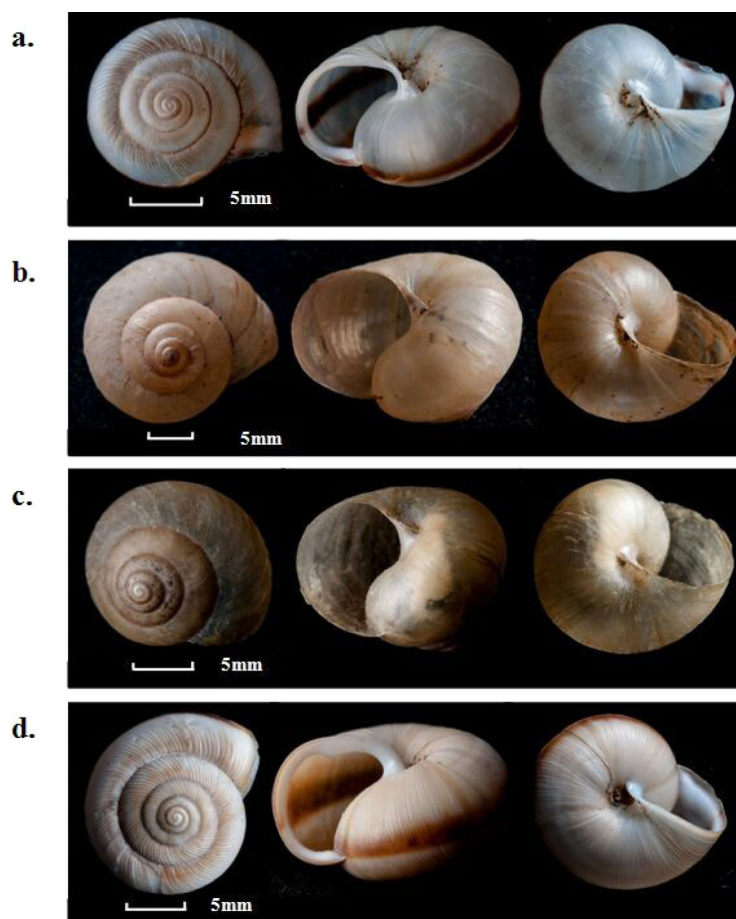
Figure 5. Photos showing the color and morphology of snail shells (*Cathaica* sp. and *Bradybaena* sp.) collected at the study sites. (a, d) *Cathaica* sp. land snails collected in Beijing and Tongchuan; (b, c) *Bradybaena* sp. land snails collected in Pucheng and Tianjin.

δ^{13}C values of the two species. In addition, some individual *Bradybaena* sp. snails may cease hibernation and become active during some cool days with abundant rain in the summer, which would decrease the δ^{18}O of the shells of *Bradybaena* sp. snails.

To further examine the seasonality of shell growth for the *Cathaica* sp. and *Bradybaena* sp. snails, we analyzed carbon and oxygen isotopes of shell segments taken by microdrilling the shells along with growth band. The two species live snails were collected from two adjacent localities (within a distance of 50 m) in Tianjin on a rainy day (22 September 2013). The seasonal patterns of isotopic variation are shown in Figure 6. For the *Cathaica* sp. snail, the δ^{13}C values vary from $-12.1‰$ to $-10.8‰$ with a mean value of $-11.5‰$ whereas the δ^{18}O values fluctuate between $-7.0‰$ and $5.1‰$ (mean

value: $-5.9‰$). By contrast, the δ^{13}C and δ^{18}O values of the *Bradybaena* sp. snail range from $-13.0‰$ to $-11.5‰$ (mean: $-12.3‰$) and from $-6.1‰$ to $-3.6‰$ (mean: $-4.8‰$). Both the δ^{13}C values of the two snails indicate that C$_3$ plants were the main dietary component for them. Here we identify the growth season using shell δ^{18}O because the δ^{18}O values of land snail shell carbonate could reflect the δ^{18}O of the ambient rainwater (δ^{18}O$_P$) [*Yanes et al.*, 2009; *Gu et al.*, 2009; *Zaarur et al.*, 2011]. The δ^{18}O values of the *Cathaica* sp. snail were more negative in the middle part of the shell (Part II, shade area in Figure 6a), representing shell deposition during the months of great rainfall. Based on the characteristics of rainfall δ^{18}O variation in this region (Figure 1b), we consider this part as summer season. Accordingly, Part I and Part III should be the parts of shell growing in autumn and spring, respectively (Figure 6a). Moreover, the latter part of the shell growing in spring has more positive δ^{18}O values than the part of shell growing in autumn, being consistent with the seasonal pattern of rainfall δ^{18}O variation in monsoonal region. If the growth rate of the shell has not undergone large changes, most part of the shell may be grown in summer. This is consistent with the relatively high Δ_{47}-derived temperatures for the *Cathaica* sp. snails (Figure 2). In addition, the δ^{13}C for the part of shell deposited in middle summer displayed more positive values than those for other parts (Figure 6a), possibly suggesting the *Cathaica* sp. snail also ate a small portion of C$_4$ plants, which have peak abundance in summer with respect to other seasons in north China [*Yang et al.*, 2012]. In comparison, the δ^{18}O values of the *Bradybaena* sp. snail showed a generally increasing trend (i.e., from $-6.1‰$ to $-3.6‰$) from the early stage of snail life cycle to the later one and the δ^{18}O values were systematically higher than those of the *Cathaica* sp. snail (Figure 6). Taking the life style of *Bradybaena* sp. snails and the seasonal pattern of rainfall δ^{18}O variation into consideration, we think the main growth season for this snail is spring. This is in accordance to the previous oxygen isotopic study on *Bradybaena* sp. snails [*Sun et al.*,
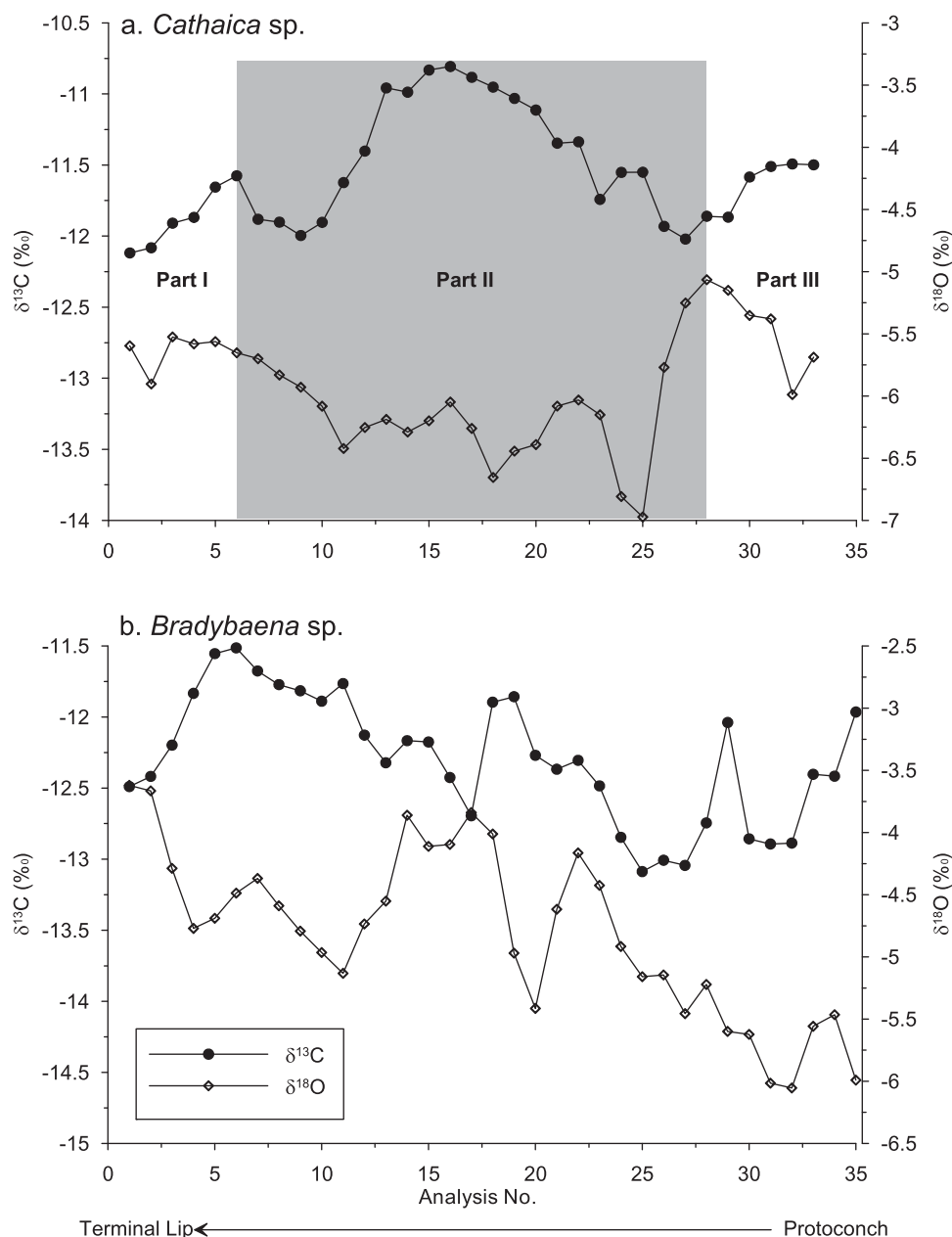
Figure 6. Carbon and oxygen isotope profiles of (a) *Cathaica* sp. snail and (b) *Bradybaena* sp. snail from the terminal lip to the protoconch. Each sample was taken at an interval of ~1 mm along with growth band using microdrilling device. The shade area may represent shell deposition in summer for the *Cathaica* sp. snail.

2009]. Several decreased intervals superimposed on the general increasing pattern of the $\delta^{18}O$ may stand for some heavy rainfall days in spring.

Similar to the previous studies [*Zaarur et al.*, 2011; *Eagle et al.*, 2013], the absolute calcification temperatures in our snail shells are uniformly higher than the local ambient mean temperatures even for the warm months. This was not attributed to a kinetic isotope effect or "vital effect" on ^{13}C–^{18}O bond in carbonate of land snail shells [*Zaarur et al.*, 2011]. Since the Δ_{47}-derived calcification temperatures for modern *Cathaica* sp. shells collected in north China were close to the average daily high temperature in summertime (JJA), *Eagle et al.* [2013] considered that the *Cathaica* sp. snails have a warm affinity for optimum growth. When our Δ_{47} temperatures of *Cathaica* sp. land snails were compared with summertime (JJA) average temperatures, we obtained a Δ_{47}-actual temperature offset ranging from 8.4°C to 9.7°C (mean value: 9.1 ± 0.5°C).
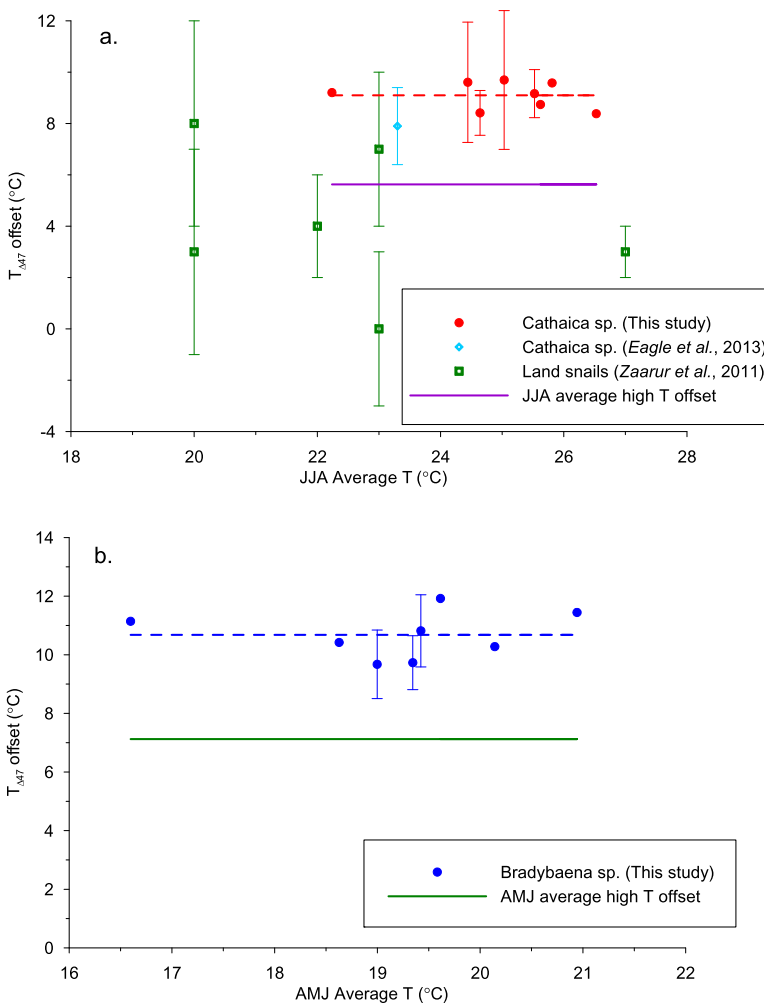
Figure 7. Plots showing (a) $T_{\Delta 47}$ offsets of *Cathaica* sp. snails relative to the local summer mean temperatures (June–August) and (b) $T_{\Delta 47}$ offsets of *Bradybaena* sp. snails relative the local spring mean temperatures (April–June). Error bars denote standard deviation (SD) for the mean $T_{\Delta 47}$ values of multiple individual shells. The $T_{\Delta 47}$ offsets for the *Cathaica* sp. snails collected in Puxian county of north China (cited from *Eagle et al.* [2013]) and the $T_{\Delta 47}$ offsets for land snails worldwide with activity season in summer (cited from *Zaarur et al.* [2011]) were also shown for comparison.

The Δ_{47}-actual temperature offset based on data provided by *Eagle et al.* [2013] is around $7.9 \pm 1.5°C$, consistent with our results within error range (Figure 7a). By contrast, the Δ_{47}-actual temperature offset calculated using the data for snails in monsoon-like regions from *Zaarur et al.* [2011] has a large scatter (range: $0–8°C$), which may be attributed to different ecophysiology for those species. This emphasizes a necessity for us to apply clumped isotope of land sail shells belonging to the same species or genera to reconstruct paleotemperature. In the same manner, we compared the Δ_{47} temperatures of *Bradybaena* sp. land snails to spring (AMJ) average temperatures because both our seasonal $\delta^{18}O$ results and a previous oxygen isotopic study [*Sun et al.*, 2009] reveal that *Bradybaena* sp. land snails almost finish their shell growth in spring. The Δ_{47}-actual temperature offset for *Bradybaena* sp. land snails varies from 9.7 to 11.9°C with a mean value of $10.7 \pm 0.8°C$ (Figure 7b). As shown in Figure 7, the Δ_{47} temperatures for both the *Cathaica* sp. and the *Bradybaena* sp. land snails were even higher than the summertime average monthly high temperature and the spring average monthly high temperature, respectively. Land snails are usually active and come out from shady microenvironments in the afternoon and preferentially at night to avoid intense sun light [*Xu et al.*, 2002; *Colonese et al.*, 2014]. The ground temperature can be very warm in the afternoon. For example, summertime ground temperature can reach up to 40°C in the afternoon in Beijing and Tianjin. Accordingly, the Δ_{47} temperatures for the *Cathaica* sp. land snails in these two places are about 35°C. Therefore, land snails may precipitate shell carbonates during the time of afternoon and night, which thus record relatively

high temperature. However, the temperature seems to extend beyond the appropriate range for these snails to live. The mechanism and timing of shell deposition for the snails deserve a further study in future work.

In summary, the Δ_{47}-actual temperature offsets are shown to be systematic for both the *Cathaica* sp. and *Bradybaena* sp. snails and can be robustly corrected for, which means a useful application of the Δ_{47} of land snail shells in paleotemperature reconstruction. Moreover, the clumped isotope of land snail shells can serve as a season-specific geothermometer in monsoonal regions because different species of land snails grow in different seasons.

4.2. Implications for Paleoclimate Studies Using Faunal Assemblages of Land Snails

In previous studies, the *Cathaica* sp. was assigned to cold-arid snail assemblages representing cold and dry climate because of its high abundance during glacial periods [*Rousseau*, 2001; *Wu and Li*, 2008]. In contrast, some *Bradybaena* sp. snails, i.e., *Bradybaena similaris* were used to infer mild climatic conditions (moderate warmth and humidity) [*Wu et al.*, 2006] and *Bradybaena duplocincta*, together with other species, indicate a warm and humid environment [*Chen and Zhang*, 1998]. By contrast, based on the inferred growing seasons of the two species using our clumped and stable isotopes (Figures 2 and 4), we conclude that *Cathaica* sp. snails prefer warm-humid conditions whereas *Bradybaena* sp. snails adapt to relatively cool and arid environments. Changes in climate seasonality probably play an important role in determining the glacial-interglacial variations in snail faunal assemblages over monsoonal climate regions. During the glacial periods, the East Asian summer monsoon is weaker, with precipitation narrowly focused mainly in the summer [*Yang et al.*, 2012], and the temperature declines, especially in spring and winter, which would produce a relatively cold/dry spring and autumn and a relatively warm/wet summer. These conditions would promote the growth of *Cathaica* sp. snails. Moreover, the habit of basking in the sun of *Cathaica* sp. snails would also favor their abundance during cold-dry glacial periods. During the interglacial periods, the intensified summer monsoon resulted in high precipitation and a long rainy season [*Yang et al.*, 2012], and the temperature also increased. In this case, the relatively warm/humid spring would promote the growth and flourishing of *Bradybaena* sp. snails. However, *Cathaica* sp. snails would not develop because of the high summer temperatures during the interglacial periods because land snails usually live within a temperature range of 10–25°C [*Chen and Zhang*, 2004]. Moreover, the flourishing of *Bradybaena* sp. snails in spring would not allow *Cathaica* sp. snails to develop due to competition for food and territory. Therefore, the ecophysiological traits of each land snail species and the influences of climatic seasonality on changes in land snail assemblages should be taken into consideration when we attempt to obtain paleoclimatic information using snail faunal assemblages.

5. Conclusions

Our clumped isotope data of snail shell carbonates reveal a robust correlation with environmental temperatures, suggesting a potential application of snail shell clumped isotope in reconstructing paleotemperatures in monsoonal climate regions. Moreover, the clumped isotope, together with the stable isotopes of the shell carbonates, indicate that the two species grow in different seasons, i.e., the *Cathaica* sp. snails prefer a warm-humid summer and the *Bradybaena* sp. snails are active in the relatively cool-arid spring and/or autumn. This result conflicts with the widely accepted view that *Cathaica* sp. snails are a cold-arid species with abundant occurrence during glacial periods and that *Bradybaena* sp. snails are thermo-humidiphilous with greater abundance during interglacial periods. In this context, seasonal changes in climate conditions (i.e., the seasonal distribution of warmth and precipitation) over glacial-interglacial cycles may determine the abundance of these two species because they have different growing seasons. This study highlights the importance of climatic seasonality in the changes in the faunal assemblages of land snails.

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