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

Xu Wang¹, Linlin Cui², Jixuan Zhai¹-³, and Zhongli Ding¹

¹Key Laboratory of Cenozoic Geology and Environment, Institute of Geology and Geophysics, Chinese Academy of Sciences, Beijing, China; ²Institute of Geology and Geophysics, Chinese Academy of Sciences, Beijing, China; ³University of Chinese Academy of Sciences, Beijing, China

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 ($\Delta_{47}$) of the shell carbonates. The $\Delta_{47}$-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 $\Delta_{47}$ in shell carbonate 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 definitively 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 $\Delta_{47}$, quantifying the excess abundance of CO₂ 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;
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 shell snails 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 clumped isotope as a proxy indicator of environmental temperatures and distinguishing differences in the ecophysiological habits of the two snail species.

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 similis 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.
Another study on *Bradybaena similaris* land snails reveals that the temperature of 20–25°C and the relative humidity of 70% 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].

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 CO₂ 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 CO₂ 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™, 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 halo-carbons with the mass 47 measurement [Eiler and Schauble, 2004]. The purified CO₂ was then collected into a small glass sample vessel using liquid nitrogen for determination. The δ¹³C, δ¹⁸O, and Δ₄₇ in the CO₂ 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 δ¹³C and δ¹⁸O are reported relative to the VPDB scale as determined using a precalibrated CO₂ tank gas as a reference working gas, and verified using NBS-19. An absolute reference frame for the Δ₄₇ measurements was defined by analyzing the CO₂ heated at 1060°C for 3 h and the CO₂ equilibrated with water at 25°C for approximately 72 h, following the method developed by Dennis et al. [2011]. The Δ₄₇ 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 Δ₄₇ 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 Δ₄₇ values of samples were finally normalized by adjusting the measured Δ₄₇ 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 Δ₄₇ values into calcification temperatures, we use the equation: Δ₄₇ = 0.0636 (10^ΔT) − 0.0047 [Dennis et al., 2011].

δ¹³C and δ¹⁸O 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% H₃PO₄ at 72°C for 1 h. The resultant CO₂ was purified by passing through two NAFION™ 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‰ for δ¹³C and 0.10‰ for δ¹⁸O, respectively.

3. Results

3.1. Variations of the Δ₄₇ Values in Shell Carbonate Along the Transect

The Δ₄₇ 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 δ¹³C and δ¹⁸O values measured on the snail shell carbonates are shown along with the sampling sites (Figure 3 and Table 1). The δ¹³C and δ¹⁸O 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 δ¹³C and −8.33‰ to −1.74‰ for δ¹⁸O (Figure 3). However, there is no systematic difference in either the δ¹³C or δ¹⁸O values between the
4.1. Clumped Isootope of Land Snail Shells as Paleothermometer in Monsoonal Regions

The robust correlation of shell carbonate $\Delta_{47}$-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 $\Delta_{47}$-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

---

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>Species</th>
<th>$\delta^{13}$C (‰) (VPDB)</th>
<th>$\delta^{18}$O (‰) (VPDB)</th>
<th>$\Delta_{47}$ (‰)</th>
<th>$\Delta_{47}$ Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BJ-B-1</td>
<td>Beijing</td>
<td><em>Bradybaena</em> sp.</td>
<td>−11.35 ± 0.007</td>
<td>−2.54 ± 0.016</td>
<td>0.688 ± 0.009</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>BJ-B-2</td>
<td>Beijing</td>
<td><em>Bradybaena</em> sp.</td>
<td>−11.65 ± 0.001</td>
<td>−3.91 ± 0.003</td>
<td>0.692 ± 0.009</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>BJ-C-1</td>
<td>Beijing</td>
<td><em>Cathaica</em> sp.</td>
<td>−11.16 ± 0.001</td>
<td>−4.69 ± 0.002</td>
<td>0.659 ± 0.007</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>BJ-C-2</td>
<td>Beijing</td>
<td><em>Cathaica</em> sp.</td>
<td>−12.17 ± 0.001</td>
<td>−3.85 ± 0.002</td>
<td>0.675 ± 0.009</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>TJ-B-1</td>
<td>Tianjin</td>
<td><em>Bradybaena</em> sp.</td>
<td>−11.90 ± 0.007</td>
<td>−5.90 ± 0.015</td>
<td>0.691 ± 0.008</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>TJ-B-2</td>
<td>Tianjin</td>
<td><em>Bradybaena</em> sp.</td>
<td>−10.74 ± 0.007</td>
<td>−4.17 ± 0.018</td>
<td>0.683 ± 0.008</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>TJ-C-1</td>
<td>Tianjin</td>
<td><em>Cathaica</em> sp.</td>
<td>−9.69 ± 0.007</td>
<td>−8.33 ± 0.012</td>
<td>0.670 ± 0.007</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>TJ-C-2</td>
<td>Tianjin</td>
<td><em>Cathaica</em> sp.</td>
<td>−11.39 ± 0.007</td>
<td>−6.66 ± 0.018</td>
<td>0.662 ± 0.010</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>TJ-C-3</td>
<td>Tianjin</td>
<td><em>Cathaica</em> sp.</td>
<td>−11.25 ± 0.007</td>
<td>−4.60 ± 0.016</td>
<td>0.669 ± 0.008</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>JX-B-1</td>
<td>Jixian</td>
<td><em>Bradybaena</em> sp.</td>
<td>−12.01 ± 0.009</td>
<td>−4.06 ± 0.018</td>
<td>0.692 ± 0.008</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>JX-C-1</td>
<td>Jixian</td>
<td><em>Cathaica</em> sp.</td>
<td>−12.38 ± 0.001</td>
<td>−5.47 ± 0.002</td>
<td>0.677 ± 0.009</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>JX-C-2</td>
<td>Jixian</td>
<td><em>Cathaica</em> sp.</td>
<td>−10.83 ± 0.005</td>
<td>−4.91 ± 0.014</td>
<td>0.663 ± 0.007</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>DZ-B-1</td>
<td>Dingzhou</td>
<td><em>Bradybaena</em> sp.</td>
<td>−10.36 ± 0.007</td>
<td>−6.54 ± 0.012</td>
<td>0.686 ± 0.008</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>DZ-C-1</td>
<td>Dingzhou</td>
<td><em>Cathaica</em> sp.</td>
<td>−13.61 ± 0.007</td>
<td>−7.00 ± 0.017</td>
<td>0.664 ± 0.008</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>TC-B-1</td>
<td>Tongchuan</td>
<td><em>Bradybaena</em> sp.</td>
<td>−11.98 ± 0.007</td>
<td>−4.35 ± 0.015</td>
<td>0.699 ± 0.008</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>TC-C-1</td>
<td>Tongchuan</td>
<td><em>Cathaica</em> sp.</td>
<td>−8.56 ± 0.001</td>
<td>−1.74 ± 0.002</td>
<td>0.682 ± 0.007</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>PC-B-1</td>
<td>Pucheng</td>
<td><em>Bradybaena</em> sp.</td>
<td>−11.99 ± 0.007</td>
<td>−6.61 ± 0.012</td>
<td>0.699 ± 0.007</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>PC-B-2</td>
<td>Pucheng</td>
<td><em>Bradybaena</em> sp.</td>
<td>−11.23 ± 0.001</td>
<td>−1.51 ± 0.002</td>
<td>0.688 ± 0.009</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>PC-B-3</td>
<td>Pucheng</td>
<td><em>Bradybaena</em> sp.</td>
<td>−11.07 ± 0.001</td>
<td>−2.25 ± 0.002</td>
<td>0.695 ± 0.009</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>PC-C-1</td>
<td>Pucheng</td>
<td><em>Cathaica</em> sp.</td>
<td>−11.58 ± 0.005</td>
<td>−8.04 ± 0.007</td>
<td>0.677 ± 0.007</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>LT-B-1</td>
<td>Lintong</td>
<td><em>Bradybaena</em> sp.</td>
<td>−10.92 ± 0.001</td>
<td>−6.97 ± 0.002</td>
<td>0.681 ± 0.008</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>LT-C-1</td>
<td>Lintong</td>
<td><em>Cathaica</em> sp.</td>
<td>−9.50 ± 0.001</td>
<td>−1.98 ± 0.002</td>
<td>0.669 ± 0.007</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>LY-B-1</td>
<td>Luoyang</td>
<td><em>Bradybaena</em> sp.</td>
<td>−12.34 ± 0.007</td>
<td>−4.70 ± 0.013</td>
<td>0.677 ± 0.008</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>LY-C-1</td>
<td>Luoyang</td>
<td><em>Cathaica</em> sp.</td>
<td>−13.27 ± 0.005</td>
<td>−3.08 ± 0.014</td>
<td>0.666 ± 0.008</td>
<td>35 ± 2</td>
</tr>
</tbody>
</table>
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, yellowish, 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 $D_{47}$ 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 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.
Southern China shows that the shells of multiple individual shells. Note the positive activity of the two species, both the possibility that display little difference from those of the shell lip samples collected in April–June. This at least excludes the more apparent in summer because most of the annual precipitation occurs in the summer at our study China usually decrease with rainfall increase [Gu et al., 2004; Liu et al., 2012]. This effect is visible as a distinct dip in 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$_r$) [Yanes et al., 2009; Gu et al., 2009; Zaarur et al., 2011]. In monsoonal climate regions, low $\delta^{18}$O$_r$ values occur in summer due to the amount effect, overshadowing the dependence of $\delta^{18}$O$_e$ 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$_e$ 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 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.

\[ Y = 0.431 \times - 9.333 \]
\[ R^2 = 0.33 \]

\[ Y = -0.264 \times - 12.711 \]
\[ R^2 = 0.31 \]
\( \delta^{13}C \) 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 \( \delta^{18}O \) 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 \( \delta^{13}C \) values vary from \(-12.1\) to \(-10.8\) with a mean value of \(-11.5\). By contrast, the \( \delta^{13}C \) and \( \delta^{18}O \) 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 \( \delta^{13}C \) values of the two snails indicate that C3 plants were the main dietary component for them. Here we identify the growth season using shell \( \delta^{18}O \) because the \( \delta^{18}O \) values of land snail shell carbonate could reflect the \( \delta^{18}O \) of the ambient rainwater \( (\delta^{18}O_p) \) [Yanes et al., 2009; Gu et al., 2009; Zaarur et al., 2011]. The \( \delta^{18}O \) 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 \( \delta^{18}O \) 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 \( \delta^{18}O \) values than the part of shell growing in autumn, being consistent with the seasonal pattern of rainfall \( \delta^{18}O \) 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 \( \Delta_{\text{at}} \)-derived temperatures for the Cathaica sp. snails (Figure 2). In addition, the \( \delta^{13}C \) 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 C4 plants, which have peak abundance in summer with respect to other seasons in north China [Yang et al., 2012]. In comparison, the \( \delta^{18}O \) 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 \( \delta^{18}O \) 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 \( \delta^{18}O \) 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.,
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 $\Delta_{47}$-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 $\Delta_{47}$ temperatures of Cathaica sp. land snails were compared with summertime (JJA) average temperatures, we obtained a $\Delta_{47}$-actual temperature offset ranging from 8.4°C to 9.7°C (mean value: 9.1 ± 0.5°C).

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.
The Δ_{27} actual temperature offset based on data provided by Eagle et al. [2013] is around 7.9 ± 1.5°C, consistent with our results within error range (Figure 7a). By contrast, the Δ_{27} 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_{27} temperatures of Bradybaena sp. land snails to spring (AMJ) average temperatures because both our seasonal δ^{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_{27} actual temperature offset for Bradybaena sp. land snails varies from 9.7 to 11.9°C with a mean value of 10.7 ± 0.8°C (Figure 7b). As shown in Figure 7, the Δ_{27} 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_{27} 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

Figure 7. Plots showing (a) T_{D_{27}} offsets of Cathaica sp. snails relative to the local summer mean temperatures (June–August) and (b) T_{D_{27}} offsets of Bradybaena sp. snails relative to the local spring mean temperatures (April–June). Error bars denote standard deviation (SD) for the mean T_{D_{27}} values of multiple individual shells. The T_{D_{27}} offsets for the Cathaica sp. snails collected in Puxian county of north China (cited from Eagle et al. [2013]) and the T_{D_{27}} offsets for land snails worldwide with activity season in summer (cited from Zaarur et al. [2011]) were also shown for comparison.
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 $\Delta_{47}$-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 $\Delta_{47}$ 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 similares 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 ecophysiolog-ical 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.

Acknowledgments

We thank Naiqin Wu and Fengjiang Li for identifying the species of land snails, Wei Zhang for taking photos of the snail shells and Lanzhen Guo and Hongwei Li for their assistances in experiments. We are greatly indebted to Jay Quade and an anonymous reviewer for their constructive comments on the early version of the manuscript. We acknowledge the financial support from the “Strategic Priority Research Program” of the Chinese Academy of Sciences (grant XDB03020500), the National Basic Research Program of China (973 Program) (2012CB821905), and the National Science Foundation of China (grant 41572163). The data of bulk stable isotopes and clumped isotope used to generate Figures 2–4 are listed in Table 1. Data for producing Figure 6 and 7 are available from the corresponding author via e-mail xuking@mail.iiggcas.ac.cn.

References


Balakrishnan, M., C. J. Yapp, D. J. Meltzer, and J. L. Theler (2005b), Paleoenvironment of the Folsom archaeological site, New Mexico, USA, approximately 10, 500–30, 000 yr B.P., as inferred from the stable isotope composition of fossil land snail shells, Quat. Res., 63, 31–44.

Bowen, G. J. (2008), The Online Isotopes in Precipitation Calculator, Version 2.2, The University of Utah, Salt Lake City, Utah, USA. [Available at http://www.waterisotopes.org.]


Heath, D. J. (1975), Colour, sunlight and internal temperatures in the land snail Cepaea nemoralis (L.), Oecologia, 19, 29–38.


