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Clumped isotopic compositions of cultured and natural land-snail shells and their implications

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Abstract

Land snails are widely distributed animals whose clumped isotope (Δ_{47}) in shell carbonates can serve as a proxy for temperature in terrestrial environments. However, it is unclear whether the Δ_{47} values of snail shells are affected by biological processes known as “vital effects.” Here, we report Δ_{47} values of whole *Achatina fulica* snail shells cultured at different temperatures at different growth ages (samples collected monthly). In general, the mean Δ_{47} values of the snail shells decrease with higher cultivation temperature, which indicates they have potential use in reconstructing temperature changes. However, the mean reconstructed Δ_{47} temperatures (T_{47}) using the calibrations from Petersen et al. (2019), Briffacé et al. (2017), and Kelson et al. (2017) are $\sim 6^\circ\text{C}$ higher than the actual growth temperatures. Interestingly, this overestimation in T_{47} disappears when applying Zhai et al.’s (2019) calibration for the *Bradybaena* snail. This calibration also yields good agreement between the T_{47} data of all field-collected snails and their growing season temperatures. The above-mentioned phenomenon indicates the existence of the vital effect in land-snail shells. The variations in Δ_{47} with snail age and the correlations between Δ_{47} , $\delta^{18}\text{O}$, and $\delta^{13}\text{C}$ values also indicate the potential influence of vital effects. Finally, we suggest that the vital effect may be explained by kinetic fractionation through CO_2 degassing associated with dehydration/dehydroxylation during the formation of snail shells.

Keywords: Vital effect; Dehydration/dehydroxylation; Growth season; Temperature;

Achatina fulica

1. INTRODUCTION

Land snails are widely distributed and abundant in various terrestrial environments, and their assemblages and shell isotopic compositions record environmental change (Goodfriend, 1992; Wu et al., 2018 and references therein). Furthermore, the fossil remains of land snails are well preserved and easily accessible as secondary carbonates in loess sediments, particularly in the loess–paleosol deposits on the Chinese Loess Plateau. Such fossils serve as excellent geological archives for reconstructing past changes in terrestrial temperature (Eagle et al., 2013a; Dong et al., 2020). Following the pioneering research of Yapp (1979) on the stable isotopes of land-snail shells, numerous studies have analyzed the climate information recorded by the isotopic compositions of land-snail shells (e.g., stable carbon ($\delta^{13}\text{C}$) and stable oxygen ($\delta^{18}\text{O}$) isotopes), of which a shell's $\delta^{13}\text{C}$ value is generally used to infer variations in the photosynthetic types (i.e., C3, C4) of local plant communities (e.g., Goodfriend and Magaritz, 1987; Goodfriend and Ellis, 2000; Goodfriend and Ellis, 2002; Stott, 2002; Balakrishnan et al., 2005; Liu et al., 2007; Yanes et al., 2009; Colonese et al., 2014; Prendergast et al., 2016; Bao et al., 2020), and a shell's $\delta^{18}\text{O}$ value indicates the isotopic composition of rainwater (e.g., Lecolle, 1985; Goodfriend and Ellis, 2002; Yanes et al., 2009; Kehrwald et al., 2010; Colonese et al., 2014). Flux balance models have also been proposed to explain the oxygen and carbon isotopic compositions of land-snail shells (Balakrishnan and Yapp, 2004). Recent studies suggest the combined investigation of $\delta^{13}\text{C}$ and stable nitrogen isotopes ($\delta^{15}\text{N}$) of snail shells may provide insights into snail foraging ecology (Yanes et al., 2018), and that

the differences in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ between snail species could roughly reflect differences in the lengths of their growing seasons (Huang et al., 2012). Snail cultivation studies indicate that food water is an important source of land-snail body water (Zhang et al., 2018a). Other recent studies have assessed the applicability of carbonate clumped isotope measurements to temperature reconstructions using modern land-snail shells (Zaarur et al., 2011; Wang et al., 2016; Zhang et al., 2018b; Guo et al., 2019; Zhai et al., 2019) and fossil shells (Eagle et al., 2013a; Dong et al., 2020).

The clumped isotopic composition of carbonates is a powerful tool in reconstructing the formation temperature of carbonate (Ghosh et al., 2006; Eiler, 2007, 2011). The abundance of the doubly substituted CO_2 isotopologue (e.g., $^{13}\text{C}^{18}\text{O}^{16}\text{O}$, mass 47) in CO_2 produced by phosphoric acid digestion of carbonates is proportional to the concentration of $^{13}\text{C}^{18}\text{O}^{16}\text{O}^{2-}$ ionic groups in the reactant carbonates (Ghosh et al., 2006). The clumped isotope signature of carbonates is generally expressed as Δ_{47} , which is defined as the enrichment of $^{13}\text{C}^{18}\text{O}^{16}\text{O}$ in CO_2 relative to the amount expected for a stochastic distribution of isotopes among all CO_2 isotopologues (Eiler and Schauble, 2004; Ghosh et al., 2006). The concentration of the $^{13}\text{C}-^{18}\text{O}$ bond in CO_2 is temperature-dependent, and is enriched at lower temperatures and depleted at higher temperatures (Wang et al., 2004) due to the lower zero-point energies of multiply substituted isotopologues (e.g. $^{13}\text{C}^{18}\text{O}^{16}\text{O}$) than their isotopically normal and singly substituted relatives (e.g. $^{13}\text{C}^{16}\text{O}^{16}\text{O}$, $^{12}\text{C}^{18}\text{O}^{16}\text{O}$) (Eiler and Schauble, 2004). Therefore, the Δ_{47} thermometer is based on thermodynamics. Moreover, it is

independent of the isotopic composition of the water from which the carbonates grew, and simultaneous determinations of Δ_{47} and $\delta^{18}\text{O}$ in carbonates can constrain the $\delta^{18}\text{O}$ value of the water from which they precipitated (Ghosh et al., 2006; Eiler, 2007, 2011).

Zaarur et al. (2011) was the first to study Δ_{47} in land-snail shells. However, they indicated that the Δ_{47} temperatures (T_{47}) of the shells, which were derived from the first Δ_{47} -temperature (Δ_{47} -T) calibration line known as the Ghosh calibration (Ghosh et al., 2006), are typically higher than the mean annual temperature and the ambient temperature of the snail activity season. The authors thus suggested that land-snail shells could not be used to directly reconstruct air temperatures, even though they are considered to form under equilibrium conditions (Zaarur et al., 2011). Later efforts also showed that the T_{47} values of natural snail shells are higher than their expected environmental temperatures or estimated snail-activity temperatures based on the Ghosh calibration (Wang et al., 2016; Zhai et al., 2019) or the Δ_{47} -T calibration of Kelson et al. (2017) (Guo et al., 2019). This higher temperature is attributed to the effects of the eco-physiological or behavioral adaptations of the snails, such as their shell color, morphology, and behavior (Zaarur et al., 2011; Guo et al., 2019).

Many studies have reported clear Δ_{47} offsets from nominal equilibrium values in biological carbonates, such as corals (Saenger et al., 2012; Kimball et al., 2016; Spooner et al., 2016), echinoid calcite (Davies and John, 2019), and brachiopod calcite (Bajnai et al., 2018). This phenomenon is known as the “vital effect”. However, land-snail culturing experiments have suggested that the shells of *Acusta despecta* are

precipitated at Δ_{47} equilibrium with no obvious vital effects, and that snail Δ_{47} values record the snail's active-season temperature (Zhang et al., 2018b). Other studies also revealed that the T_{47} values of land-snail shells are strongly correlated with environmental (snail active season) temperatures (Wang et al., 2016). Snail-specific Δ_{47} – T calibration lines have been established from estimated snail-shell formation temperatures (Zhai et al., 2019). However, the mean T_{47} values of modern snails measured by Eagle et al. (2013a) and Dong et al. (2020) (~31 °C and 28 °C, respectively) are still higher than the expected temperature during the snail growing season (i.e. the warm season from June to August), although the reconstructed temperature differences between glacial and modern periods may be less affected by the high-than-expected T_{47} .

The unexpectedly high temperatures derived from T_{47} measurements of snails remain an intriguing topic because experimental studies have shown that snails are generally not active at temperatures above 27 °C (Cowie, 1984; Thompson and Cheny, 1996), and that high temperatures (>30 °C) are lethal to snails if the relative humidity (RH) is low (Xu et al., 2002). Recent laboratory culturing experiments have shown that a narrow RH range of $\geq 55\%$ –70% is needed to prevent snail aestivation (Rangarajan et al., 2013). Thus, a persistent water supply is required to keep a favorable RH level for snails under high temperatures, which is difficult to achieve in a natural environment.

Since the publication of the Ghosh calibration, many empirical Δ_{47} – T calibrations have been established based on laboratory synthetic carbonates (Dennis and Schrag,

2010; Zaarur et al., 2013; Fernandez et al., 2014; Tang et al., 2014; Defliese et al., 2015; Kluge et al., 2015; Staudigel et al., 2016; Winkelstern et al., 2016; Bonifacie et al., 2017; Kelson et al., 2017; Levitt et al., 2018). Among these studies, the Δ_{47} -T calibrations developed by Kelson et al. (2017) and Bonifacie et al. (2017) may be considered as the nominal equilibrium calibration under the absolute reference frame, which is consistent with a large composite dataset ($n = 1253$ sample replicates) from 11 different labs (Petersen et al., 2019). Various biological carbonates have been cultured under controlled temperatures or collected in the field with known or well-constrained growth temperatures and analyzed to study the relationship between their Δ_{47} values and growth temperatures, including fish otoliths (Ghosh et al., 2007), foraminifera (Tripathi et al., 2010; Gräbe et al., 2013; Wacker et al., 2014; Breitenbach et al., 2018; Peral et al., 2018), corals (Thiagarajan et al., 2011), mollusks (Eagle et al., 2013b; Henkes et al., 2013; Petrizzi et al., 2014; Wacker et al., 2014; Zhang et al., 2018b), brachiopods (Henkes et al., 2013; Came et al., 2014; Wacker et al., 2014), ostrich egg shells (Wacker et al., 2014), coccoliths (Katz et al., 2017), and echinoids (Davies and John 2017). However, it remains unclear which calibration is suitable for the Δ_{47} values of snail shells.

Furthermore, it is difficult to evaluate the applicability of these calibration lines to snail shells by only studying natural land snails, since there is a large uncertainty in the estimation of shell-formation timing and temperature for different species owing to seasonal temperature variations, particularly in monsoonal regions. Moreover, the non-equilibrium effect in Δ_{47} measurements has been observed in biological

carbonates and inorganic carbonates of speleothems (Affek et al., 2008, 2014; Daëron et al., 2011; Kluge and Affek, 2012; Kluge et al., 2013). However, the importance of non-equilibrium effect during the formation of snail shells remains unclear.

Therefore, further research is needed on the Δ_{47} values of snail shells precipitated at known temperatures for their use in paleotemperature reconstructions. We analyzed the Δ_{47} compositions of snail shells cultured under well-controlled temperatures and modern shells collected in the field from two different climatic regions. First, we compared the laboratory snail-growth temperatures with the reconstructed T_{47} using different calibration lines to investigate which calibration applies best to snails. Second, the selected calibration line was applied to the field-collected land-snail shells from different climatic regions and to previously published data to test its reliability. Finally, we discuss the evidence and possible underlying factors for the vital effect in land-snail shells.

2. MATERIALS AND METHODS

2.1. Cultured land snails

Three snail species (*Achatina fulica* “white”, *Bradybaena ravida redfieldi*, and *Rumina decollata*) were used for culturing experiments at the Institute of Earth Environment, Chinese Academy of Sciences, Xi’an, China. The culturing temperature and RH were monitored using data loggers (Hobo(@) Pro v2). Parent snails were raised in a plastic box in an incubator and their eggs were hatched at controlled temperatures. The hatched snails were raised at 22.9 ± 0.3 °C, 27.7 ± 0.4 °C, or 29.3 ± 0.8 °C (mean $\pm$ standard deviation) (Table 1), in an average RH of ~90%. Fresh

lettuce ($\delta^{13}\text{C}$ = approximately -27.5‰) was the only food supplied and loess deposits from the penultimate glaciation were provided as a calcium source during the experiments. The $\delta^{18}\text{O}$ of the supplied water was relatively stable (approximately -10.5‰); the $\delta^{18}\text{O}$ of the water in the lettuce was not constrained.

Achatina fulica “white” is a variant species of *A. fulica* and is also known as the white jade snail. This species was selected for study because their large shells provide substantial material for Δ_{47} analysis. In our experimental treatments, whole shells of *A. fulica* “white” were harvested one month after hatching, which were treated as first-month shells, and so on. Five shells of *A. fulica* “white” were sampled and crushed each month for the first five months for snails raised at 27.7 °C . Five shells were harvested for the first, third, and fifth months for those raised at 22.9 °C because of slow growth at this temperature (Fig. 1). *Bradybaena ravida redfieldi* and *R. decollate* are readily available around the Institute of Earth Environment, CAS; they were cultured only at 29.5 °C to compare the Δ_{47} values between the three different species.

2.2. Modern land snails collected in the field

Modern snail shells were collected from three locations: *A. fulica* and *Bradybaena sp.* on Hainan Island, *Bradybaena sp.* in Kunming city in China, and Helicidae *Helix aspersa* and *Oxychilus draparnaudi* on the campus at the California Institute of Technology (Caltech) in Pasadena, CA, USA (Table 2). The first two locations are influenced by the East Asian monsoon whereas the third is dominated by a Mediterranean climate. Hainan Island is characterized by a warm–wet summer,

while Pasadena is dominated by winter precipitation. The difference in monthly average instrumental temperatures between Hainan Island and Kunming is almost constant at ~ 8 °C throughout the year, with minor variations in RH (Fig. S1). Thus, the two locations may be regarded as a natural laboratory for studying the Δ_{47} values of snails.

2.3. Sample preparation and isotopic analysis

To prepare samples for Δ_{47} measurement, all cultured snail shells and fresh modern shells were treated with 3% H_2O_2 solution (buffered to neutral) overnight to remove adhered organic components. The shells were then rinsed in distilled water and air-dried at room temperature. The whole shells were then crushed into a homogeneous powder using an agate mortar.

All the Δ_{47} measurements were performed at Caltech in 2017 using an automated acid digestion and gas purification device coupled to a dual-inlet Thermo MAT253, as described by Passey et al., (2010). In brief, ~ 8 mg samples were placed into Ag capsules and reacted in a common phosphoric acid bath for 20 min at 90 °C. The extracted CO_2 was passed through an ethanol/dry ice U-trap at ~ 80 °C before being collected on a liquid nitrogen U-trap. Following the 20 min reaction, 15 min was given for the CO_2 sample to pass completely through a Porapak Q 120/80 mesh gas chromatography column held at -20 °C using He as the carrier gas. The purified CO_2 was analyzed using a Thermo Scientific MAT253 mass spectrometer configured to collect masses 44–49. Each measurement consisted of eight acquisitions (16 V on $m/z = 44$) of seven cycles of the unknown CO_2 sample versus the Oztech working

CO₂ gas [$\delta^{18}\text{O} = 24.97\text{‰}$ Vienna Standard Mean Ocean Water (VSMOW), $\delta^{13}\text{C} = -3.62\text{‰}$ Pee Dee Belemnite (PDB)]. The signal integration time of each cycle was 26 s, resulting in a total integration time of 1456 s for each sample. This corresponds to a Δ_{47} precision of $\sim 0.01\text{‰}$ at the shot-noise limit.

All Δ_{47} values are reported on the carbon dioxide equilibrium scale (CDES) proposed by Dennis et al. (2011) using the theoretical equilibrium values of heated and equilibrated gases at 1000 °C and 25 °C (Wang et al., 2004; table 1 in Dennis et al., 2011). The heated and equilibrated gases were purified and measured regularly throughout the analysis period using the same method as for the carbonate-derived CO₂ samples. Furthermore, all data were calculated by applying ^{17}O correction parameters following Brand et al. (2010), as suggested by Daëron et al. (2016) and Schauer et al. (2016). When necessary, an acid fractionation factor (AFF) of 0.088 was applied to all data at the CDES scale for comparison with the samples digested at 25 °C, as proposed by Petersen et al. (2019). For $\delta^{18}\text{O}$ calculation from aragonite snail shells, an acid digestion fractionation factor of 1.00854 was used, calculated by extrapolation from a published calibration (Kim et al., 2007). The standard errors of the in-house standards (TV04 and CIT Carrara) are 0.01‰, 0.02‰ and 0.06‰ for Δ_{47} , $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$, respectively.

3. RESULTS

3.1. Clumped isotopic compositions of cultured land-snail shells

Table 1 summarizes the isotopic results ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and Δ_{47}) of all cultured snail shells. The average Δ_{47} values are $0.588 \pm 0.005\text{‰}$ ($n = 9$), $0.580 \pm 0.003\text{‰}$ ($n = 15$),

and $0.573 \pm 0.003\text{‰}$ ($n = 15$) for snails grown at $22.9 \pm 0.3\text{ °C}$, $27.7 \pm 0.4\text{ °C}$, and $29.3 \pm 0.8\text{ °C}$, respectively, where n is the number of replicates, with no fractionation correction for the acid digestion at 90 °C . Shell Δ_{47} values decreased as the snail growth temperature increased. However, the reconstructed T_{47} are on average $\sim 6\text{ °C}$ higher than the measured temperatures when adopting the Δ_{47} – T calibrations of Petersen et al. (2019), Bonifacie et al. (2017), and Kelson et al. (2017) (Table 1). In contrast, T_{47} is consistent with the monitored growth temperatures when adopting the Δ_{47} – T calibration for *Bradybaena* species from Zhou et al. (2019); however, the calibration for *Cathacia* species produced an underestimate in T_{47} (Table 1).

Notably, the Δ_{47} values of *A. fulica* decreased from $0.603 \pm 0.005\text{‰}$ in the first month to $0.585 \pm 0.003\text{‰}$ in the third month and finally to $0.577 \pm 0.008\text{‰}$ in the fifth month for snails cultured at 22.9 °C (Table 1; Fig. 2A); similar Δ_{47} variations were observed for snails grown at 27.7 °C . However, for snails grown at 27.7 °C , the decrease occurred only for the first two months, from $0.591 \pm 0.004\text{‰}$ to $0.574 \pm 0.006\text{‰}$, and the Δ_{47} values appeared to level off for the later months (Table 1; Fig. 2B). For snails grown at 29.3 °C , the Δ_{47} values of *A. fulica* “white” and *B. ravidula redfieldi* were almost identical, whereas those of *R. decollata* were slightly lower but still within analytical error (Table 1).

3.2. Stable carbon and oxygen isotopic compositions of cultured land-snail shells

For the snails cultured at 22.9 °C and 27.7 °C , the variability of $\delta^{13}\text{C}$ is 1.402‰ and 2.984‰ , respectively, and for $\delta^{18}\text{O}$ is 1.222‰ and 1.942‰ , respectively (Table 1). For shells grown at 22.9 °C and 27.7 °C , a negative correlation is observed between

$\delta^{18}\text{O}$ and Δ_{47} , with $R^2 = 0.97$ and 0.78 , and slopes of -0.024 and -0.009 , respectively (Fig. 3). Similar negative correlations between $\delta^{13}\text{C}$ and Δ_{47} are observed for snail shells grown at $22.9\text{ }^{\circ}\text{C}$ and $27.7\text{ }^{\circ}\text{C}$, with $R^2 = 0.84$ and 0.57 , respectively (Fig. 3). Generally, the correlation is weaker for snail shells cultured at $27.7\text{ }^{\circ}\text{C}$. Moreover, there is a weak positive correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ for *A. fulica* “white” cultured at $22.9\text{ }^{\circ}\text{C}$ and $27.7\text{ }^{\circ}\text{C}$ (Fig. S2), suggesting the existence of the kinetic effect.

3.3. Stable and clumped isotopic compositions of land- snail shells from different climatic regions

Table 2 presents the $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and Δ_{47} compositions of modern snail shells collected from monsoonal and Mediterranean climatic regions. The $\delta^{13}\text{C}$ values vary from -9.681‰ to -15.994‰ and the $\delta^{18}\text{O}$ values from -0.316‰ to -7.671‰ . The average Δ_{47} values for snail shells collected from Hainan Island, Kunming, and Pasadena are $0.581 \pm 0.002\text{‰}$, $0.606 \pm 0.005\text{‰}$, and $0.622 \pm 0.003\text{‰}$, respectively. Similarly, T_{47} derived from the Δ_{47} – T calibration for *Bradybaena* species from Zhai et al. (2019) agrees with the measured growth temperature, but T_{47} is slightly lower when the calibration for *Cathacia* species is used (Table 2) and higher when the calibration line of Petersen et al. (2019; Table 2) is used.

4. DISCUSSION

4.1 Δ_{47} – T calibrations for land snails

The first Δ_{47} study on land snails suggested that their Δ_{47} values could not be used to directly reconstruct air temperatures (Zaarur et al., 2011). However, Eagle et al.

(2013a) observed growing season temperature (GST) differences between modern snails and those from the Last Glacial Maximum in central China, based on Δ_{47} values. Further studies reported a robust correlation between snail-shell aragonite Δ_{47} and environmental temperature, and suggested the potential for temperature reconstructions, even though the T_{47} values were higher than the temperatures expected from the GST (Wang et al., 2016; Zhai et al., 2019). A recent culturing study also suggested that snail-shell Δ_{47} values followed the Δ_{47} - T calibration line of Kelson et al. (2017), indicating that the aragonite shells of land snails are precipitated at Δ_{47} equilibrium with no obvious Δ_{47} vital effect (Zhang et al., 2018b). Our land-snail culturing experiment further demonstrates that the Δ_{47} values of snail shells decrease when the growth temperature is increased. Thus, we suggest that the Δ_{47} values of snail shells may be used to reconstruct their formation/environmental temperature.

However, when applying the Δ_{47} - T calibration line of Kelson et al. (2017) to our results, the reconstructed T_{47} values are much higher than the monitored growth temperatures. Similar high T_{47} values are generated using the Δ_{47} - T transfer functions from Petersen et al. (2019) and Bonifacie et al. (2017). Recently, snail-specific Δ_{47} - T transfer functions have been developed for *Bradybaena* and *Cathacia* species, based on large sampling areas in China and estimated growing-season temperatures (Zhai et al., 2019). In contrast to the above-mentioned phenomenon, the Δ_{47} - T calibration function for *Bradybaena* species produces more reasonable T_{47} values, but the reconstructed T_{47} are lower using that for *Cathacia* species (Table 1). This indicates that snail-specific Δ_{47} - T transfer functions may be necessary to accurately reconstruct

the calcification temperature of land-snail shells.

To further evaluate the applicability of the transfer functions of Petersen et al. (2019) and Zhai et al. (2019), we applied them to the Δ_{47} results from our field-collected snails from the monsoonal and Mediterranean regions. The growing season of land snails depends on seasonal temperature and precipitation in specific regions (Zhai et al., 2019). The derived T_{47} values are consistent with the expected GSTs from both climatic domains when using the Δ_{47} - T function for *Bradybaena* species (Table 2; Fig. 4). However, the derived T_{47} values are lower or higher when using the functions for the *Cathacia* species from Petersen et al. (2019). We also applied the transfer function for the *Bradybaena* species to previous results (Dong et al., 2020). The mean recalculated T_{47} values are closer to the instrumental GSTs and are ~6 °C lower than those from the Peterson calibration (Fig. S3; Table 3). Therefore, a snail-specific calibration may provide reasonable T_{47} values, indicating the existence of the vital effect in land snails.

Future studies will need to confirm whether the proposed functions for the two snail species (*Bradybaena* and *Cathacia*) are related to their microenvironments or species-specific vital effects. For example, the *Cathacia* species prefer to live on trees, while *Bradybaena* are ground-dwelling species (Zhai et al., 2019). Our monitored temperature is several degrees (Celsius) higher at ~2 m on the tree than at the plant-covered ground surface in the Xi'an region in China (Fig. S4). This may explain the higher observed T_{47} for *Cathacia* species than for *Bradybaena* species, as reported by Zhai et al. (2019). Therefore, there may be no need for species-dependent

calibrations.

4.2 Comparison of cultured land-snail Δ_{47} values and other calibrations

Carbonate Δ_{47} -T calibration lines with similar shallow slopes have been on the basis of large sample populations and a wide temperature range (Bonifacie et al., 2017; Kelson et al., 2017; Petersen et al., 2019) (Fig. 5). Here, we compared our cultured land-snail Δ_{47} values with the Petersen calibration, because it represents many mineralogies, bulk compositions, sample types, reaction temperatures, and sample preparation and analysis methods. It may thus be treated as an experimental “universal” Δ_{47} -T calibration. All our data were directly transferred to the CDES frame and processed using the updated ^{17}C correction parameters, as suggested by Daëron et al. (2016) and Schauer et al. (2016). These are the same as used in the comprehensive compilation study of Petersen et al. (2019) and the experimental study of Kelson et al. (2017). Thus, our data can be directly compared with these two calibrations (Fig. 5). The calibration line developed by Bonifacie et al. (2017) is also shown for comparison, although they used different ^{17}O correction parameters for data processing (Fig. 5).

Almost all of our Δ_{47} values sit below the Petersen calibration, with a mean depletion of $\sim 0.016\text{‰}$. Additional replicates would be helpful to clarify this offset, as the Δ_{47} values of the first-month shells are closest to this calibration (filled triangles in Fig. 5). In contrast, the recently proposed calibration function for *Bradybaena* fits well with our results within the studied temperature ranges (Fig. 5). Besides, low Δ_{47} values have also been reported for mollusks in other labs (Henkes, et al., 2013;

Petrizzo et al., 2014; Wacker et al., 2014; Fig. 5). Thus, the depletion of land snail shell's Δ_{47} values from Petersen calibration may be truly exist.

4.3 Validation of the lower Δ_{47} values

The disagreements in Δ_{47} values/ Δ_{47} -T calibrations between labs have been attributed to instrument-specific effects (Petrizzo et al., 2014), methodology issues (Defliese et al., 2015; Kelson et al., 2017), or small sample sizes and narrow temperature ranges of carbonate formation (Fernandez et al., 2017). The parameters of Brand et al. (2010) for ^{17}O correction are recommended to eliminate or reduce such inter-laboratory discrepancies (Daëron et al., 2016; Schauer et al., 2016). However, the new Brand parameters are possibly not the only reason for interlaboratory differences, the cause of which remains enigmatic (Bernasconi et al., 2018).

The Δ_{47} values for all snail shells in this study were calculated using the updated set of parameters from Brand et al. (2010), including $R^{13}_{\text{VPDB}} = 0.0112372$, $R^{17}_{\text{VSMOW}} = 0.038475$, $R^{18}_{\text{VSMOW}} = 0.0020052$, and $\lambda = 0.528$, and were translated into the absolute reference frame proposed by Dennis et al. (2011). The same calculations were used for the data of Petersen et al. (2019) and Kelson et al. (2017). This enabled us to discount the differences in data processing as a potential cause of the deviation in snail shell Δ_{47} values. This enables us to directly compare our results with these calibration lines.

It is also unlikely that the instrument-specific effects are responsible for the observed offset. First, all the cultured shells were analyzed multiple times and the standard error in mean Δ_{47} value for each month was $<0.01\%$ (Table 1), which is less

than the offset reported here (although our sample size is small). Second, our Δ_{47} values are lower than those calculated from the calibration of Bonifacie et al. (2017), a portion of whose results were also obtained from Caltech, thereby removing possible inter-lab differences. The snail-specific calibration function established by a different lab (in Institute of Geology and Geophysics, Chinese Academy of Sciences) could provide reasonable T_{47} values that are consistent with our controlled cultivation temperature and estimated growing-season temperature (Tables 1, 2; Fig. S3). Thus, instrument artifacts cannot fully account for the observed offset to low Δ_{47} values. Furthermore, a similar magnitude of offset has been reported in brachiopod and echinoid calcites and attributed to kinetic/vital effects, although the offset was in the opposite direction to that in the present study (Bajnai et al., 2018; Davies and John, 2019).

The variations in Δ_{47} values could also have been induced by mixing effects, as suggested by Defliese and Lohmann (2015), because whole shells were used for measurements in this work, and newly formed aragonite may precipitate on the young spirals of juvenile shells as the snail grows (Fig. 1). The mixing offsets are usually not significant unless the variability in both $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ exceeds 2‰, and a positive correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values will cause a positive Δ_{47} offset (Defliese and Lohmann, 2015). However, the observed variations in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were <2‰ (Table 1), and we found a weak positive correlation in the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values and lower Δ_{47} values (Figs. 3, S2). Therefore, we suggest that the mixing effect made an insignificant contribution to our snail-shell Δ_{47} values.

On the basis of the above discussions, we argue that the low Δ_{47} values of our cultured land snails do not reflect analytical or methodological artifacts. The offset of shells' Δ_{47} values from Petersen calibration is real and may be related to the vital effect. Importantly, it appears that the offset/vital effect in land-snail shells is relatively constant in the studied temperature range. Thus, it can be corrected for using an independent snail-specific calibration line. This would enable the accurate reconstruction of snail-shell calcification temperatures.

4.4 Evidence of the vital effect

If the land-snail shells were precipitated at equilibrium, the Δ_{47} values of cultured snail shells should be evenly distributed around the “true” equilibrium Δ_{47} - T calibration line. However, most of the Δ_{47} values of our snail shells and other mollusks from different labs lie below the Peterson calibration line, especially at low temperatures (Fig. 5). Our Δ_{47} values are also lower than the measured temperature when employing the Δ_{47} - T relationships of Kelson et al. (2017) and Bonifacie et al. (2017) (Fig. 5). Moreover, the Δ_{47} values vary with the age in months of the shells, particularly for low growth temperatures (Fig. 2). All of these observations may be attributed to the vital effect in snail shells, which contrasts with the conclusions of previous studies (Zarrur et al., 2011; Zhang et al., 2018b).

4.4.1 Clumped isotope values versus snail growth ages

The Δ_{47} values of the first-month shells (juvenile shells) may represent the equilibrium values at their corresponding temperatures, because the shell weight increments are smallest (Figs. 1, S5) and their Δ_{47} and T_{47} values are closest to the

expected values based on the Petersen calibration (Fig. 5). Moreover, the mean Δ_{47} values of the whole shells become smaller from the first month to the fifth month with a magnitude of about $\sim 0.026\text{‰}$ for *A. fulica* raised at 22.9 °C (Table 1; Fig. 2A), although this magnitude may be complicated by the analytical error, and needs further confirmation. Similar Δ_{47} variation of $\sim 0.017\text{‰}$ is observed at 27.7 °C , with the lowest value in the second month and values leveling off after that (Fig. 2B). Given that the timescale for dissolved inorganic carbon (DIC) equilibration in solution is longer at lower temperatures (Zeebe and Wolf-Gladrow, 2001), it is reasonable that kinetic/vital effects might be well expressed at lower snail-growth temperatures. Thus, this phenomenon (larger offsets in Δ_{47} values at 22.9 °C compared with that at 27.7 °C) supports the existence of the vital effect.

Shell thickness increased as snails grew from juveniles to adults (Fig. 1). This implies that newly formed aragonite is incorporated into earlier spirals and juvenile shells, which may dilute or even overwrite the Δ_{47} signals of the original shells. This could explain why earlier studies failed to detect differences in Δ_{47} values by analyzing different parts or spirals of the same adult shells (Zaarur et al., 2011; Zhang et al., 2018b), even though the upper and lower parts of snail shells are traditionally assigned to younger and older ages, respectively. Therefore, it is vital to analyze shells that genuinely represent different growth stages to reveal the corresponding changes in Δ_{47} values.

Our results are also supported, to some extent, by the shell-size-dependence of Δ_{47} , whereby lower Δ_{47} values (i.e., higher temperatures) are found in larger snail

shells (Zhang et al., 2018b). Growth-related kinetic effects have also been reported in brachiopod calcite (Bajnai et al., 2018). Davies and John (2019) documented a offset of 0.014‰ in the Δ_{47} -T calibration line in echinoid calcite, although it was in the positive direction. Thus, we suggest that the vital effect may influence the Δ_{47} values of land-snail shells, and may be responsible for the ~6 °C higher apparent T_{47} values obtained when the calibration line of Petersen et al. (2019) (Tables 1, 2) is applied. The offset observed in our cultured snails (average ~0.016‰) may represent a conservative estimate of the vital effect, because the adult shell includes the clumped isotope signature of the juvenile shells, which have higher Δ_{47} values (Table 1; Fig. 2).

4.4.2 Correlation between clumped isotope values and oxygen and carbon isotopes

Affek (2013) suggested that the time required to reach equilibrium is indistinguishable between $\delta^{18}\text{O}$ and Δ_{47} values during the isotopic exchange between CO_2 and water, and that when one isotopic system shows disequilibrium, so will the other. In this case, we would expect correlation between the $\delta^{18}\text{O}$ and Δ_{47} values of snail shells if they were both affected by the vital effect. However, the results of Affek (2013) might not be applicable to snails, because that study considered isotope fractionation in a CO_2 - H_2O system rather than the DIC fluid in which a snail shell forms. Notably, a recent study showed decoupled equilibrium behavior of $\delta^{18}\text{O}$ and Δ_{47} in carbonates rapidly formed from a DIC solution (Staudigel et al., 2018). This might partly explain the weak correlations observed in the plots of $\delta^{18}\text{O}$ - Δ_{47} (Fig. S2) and the lack of a trend between the excesses in Δ_{47} and $\delta^{18}\text{O}$ in the first study of Δ_{47}

values of cultured land-snail shells, which was originally used to demonstrate the absence of the Δ_{47} vital effect (Zhang et al., 2018b).

Many factors might affect the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ signatures of snail shells; e.g., the $\delta^{18}\text{O}$ of water and water vapor, relative humidity, growth temperature, and the $\delta^{13}\text{C}$ values of carbonates and the snail's diet (Balakrishnan and Yapp, 2004). For example, a higher temperature will produce lower Δ_{47} values and lower shell $\delta^{18}\text{O}$ if the supplied water $\delta^{18}\text{O}$ is kept constant, and less carbonate consumption by a snail may lower the $\delta^{13}\text{C}$ value of its shell. Sample pretreatments may also influence a shell's $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ signatures (Guo et al., 2019; Key Jr et al., 2020; Zhang et al., 2020;). Thus, the weak $\delta^{18}\text{O}$ – Δ_{47} , $\delta^{13}\text{C}$ – Δ_{47} , and $\delta^{18}\text{O}$ – $\delta^{13}\text{C}$ correlations may be partly affected by these factors (Figs. 3, S2). In this study, the amount of carbonate consumed and the $\delta^{18}\text{O}$ of the water in the fresh lettuce and water vapor were not constrained, whereas the growth temperature, supplied water, and carbonate and diet were controlled during the experiment. Therefore, the relationships among $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and Δ_{47} in our culturing experiment cannot fully support the existence of the vital effect in land-snail shells.

4.4.3 Clumped isotope values of different species

Discrepancies in Δ_{47} values in land snails were first interpreted as biological adaptations arising from inter-species differences rather than non-equilibrium isotope effects (Zaarur et al., 2011). Wang et al. (2016) and Zhai et al. (2019) attributed the difference in Δ_{47} values between *Cathaica* sp. and *Bradybaena* sp. to different growing seasons, but they could not fully exclude the influence of microenvironments,

which may be the underlying reason for the two different calibration functions (Fig. S4). In contrast, Guo et al. (2019) found no significant inter-species difference because the shell Δ_{47} values of different species from the same location overlapped within analytical error. Our results also show that the mean Δ_{47} values of different species collected in the same location are indistinguishable within analytical precision (Table 2). Moreover, the Δ_{47} values of cultured *A. fulica* “white” and *B. ravidula redfieldi* are almost identical at the same cultivation temperature (Table 1). Therefore, our results suggest that the species-related vital effects may be insignificant. However, the Δ_{47} values of cultured snails from Zhang et al. (2018b) are slightly different from ours. The underlying reason is still unclear, and more data/studies are needed to answer the discrepancy in the future, such as the Δ_{47} values of same snails or different species under well controlled temperatures from different labs.

4.5 Origin of clumped isotope vital effect in land snails

4.5.1 CO₂ diffusion into calcifying space

Previous studies have reported clear Δ_{47} offsets from equilibrium values in corals (Saenger et al., 2012; Kimball et al., 2016; Spoonser et al., 2016). Thiagarajan et al. (2011) suggested that CO₂ diffusion into the calcifying space could cause kinetic fractionation of Δ_{47} . CO₂ diffusion will lead to an increase in Δ_{47} but a decrease in $\delta^{18}\text{O}$ (Eiler and Schauble, 2004; Thiagarajan et al., 2011; Saenger et al., 2012). Thiagarajan et al. (2011) predicted a 0.7‰ decrease in $\delta^{18}\text{O}$ and a 0.036‰ increase in Δ_{47} for liquid phase diffusion of CO₂. Our cultured snail Δ_{47} and $\delta^{18}\text{O}$ offsets display opposite signs when the values of the first-month shells are treated as the equilibrium

state (Fig. 3). Thus, CO₂ diffusion is ruled out as a possible mechanism for the vital effect in land-snail shell Δ_{47} .

4.5.2 Growth rate

The manifestation of kinetic effects is dependent on the timescale for equilibration relative to the rate of precipitation. A process-based model prediction study demonstrated that the Δ_{47} value is equal to the specified equilibrium composition in the case of low growth rates, and approaches a kinetic limit with increasing growth rate (Watkins and Hunt, 2015). This view is supported by the apparent Δ_{47} equilibrium in slow-growing brachiopods and positive Δ_{47} offset in fast-growing brachiopods (Bajnai et al., 2018). A general increase in the Δ_{47} offset from equilibrium with increasing calcification rate has also been demonstrated in corals (Saenger et al., 2012). Thus, growth rate may also cause the vital effect in land snails. The apparent Δ_{47} equilibrium in the first-month shells seems consistent with the above studies, given the low growth rate estimated from the shell size and weight increments (Figs. 1, S5) and body weight (Figure 9.1 in Baker, 2001), as well as the low rate of weight gain in the snails (Egonmwan, 2008). However, the Δ_{47} values of the cultured land-snail shells became lower during each growth stage and leveled off for snails grown at 27.7 °C, even though the shell size/weight increments continued to increase (Figs. 1, 3, S5). This is opposite to the model prediction that Δ_{47} values increase with growth rate (at pH 7–9) (Watkins and Hunt, 2015). Furthermore, our results highlight the different growth-rate-related Δ_{47} variations of brachiopods and corals (Saenger et al., 2012; Bajnai et al., 2018). Therefore, our data may not fully

support the presence of a growth-related vital effect in land-snail shells. A different growth-related model may be needed to describe the phenomena observed in land snails.

4.5.3 Effect of pH

The relative abundances of DIC species are determined by the pH of the solution. Theoretical modeling has suggested that each of the three DIC species has a distinct equilibrium Δ_{47} signature, such that $\Delta_{47}(\text{H}_2\text{CO}_3) > \Delta_{47}(\text{HCO}_3^-) > \Delta_{47}(\text{CO}_3^{2-})$ (Hill et al., 2014; Tripathi et al., 2015). In addition, $\Delta_{47}(\text{HCO}_3^-)$ is predicted to be more enriched than $\Delta_{47}(\text{CO}_3^{2-})$ at equilibrium for $\sim 0.02\%$ (Guo et al., 2008) and $\sim 0.03\%$ (Hill et al., 2014) at 25 °C. Thus, the pH of the parent DIC pool can affect the Δ_{47} composition of the carbonate mineral precipitated from the pool.

An increase in pH favors the formation of CO_3^{2-} over HCO_3^- at equilibrium, and thus induces lower Δ_{47} values in carbonates. The effect of increased pH on Δ_{47} is in the same direction as that observed in our land snails. However, the pH in the hemolymphs of active snails at 20 °C is about 7.6–8.0 (Burton, 1976). This is lower than the Δ_{47} crossover pH for aragonite (Hill et al., 2014). Therefore, the Δ_{47} value should be higher than the equilibrium aragonite Δ_{47} value. Moreover, an increase in pH results in $\delta^{18}\text{O}$ depletion (Adkins et al., 2003). This contrasts with the observed trend of more positive $\delta^{18}\text{O}$ with lower Δ_{47} values in snail shells, assuming the first-month shells were precipitated at equilibrium (Figs. 3, S2). Although no exact pH data are available for the calcifying space in land snails, available data do not support the pH effect as the underlying mechanism of the vital effect in land snails.

4.5.4 Kinetic effects associated with dehydration/dehydroxylation

McConnaughey (1989a, 1989b) proposed that kinetic effects associated with hydration and hydroxylation could explain the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ vital effects in corals. Affek (2013) further demonstrated that hydration/dehydration of CO_2 occurs at the same rate for Δ_{47} and $\delta^{18}\text{O}$, which indicates that disequilibrium in one system will also occur in the other. Therefore, these effects are considered to contribute to the measured positive Δ_{47} offsets in surface corals (Saenger et al., 2012). However, the kinetic effects during hydration/dehydration result in an enrichment in Δ_{47} and depletion in $\delta^{18}\text{O}$, which is the opposite of the increased $\delta^{18}\text{O}$ and lower Δ_{47} values found in our cultured land-snail shells when the data of the first-month shells are regarded as equilibrium values (Figs. 3, S2). Therefore, we suggest that the kinetic effects associated with dehydration/dehydroxylation may cause the vital effect in land-snail shells.

The kinetic effect associated with dehydration and dehydroxylation was originally proposed to explain the lower Δ_{47} values ($\sim 0.08\text{‰}$) in speleothems, which precipitate from super-saturated dripping water via rapid degassing of CO_2 through either bicarbonate dehydration or dehydroxylation reactions (Affek et al., 2008; Daëron et al., 2011; Kluge and Affek, 2012). Snail-shell calcification occurs from the extrapallial fluid (McConnaughey & Gillikin 2008), a thin layer of fluid between the mantle and the inner shell surface. Similarly, land snail hemolymphs are supersaturated with respect to aragonite by a factor of 15–20 (Burton, 1976). This supersaturation state undoubtedly facilitates the rapid degassing of CO_2 and the

formation of aragonite. In addition, CO₂ is periodically or intermittently released in dormant land snails (Barnhart and McMahon, 1987), which may also occur during the quiescent intervals of active snails. Unfortunately, the sources of snail CO₂ are still unclear. Taken together, these findings suggest that the kinetic effect in land-snail shells may mimic that of speleothems, although the effect in land snails is subtle at ~0.016‰ on average. This weak kinetic effect may be related to the presence of carbonic anhydrase at the calcification site (McConnaughey & Gillikin 2008).

According to the respiratory gas-exchange model of McConnaughey et al. (1997), respired CO₂ is an important constituent of the carbonate of most air-breathing animals (e.g., land snails), but is much less important in the carbonate of most aquatic animals (e.g., corals). For corals, the diffusion of CO₂ dissolved in seawater into the calcifying fluid is likely to result in kinetic fractionation in Δ_{47} values (Thiagarajan et al., 2011; Saenger et al., 2012). We similarly propose that in land snails, CO₂ degassing out of the calcification space may explain the vital effect in Δ_{47} values. However, the complex nature of the bio-mineralization strategies of different animals means that more research is needed.

The results of theoretical calculations indicate that isotopic fractionation during CO₂ degassing from aqueous solutions leads to enrichment of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ and depletion of Δ_{47} in the residual DIC species and in carbonate minerals that precipitate from the DIC pool (Guo, 2008). The predicted kinetic isotope fractionation associated with dehydration and dehydroxylation for Δ_{47} in carbonates is a decrease by 0.017‰–0.026‰ for every 1‰ of kinetic enrichment in $\delta^{18}\text{O}$ at 25 °C, but depends on the

exact pathway for CO₂ degassing and carbonate formation. This generally agrees in both sign and magnitude with the vital effect observed in our cultured land snails (Figs. 3, S2). For example, the observed slopes in the $\delta^{18}\text{O}$ - Δ_{47} plots are -0.022 and -0.009 for snails growing at 22.9 °C and 27.7 °C, respectively (Fig. 3). Therefore, we conclude that the kinetic isotope fractionation associated with dehydration and dehydroxylation during CO₂ degassing may play a major role in producing the vital effect during the growth of land-snail shells.

5. CONCLUSION AND OUTLOOK

This study investigated the Δ_{47} values of land-snail shells cultivated at controlled temperatures. The Δ_{47} values of the shells decreased with increasing temperature, suggesting that a snail's Δ_{47} signature can be used to reconstruct its shell-formation temperature. The T_{47} values calculated from the Δ_{47} - T calibration function for *Bradybaena* species are similar to our cultivation temperatures. Notably, almost all our cultured-snail Δ_{47} values are lower (average ~0.016‰ lower) than those calculated from the Petersen calibration function. Combined with the observed relationships among $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and Δ_{47} and the signature of cultured shell Δ_{47} values versus snail growth age, this offset is attributed to the vital effect. We suggest that the vital effect can be explained by kinetic fractionation due to CO₂ degassing from calcification space associated with dehydration/dehydroxylation during the precipitation of snail shells.

In view of the limitations of this and previous studies, the following points should be considered in future studies. (1) More experiments should focus on the Δ_{47}

variations with different stages of snail growth, especially for the juvenile periods. (2)

Future investigations should examine Δ_{47} changes among different species in the same environment. (3) Experiments with strict controls on the sources of $\delta^{13}\text{C}$ (food, CO_2) and $\delta^{18}\text{O}$ (water) could identify the vital effect on the isotopic compositions of snail shells.

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Tables and Figures

Table 1. Compiled clumped (Δ_{47}) and stable isotope compositions ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) of cultured snail shells.

Species	Sample No.	Month	$\delta^{13}\text{C}$	$\delta_{13}\text{C}$	$\delta^{18}\text{O}$	$\delta_{18}\text{O}$	$\Delta_{47}\text{CDE S90}^a$	$\pm 1\text{SE}$	Average $\Delta_{47}\text{CD ES 90}^b$	Average $\Delta_{47}\text{CD ES 90}^b$	Monitored growth temperature (°C)	Calculated growth temperature ^e (°C)				
			(VPDB, ‰)	std dev	(VPDB, ‰)	std dev						c	d	e	f	g
<i>Achatina fulica</i> "white".(White Jade Snails)	T2 4-1 -1	1		0		0		0								
			-12.990	.007	-7.199	.004	0.610	.006								
									0.603±							
			-12.829	.004	-6.963	.000	0.605	.001								
	T2 4-1 -2		-13.218	.001	-7.186	.005	0.593	.001		0.588±	22.9±0.3	29.5±.8	29.1±.6	31.4±.7	23.5±.3	20.3±.2
T2 4-3 -1	3															
		-11.855	.002	-6.603	.004	0.579	.005		0.585±							
		-11.828	.000	-6.493	.000	0.588	.000									

				0	0	1										
				2	3	7										
				0	0	0										
T2 4-5 -1	5		-11.	·	-6.0	·	0.5	·								
			921	0	33	0	87	0								
				0		0		1								
				5		8		3								
				0		0		0	0.5							
			-11.	·	-5.9	·	0.5	·	77±							
			953	0	76	0	83	0	0.0							
				0		0		1	0.8							
				2		4		4								
				0		0		0								
				-11.	·	-6.0	·	0.5	·							
	915	0	92	0	60	0										
		0		0		1										
		4		7		3										
Achaetina fulicaria "white" (White Jade Snails)	T2 8-1 -2	1		0	0	0										
			-14.	·	-7.5	·	0.5	·								
			220	0	57	0	99	0								
				0		0		·								
				4		5		4								
				0		0		0	0.5							
			-14.	·	-7.5	·	0.5	·	91±							
			206	0	20	0	87	0	0.0							
				0		0		1	0.4							
				3		6		9								
				0		0		0								
	-14.	·	-7.6	·	0.5	·	0.5	27.	32	31	34	25	22			
	207	0	47	0	88	0	80±	7±0	.5	.8	.1	.5	.2			
		0		0		1	0.0	.4	±1	±1	±1	±0	±0			
		1		5		0	0.3		.1	.0	.0	.8	.7			
T2 8-2 -2	2			0	0	0										
			-12.	·	-5.7	·	0.5	·								
			648	0	20	0	87	0								
				0		0		1								
				3		5		2	0.5							
				0		0		0	74±							
			-12.	·	-5.7	·	0.5	·	0.0							
			671	0	62	0	67	0	0.6							
				0		0		0								
				2		8		9								
				-12.	·	-5.7	·	0.5	·							
	654	·	05	·	69	·										

			0	0	0	
			0	1	1	
			3	0	6	
			0	0	0	
		-11.	·	-6.5	·	0.5
		285	0	53	0	72
			0		0	1
			4	7		1
			0	0		0
T2						0.5
8-3	3	-11.	·	-6.4	·	78±
-2		336	0	73	0	85
			0		0	1
			2	7		5
			0	0		0
		-11.	·	-6.5	·	0.5
		321	0	39	0	77
			0		0	0
			3	5		9
			0	0		0
		-12.	·	-6.5	·	0.5
		322	0	06	0	71
			0		0	1
			3	6		6
			0	0		0
T2						0.5
8-4	4	-12.	·	-6.4	·	75±
-2		004	0	97	0	65
			0		0	1
			2	6		0
			0	0		0
		-11.	·	-6.5	·	0.5
		972	0	10	0	89
			0		0	1
			2	8		1
			0	0		0
		-12.	·	-6.4	·	0.5
		379	0	70	0	90
			0		0	1
			3	7		2
T2						0.5
8-5	5		0	0		0
-2						0.0
		-12.	·	-6.4	·	0.5
		436	0	77	0	78
			0		0	1
			3	8		3
		-12.	0	-6.4	0	0.5
					0	0

		0	0	0	
	-10.	·	-8.3	·	·
	588	0	36	0	0
		1		2	1
		3		3	0
		0	0	0	
	-11.	·	-8.4	·	·
	549	0	00	0	0
		0		0	1
		2		4	6
		0		0	0
					0.5
T3	-11.	·	-8.3	·	·
0-1	490	0	62	0	0
(R)		0		1	0
		3		1	1
		0		0	2
					0
	-11.	·	-8.3	·	·
Bra	469	0	37	0	0
dyb		0		0	1
aen		2		6	4
a		0	0	0	0
ravi		0	0	0	0
da		0	0	0	0
redfi	-10.	·	-8.3	·	·
eldi	955	0	14	0	0
		0		0	1
		3		3	8
		0		0	0
					0.5
T3	-10.	·	-8.3	·	·
0-3	867	0	62	0	0
(R)		0		1	0
		3		2	9
		0		0	0
	-10.	·	-8.2	·	·
	995	0	88	0	0
		0		0	1
		3		3	2

- Δ_{47} data are reported relative to the absolute carbon dioxide equilibrium scale (CDES) defined by Dennis et al. (2011) with no acid fractionation correction for acid digestion at 90 °C. The Δ_{47} values have been calculated using the ^{17}O correction parameters of Brand et al. (2010).
- Uncertainties in average Δ_{47} values are given as one standard error of the mean (i.e., $1 \text{ SD}/\sqrt{n}$). Only one shell was analyzed three times for each month divided by solid lines except for the first month at 22.9 °C, in which two shells were used.
- Growth temperature calculated using Petersen et al. (2019) calibration and their suggested

acid fractionation of 0.088‰.

- d. The calculated growth temperature using the calibration from Bonifacie et al. (2017).
- e. The calculated growth temperature using the calibration from Kelson et al. (2017).
- f. The calculated growth temperature using the calibration for *Bradybaena* species from Zhai et al. (2019).
- g. The calculated growth temperature using the calibration for *Cathaica* species from Zhai et al. (2019).

Table 2. Compiled clumped (Δ_{47}) and stable isotope compositions ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) of field-collected snail shells and estimated growth temperatures.

Loc atio n	Spec ies	Sam ple No.	$\delta^{13}\text{C}$ C	$\delta^{13}\text{C}$ C	$\delta^{18}\text{O}$ O	$\delta^{18}\text{O}$ O	$\delta^{18}\text{O}$ water	Δ_{47} CD ES 90 ^a	$\pm$ 1 SE	Ave rage Δ_{47} for sa me sp eci es CD ES 90 ^b	Ave rag e Δ_{47} CD ES 90 ^b	Esti mat ed gro win g sea son tem per atur e ^e (°C)	Calculated growth temperature (°C)
			(VP DB, ‰)	st d e v	(VP DB, ‰)	st d e v	(SM OW, ‰)						d e f
Hai na n	<i>Acha tina fulica</i>	2015	0.	0.	0.	0.	0.	0.	0.	0.	0.		
		-27A	-14.296	0	-4.137	0	-1.161	0.5	1	0.5	1		
		-2		4		4		83	4				
		2015	0.	0.	0.	0.	0.	0.	0.	0.5			
		-27A	-15.994	0	-5.539	0	-2.703	0.5	1	0.5	1	84	
		-3		3		5		85	0				
	<i>Brad ybae na sp.</i>	2015	0.	0.	0.	0.	0.	0.	0.	0.5		3 2 21	
		-30A	-10.981	0	-4.011	0	-1.034	0.5	1	81±	25	± ± ±	.9
		-2		4		8		83	2	0.0		1. 0. ±0	
										03		1 8 .7	
		2015	-12.0	0	-1.9	0	0.96		0				
		-27B	-11.611	0	48	0	7	0.5	1				
Ku nmi ng, Yu nn an	<i>Brad ybae na sp.</i>	-1		2		3		84	3	0.5	78		
		2015	-12.0	0	-3.6	0	0.15		0				
		-27B	-9.74	0	45	0	8	0.5	2				
		-2		2		6		71	0				
		2015	-9.683	0	-3.178	0	-1.079	0.5	1	0.6		2 1 16	
		-42-1		0		4		96	6	06±	17	± ± ±	.3
	<i>Brad ybae na sp.</i>	2015	-9.681	0	-2.960	0	-2.058	0.6	1	0.0		1. 1. ±1	
				2		6		14	0	05		7 2 .1	
		2015	-10.0	0	-7.5	0	-7.1	0.6	0.				

- a. Δ_{47} data are reported relative to the absolute carbon dioxide equilibrium scale (CD_{ES}) defined by Dennis et al. (2011) with no acid fractionation correction for acid digestion at 90 °C. The Δ_{47} values have been calculated using the ^{17}O correction parameters of Brand et al. (2010).
- b. Uncertainties on average Δ_{47} values are given as one standard error of the mean (i.e., $1 \text{ SD}/\sqrt{n}$). Five shells were analyzed to obtain the average Δ_{47} for Hainan and Kunming, and several shells were powdered together and were analyzed two and three times for *O. draparnaudi* and *H. aspersa*, respectively.
- c. Mean annual temperature for Hainan and Kunming and winter temperature (December, January, and February) for Pasadena.
- d. The calculated growth temperature using the calibration from Petersen et al. (2019) and their suggested acid fractionation of 0.088‰.

- e. The calculated growth temperature using the calibration for *Bradybaena* species from Zhai et al. (2019).
- f. The calculated growth temperature using the calibration for *Cathaica* species from Zhai et al. (2019).

Table 3. Summary of field collected land snail studies

Study	Correction of Δ_{47}	Calibration line	M AT	GS T	Summer T
Zaarur et al., 2011	Heated gases	Ghosh et al., 2006	>	>	>
Eagle et al., 2013	Heated gases	Ghosh et al., 2006	>	>	>
Wang et al., 2016; Zhai et al., 2019	Equilibrium & heated gases	Ghosh et al., 2006	>	>	>
Guo et al., 2019	Heated gases and carbonate standards	Kelson et al., 2017	>	>	>
Dong et al., 2020	Heated gases and carbonate standards	Peterson et al., 2019	>	$\approx$	$\approx$
This study	Equilibrium & heated gases	Calibration for Bradybaena.#	=	=	=

#. T_{47} is calculated using the proposed calibration function for Bradybaena species (Zhai et al., 2019). For land snails collected in southern China, e.g. Famen and Kunming, T_{47} are consistent with MAT, while for land snails collected at Caltech (winter precipitation) and on the Chinese Loess Plateau (summer precipitation), T_{47} are consistent with winter and summer temperature, respectively (Figure S3). MAT: mean annual temperature, GST: growing season temperature.

Fig. 1. *A. fulica* "white" snail shells of different month ages grown at 22.9 °C and 27.7 °C. It is clear that the snails grew slower at 22.9 °C than that at 27.7 °C, and the shells grow larger and thicker, suggesting that newly formed aragonite was precipitated on former/ juvenile shells. The ruler is shown as a scale.

Fig. 2. Δ_{47} values versus months for *A. fulica* snails cultured at 22.9 °C (A) and 27.7 °C (B). The Δ_{47} values of the first month shells were the highest at both culturing temperatures.

Fig. 3. Relationship among $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and Δ_{47} for snails cultured under temperatures of 22.9 °C (A) and 27.7 °C (B). The correlation coefficients (R^2) of $\delta^{13}\text{C}$ - Δ_{47} and $\delta^{18}\text{O}$ - Δ_{47} are 0.84 and 0.97 for snails grown at 22.9 °C, respectively, and the values are 0.57 and 0.78 for snails cultured at 27.7 °C. The slopes of $\delta^{18}\text{O}$ versus Δ_{47} (yellow line) are -0.022 and -0.009 for snails growing at 22.9 °C and 27.7 °C, respectively. Each symbol represents the average value of different month old shell (Table 1). The numbers in brackets represent the corresponding months.

Fig. 4. T_{47} of modern snail shells (yellow filled circles) obtained from Hainan Island (A), Kunming (B), and Pasadena (C) versus the monthly average temperature (black filled circles, left axis) and precipitation (blue bars, right axis), respectively. The yellow, purple and black dashed lines show the mean T_{47} , mean seasonal temperature of concentrated rainfall (May–October for Hainan and Kunming, December to next March for Pasadena) and mean annual temperatures, respectively. The T_{47} values were derived from the calibration for *Bradybaena* species from Zhai et al. (2019). The error bars of T_{47} denote one standard error of single measurement.

Fig. 5. Comparison of Δ_{47} values of cultured land snails from this study (sky blue triangles) and previous calibration lines from Bonifacie et al. (2017), Kelson et al (2017), Petersen et al (2019) and the calibration for *Bradybaena* land snails from Zhai et al. (2019), as well as other results from mollusks. For comparison, all samples reacted at 90 °C were corrected using an acid fractionation factor of 0.088‰ (Petersen et al., 2019). Filled triangles show data of first month cultured snail shells.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights:

1. Land snail shells may suffer from the influence of subtle vital effect.
2. Dehydration/dehydroxylation may cause vital effect or the depletion of Δ_{47} .