

## Response to the referees

N. Zhang et al.

We thank both referees for their careful considerations and constructive comments on this manuscript. We will response these comments point by point. The words in blue colour are the revised part in the manuscript.

### Anonymous Referee #1

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#### General comment:

R1-G1 This work presents interesting and new data, but is in need of some revisions and clarifications. My major concern is that the authors claim that the atmospheric CO<sub>2</sub> influences significantly the carbon isotope composition of the shell, but they did not monitor this variable.

Response: We thank the referee to point out this important issue, and we agree that more discussions and observations are important for accurate estimations of atmospheric CO<sub>2</sub>. We will show our revised discussions on this issue in the following comments.

#### Specific comments:

R1-S1. Page 6556. Lines 10-20. Why do you think that the relative contribution of different carbon sources vary so much between snails fed with either C<sub>3</sub> or C<sub>4</sub> plants? In other words, why C<sub>4</sub>-fed snails require significantly higher consumption of limestone?

Response: Many thanks for this good question. We can show some reasonable hypotheses here.

One possibility is, the higher contribution of limestone in C<sub>4</sub> fed snails observed in this study means relative higher proportion contributed from limestone compared with food rather than absolute consumption amount. As we mentioned in the manuscript, the food quality such as water content or physical structure can cause the diet preference of snails. We think the snails consumed less amount of corn compared with cabbage so that they presented a lower growth rate, meanwhile, the absolute amount of ingested limestone may not vary too much among those snails fed different food. In this study, we have observed that the snails fed corn at 20 °C have slightly smaller size compared with those fed cabbage at the same temperature; and if we compared those snails with same size, the corn fed snails always presented lighter mass, both in total body mass and in dry tissue mass. We will add a table in the supplement (Table S2), you can find the information of total body mass, dry tissue mass, shell mass, length, height and spiral numbers for each snail.

Another possibility is the pH difference in cabbage tissue and corn tissue. We could not find the literature which reported the pH of such plants, but we assume that the C<sub>4</sub> plant tissue water may have lower pH values because of the pathways of C<sub>4</sub> photosynthesis (contribution from organic acid). Thus, if the corn tissue has a lower pH value compared with cabbage tissue (both of them are lower than the suitable pH~7.75 in land snail fluid), the snails would prefer ingesting more CaCO<sub>3</sub> to adjust the stomach pH to a suitable value. This phenomenon may depend on the irritability of the land snail species itself.

R1-S2. Page 6556. Line 25. The authors may want to clarify that most “Quaternary” land snail species are extant. However, many older taxa (note that snails first appeared in the Carboniferous) are extinct.

Response: We agree. We rearranged this sentence as, 'Land snail shells are widely applied for studying paleo and present environment characteristics because most of those species which can be well preserved in Quaternary fossils are still extant today.'

R1-S3. Page 6557. Lines 10-25. The great variability in the proportional contribution of different carbon sources from the published literature is partially explained because (1) the variability of species investigated with differing ecological requirements, ethology and life cycles, and (2) the variability of environmental settings examined (e.g., carbonate rich areas against carbonate-poor areas, wet versus dry locales). This should probably be clarified.

Response: Thanks for the kind advice. We have added this discussion in the last paragraph of Page 6558.

Page 6558, Line 17-19.

'These great discrepancies from published literatures are probably generated by: (1) the variability of land snail species studied with different ecological requirements, ethology, and other species-dependent-behaviors; (2) the variability of environmental conditions where these snails were living (e.g., CaCO<sub>3</sub>-rich areas vs. CaCO<sub>3</sub>-poor areas, wet areas vs. dry areas, hot/warm areas vs. cold/cool areas, etc.); (3) the limitation of calculations. Therefore, a better understanding of the contribution of each carbon source and related environmental controlling factors can promote this isotopic tool in the field of paleo environment reconstruction. In this study, we cultured one land snail species (*Acusta despecta sieboldiana*) under different controlled conditions.'

R1-S4. Page 6559. I have noticed that relevant information about the lifespan and life cycle of the studied species (*Acusta despecta sieboldiana*) in the wild and/or in the lab is missing. How long does this species live? What food resources consume? Only living plant tissue? What is the range of sizes this species reaches when adult? From figure 1 I assume you only measured the juvenile stages (a few months old) of the shell, right? What type of environment this species occupies today and what is its geographical distribution? Is there a good fossil record of this taxon? Please, expand.

Response: We have read some reports of this species, however, almost all of them are written in Japanese. We summarized the useful information as following:

*Acusta despecta sieboldiana* (Pfeiffer, 1850), with a Japanese name 'Usukawa-maimai', is belonging to the species *Acusta despecta*. The lifespan of this species is around 1 year. The observations of this species in the wild of Northern Kyushu (Sumikawa, 1962) shown that after waking up from hibernation, the adult snails mate and lay eggs in April and May for several times and then most of them die in June. The eggs will hatch around 20-25 days. Till September, the length of new generation could reach 10mm and the spermatogenesis will begin. They will go into hibernation in November, at this time, most of them have become adult and the length could reach as large as 15mm. In next March or April, these adults will wake up from hibernation. Another observation in Tokyo (Okuma, 1982) shown that the most frequent copulation period of *Acusta despecta* is May and June, and some of them can mate in November. The author pointed that the reason why no copulation observed in summer is, all of the adult snails born last year have died, which means that no adult snail is existing during summer. This inference is supported by in-lab observation (Takahashi et al., 1992), which reported *Acusta despecta* could mate from March to December.

This species usually eat fresh plants as food, and is regarded as a kind of pest to crops. However, it has preference to eat different plant species. Suzuki and Yamashita (1967) reported that among the 50 families 267 species of plant they tested, *Acusta despecta* most prefer 28 families 149 species; can eat 16 families 43 species; a little eat 8 families 21 species; and cannot eat 17 families 54 species at all. The preference is not related to phytotaxonomy, but probably related to some physical structure or water content. Similar observation of food preference was reported by Takeuchi and Tamura (1995), for example, *Acusta despecta*

cannot eat *Oxalis corniculata* (C<sub>3</sub> plant), *Commelina communis* (C<sub>3</sub> plant), *Yoshinagella japonica* (Fungi) at all.

According to the culture experiment by Kohno (1976), the optimum temperature for the growth of *Acusta despecta* ranged from 25 °C to 30 °C. The higher the temperature is, the faster the snails grow up. However, higher temperature also related to higher mortality, which is consistent to our observation in this study (Sect. 3.1).

This species is widely distributed all around Japan excepted Northern Hokkaido (Azuma, 1995), and Korea (Lee and Kwon, 1996). Their fossils have been found in Okinawa (3,370 B.P., Takamiya and Meighan, 1992) and other islands in Southern Japan (2,000-3,000 B.P., Fujie, 2000a; 38,000-35,000 B.P., Fujie, 2000b), suggesting this species could be used for reconstructing the paleo-environment of Japan from late Pleistocene Epoch.

Finally, we have checked the growth stage of one snail cultured at 25 °C (S43), whose length is 10.1mm, and the result shown that it has already become adult (genitalia became matured enough to reproduce). Most of other snails have longer length and similar culture period in this study (Table S1), suggesting they probably have reached adult stage.

We summarized and added this information in Page 6559, Line 3-19.

‘Land snail *Acusta despecta*, with a Japanese name ‘Usukawa-maimai’, is widely distributed around Japan except Hokkaido (Azuma, 1995), and Korea (Lee and Kwon, 1996). The main food of this species are fresh plants (Suzuki and Yamashita, 1967; Takeuchi and Tamura, 1995) and the optimum temperature for their growth ranges from 25 °C to 30 °C, while they can live from 15 °C to 30 °C (Kohno, 1976). Typically, the lifespan of *Acusta despecta* is around 1 year and the individual could become adult in 6 months from birth (Sumikawa, 1962; Okuma, 1982; Takahashi et al., 1992).

In this study, eight adult snails of *Acusta despecta sieboldiana* (a subspecies of *Acusta despecta*) were collected in Suzukakedai, Yokohama, Japan and were cultured at room temperature (ca. 25 °C) from January, 2012. These snails began to lay eggs in March, 2012. Then the eggs were transferred into a stainless steel container and were covered with moist cloth in a 25 °C incubator. Most eggs hatched at around 3–4 weeks.

Larvae were distributed randomly into around 30 small transparent plastic boxes that had been perforated to allow air and vapor exchange: Each box contained 3–5 snails. Some later hatched larvae were added to May and June, 2012. Then four small boxes were put into semi-sealed big plastic boxes: two parallel groups were fed cabbage (*Brassica oleracea* var. *capitata*, green cabbage, C<sub>3</sub> plant,  $\delta^{13}\text{C} = -28.4 \pm 1.2\text{‰}$ ,  $n=12$ ) that had been sprinkled with fine calcium carbonate powder ( $\text{CaCO}_3$ ,  $\delta^{13}\text{C} = 4.0\text{‰}$ ). Another two were fed cabbage sprinkled with calcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ). A NaCl saturated solution was used to produce high humidity conditions in each large box. The snails grew under natural light/dark cycles. The air and food were changed every two days. Then the big plastic boxes were put into three incubators with respective temperatures of 20 °C, 25 °C, and 30 °C, until January, 2013; some snails from 20 °C group were cultured until May, 2013. At the end of culturing period, we recorded the length and height for each snail (Table S2). Additionally, we have checked the growth stage of one individual cultured at 25 °C (S43), whose length is 10.1mm. The result showed that this snail has already become an adult. Consequently, the lengths for most of other snails were larger than 10.0mm, suggesting they probably have reached adult stage. The growth phases of snails are presented in Fig. 1.

We also cultured some snails using corn (C<sub>4</sub> plant,  $\delta^{13}\text{C} = -12.0 \pm 0.7\text{‰}$ ,  $n=4$ ; first we used *Miscanthus sinensis*, but the snails did not eat it; then we changed the food to corn) sprinkled with fine calcium carbonate powder in a 20 °C incubator.’

Page 6582, Fig. 1, ‘(E) juvenile/adult (> 6 months, > 10mm)’

## References:

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Kohno, M.: Rearing of SIEBOLD's globular snail *Acusta despecta* (SOWERBY) on a semi-synthetic diet (in Japanese), *The Japanese Journal of Malacology*, 35, 50-62, 1976.

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Suzuki, M. and Yamashita, Y.: On the Food Plants of Siebold's globular snail, *Acusta despecta* Gray (Gastropoda, Mollusca) (in Japanese), *Memoirs of the Institute of Sciences and Technology, Meiji University*, 6, 211-227, 1967.

Takahashi, K., Asakura, A. and Kurozumi, T.: Copulation frequency and mating system of the land snail *Acusta despecta sieboldiana* (Pfeiffer) (in Japanese), *The Japanese Journal of Malacology*, 51, 323-326, 1992.

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Takeuchi, M. and Tamura, M.: Seasonal occurrence, daily activity and food utilization of three sympatric terrestrial Gastropods (in Japanese), *House and Household Insect Pests*, 17, 11-23, 1995.

Literatures in blue colour will be cited in the revised manuscript.

R1-S5A. Page 6560. Line 15-20. I assume you homogenized and analyzed the entire shell of each individual, right? Please, clarify. I suspect that when you separated the body tissue from the shell, you probably lost some shell material in the process. If you want to separate the body from the shell, without breaking the shell, the snails should be drawn in water for a few days.

Response: Yes, we agree. After being dried from a cryogenic vacuum line, all of the snails were put into distilled water for around 10 min, and then the tissue can be easily separated from the shell without any damage. During the preparation of shell samples, we removed the piece of inside 2 spirals (length, ca. 2mm) which was contributed from their parents and then crushed all of the left part homogenously. Based on our calculation, this piece (inside 2 spirals) only accounted for less than 5% of the total shell mass, and was related to a shift of  $\delta^{13}\text{C}$  values less than 0.1‰ (e.g., Table 3). We added this information in the revised manuscript.

Page 6560, Line 2-4, 'They were then washed with distilled water and kept in the water for 10 min. The soft body tissues of respective snails were separated from shells using a nipper without any damage.'

Page 6560, Line 20-23, 'Finally, all the shells were dried using lyophilization and were crushed into powder homogenously (removed the piece of inside 2 spirals, which was contributed from their parents and accounted for less than 5% of the total shell mass) using an agate mortar for isotopic analyses.'

R1-S5B. Also, I noticed that you did not mention how you monitored the atmospheric CO<sub>2</sub> from each terrarium. Do you think the CO<sub>2</sub> concentration and isotopic composition was constant? Because the snails were kept in sealed systems (which were refresh with new air every 2days), I wonder if significant variations of CO<sub>2</sub> occurred throughout the experiment. Note that in the wild and away from anthropogenic sources, I anticipate that atmospheric CO<sub>2</sub> will vary minimally.

Response: Please see our response to comment R2-G2.

R1-S6. Page 6563. Line 5-10. The dashed-line in figure 3 by Balakrishnan and Yapp (2004) is based on a phi value = 0.00? What assumptions did you use to conduct model calculations and why?

Response: Yes, we cited the extremely condition as suggested by Balakrishnan and Yapp (2004) here. When phi value = 0, the input flux of CO<sub>2</sub> is equal to respired flux of CO<sub>2</sub>, and the  $\delta^{13}\text{C}$  fractionation between metabolic CO<sub>2</sub> and shell carbonate totally depend on the diffusion process. However, in our opinion, during the derivation of this model, the authors contained the information from atmospheric CO<sub>2</sub>; in other words, we think this model combined the information from mix and exchange of metabolic CO<sub>2</sub> and atmospheric CO<sub>2</sub>. Our comprehension may not be exactly correct, however, an important defect of this flux balance model should be concerned, that is, if the respired flux of CO<sub>2</sub> is 0 (phi = 1), which means all of the metabolic CO<sub>2</sub> (here we neglect the contribution from limestone) dissolved into the bicarbonate pool and precipitated into shell carbonate, the carbon isotopic fractionation should obey the isotopic exchange rule of CO<sub>2</sub> (aq) - HCO<sub>3</sub><sup>-</sup> - aragonite. For instance, at 20 °C,  $\delta^{13}\text{C}$  fractionation between snail shell carbonate and snail organic matter is 12.4‰ (Stott, 2002), but based on the flux balance model, the fractionation is less than 5‰ (see Fig. 7, Balakrishnan and Yapp, 2004). In other words, the authors didn't consider the isotopic fractionation between metabolic CO<sub>2</sub> and bicarbonate in this model. To be honest, we worry that whether the diffusion model could present the carbon isotope behavior well for the whole body fluid. We agree that the isotopic fractionation may occur in the blood capillary of lung; nevertheless, its contribution to the total amount of blood dissolved CO<sub>2</sub> can be ignored for the animals because the production of metabolic CO<sub>2</sub> derived from food will not be stopped during the lifespan of animals. Overall, here we just want to show that the observed enriched  $\delta^{13}\text{C}$  values than the predicted ones from both models can reveal the contribution of limestone, but not discuss the model itself.

Similar to other published works, in this study we choose the mass balance model for calculating the contribution of each source.

For avoiding being misunderstood by the readers, we revised the following sentence. Page 6563, Line 7-9.

‘whereas the dashed line shows a flux balance model ~~considering both metabolic CO<sub>2</sub> and atmospheric CO<sub>2</sub>~~ based on CO<sub>2</sub> diffusion when the input flux of CO<sub>2</sub> is equal to respired flux of CO<sub>2</sub> in the body fluid of land snails (Balakrishnan and Yapp, 2004).’

R1-S7. Page 6566. Lines 5-10. The present study documented that snails fed with corn exhibited were heavier (thicker?) than snails fed with cabbage, which reflects that corn-fed snails ingested more carbonate than cabbage-fed snails. This finding may contradict the results reported by Metref et al (2003) who observed larger shell size in C<sub>3</sub>-fed snails rather than C<sub>4</sub>-fed snails. Did you mean that snails fed with C<sub>4</sub> plants were smaller but thicker?

Answer: Yes, that's right. Metref et al. (2003) reported smaller shell size in C<sub>4</sub> food (corn) fed snails compared with C<sub>3</sub> food (lettuce) fed snails. Similarly, in this study, we also observed smaller size in C<sub>4</sub> food (corn) fed snails compared with those snails fed C<sub>3</sub> food (cabbage) at the same temperature, however, these C<sub>4</sub> food fed snails showed higher shell



weight proportion, suggesting thicker shells compared with C<sub>3</sub> food fed snails. Moreover, if we compared those snails with a same size, C<sub>4</sub> food fed snails would present lighter mass, both in total body mass and in dry tissue mass, compared with those fed C<sub>3</sub> food. See also the response of R1-S1.

R1-S8. Page 6567. Lines 20-25. Do you have any supporting references that postulate that C<sub>4</sub> plant diet stresses snail growth rates? In nature, snails from the same species that inhabit a C<sub>3</sub>/C<sub>4</sub>-CAM mixed habitat would ingest different photosynthetic pathways indiscriminately, in relation to their apparent abundance (Baldini et al., 2007; Yanes et al., 2008, 2013).

Response: We combined the answer to this comment with R1-S10.

R1-S9. Page 6568. Lines 10-20. I suspect that the inconclusive degree of isotopic fractionation between snail shell and diet might be more the consequence of poor analytical precision.

Response: We think the referee probably means the isotopic fractionation between snail body tissue and diet? If so, we added a clarification in Page 6568, Line 17-20,

‘However, this enrichment is negligible when considering measurement precision among both snail individuals and vegetable samples. We suspect that the small discrepancies of the  $\delta^{13}\text{C}$  values between snail body tissue and their diet observed in the literatures and this study may depend on the analytical precision and/or limitation of samples (e.g., sample size, growth condition, etc.). Therefore, we infer that the  $\delta^{13}\text{C}$  values of snail body tissue are similar and that they should directly reflect the  $\delta^{13}\text{C}$  values of their food.

R1-S10. Page 6569. Lines 10-15. I think the inferred food preference by cabbage over corn under laboratory control cannot directly translate to the natural landscape. In C<sub>3</sub>/C<sub>4</sub>-CAM mixed systems, some individuals would preferentially ingest C<sub>4</sub> plant only, as demonstrated in published field studies (Baldini et al., 2007; Yanes et al., 2008, 2013).

Response: Many thanks for the comments and we are sorry for the insufficiency in our discussion. We have not found the literatures discussing this kind of diet stress of land snails in nature yet, however, based on the laboratory cultured conditions reported by Stott (2002, fed sour orange tree leaves) and Metref et al. (2003, fed corn), which were similar to this study, we found the snails fed corn (C<sub>4</sub> plant) tend to have a lighter total body mass compared with those snails fed cabbage (C<sub>3</sub> plant) in the same size, probably reflecting ‘food stress’ to some land snail species, and we concerned this phenomenon may result from food quality such as water content or physical structure (e.g. fresh leaves vs. dry powder; succulent leaves vs. hard leaves).

In this study, we admit that food preference observed under the laboratory controlled conditions might not directly reveal the natural conditions. According to the literatures and this study, we quite agree that the carbon isotopic composition in natural land snail shell could reflect their diet, which can tell us the vegetation distribution where they are living, but it still hard to demonstrate whether it could reflect a correct C<sub>3</sub>/C<sub>4</sub> plant distributed situation indiscriminately. For instance, Goodfriend and Magaritz (1987) reported an average  $\delta^{13}\text{C}$  difference of ca. 2-3‰; while Baldini et al. (2007) observed this value of ca. 1‰ between snails living in C<sub>4</sub>/CAM predominant environment and C<sub>3</sub> predominant environment, despite the difference of  $\delta^{13}\text{C}$  values between C<sub>4</sub> and C<sub>3</sub> plant can be around 14‰, probably suggesting that the land snails may prefer one kind of plant from another. Moreover, the discussion of Baldini et al. (2007) in Page 183 told us that the feces of land snails collected from C<sub>4</sub> plant exhibited  $\delta^{13}\text{C}$  values more typical of a C<sub>3</sub> plant, suggesting the preference of C<sub>3</sub> plant as food of these snails. In addition, Figure 9 of Baldini et al. (2007) showed that intrashell carbon isotopic variations of a land snail individual reflected a change in food source between C<sub>3</sub> and C<sub>4</sub> plants in its habitat and the higher frequency of lighter  $\delta^{13}\text{C}$  values

presented the preference of C<sub>3</sub> plants, too. In another paper suggested by the referee, Yanes et al. (2008) claimed that the  $\delta^{13}\text{C}$  values of body tissues from collected living snails varied from -12‰ to -27‰, indicating the land snails consumed C<sub>3</sub> and C<sub>4</sub> plants indiscriminately. However, we found among their observed 17 snails, only 3 individuals have  $\delta^{13}\text{C}$  values heavier than -17‰, and only 1 individual heavier than -15‰, while most of the others are lighter than -20‰. We suspect whether this observation reflected that the snails consumed C<sub>3</sub> and C<sub>4</sub> plants evenly. For example, if there are only C<sub>4</sub> plants (or limited C<sub>3</sub> plants) in the habitat of land snails, their tissue  $\delta^{13}\text{C}$  values could totally reflect the contribution from C<sub>4</sub> plants, similar to what we have shown in the corn fed groups, and once the C<sub>3</sub> plants reach an enough proportion (e.g., 10%) when the snail can find them easily, the preference of C<sub>3</sub> plants may occur, and we may possibly obtain incorrect proportion information of C<sub>3</sub> plant (e.g., 45%, Yanes, et al., 2013). The diet preference issue is also supported by Hatzioannou et al. (1994) and Iglesias and Castillejo (1998), where they observed land snails do not eat plant species at random. Similarly, to the species *Acusta despecta*, diet preference was also observed (see response to R1-S4).

Overall, we quite agree that the  $\delta^{13}\text{C}$  values of the land snail shells could reflect the trend of C<sub>3</sub>/C<sub>4</sub> distribution, but we suspect the accuracy of its indication. According to the literatures and this study, we think that the food preference is not directly correlated to the plant type (C<sub>3</sub>/C<sub>4</sub>), but probably depends on the food quality (water content or physical structure) from different plant species. Therefore, we consider that the further studies of food quality are needed. Moreover, we suggest that the pre-investigation of food preference on living snails is import before applying shell carbon isotopic values in the plaeo-environmental reconstruction of a certain species.

Based on the literatures suggested by the referee, we added/revised some discussions in the manuscript.

Page 6556, Line 16-17,

‘Moreover, we present new evidence that snails have discrimination to [choose different plant species](#) as food.’

Page 6569, Line 10-15,

‘However, Stott (2002) reported a lower growth rate of snails cultured with dried sour orange tree leaves (C<sub>3</sub> plant) compared to those cultured by lettuce and corn, suggesting food quality such as water content or physical structure as the reason for food preference. [To land snail \*Acusta despecta\*, food preference has been reported by Suzuki and Yamashita \(1967\), Takeuchi and Tamura \(1995\), for instance, this species could not eat \*Oxalis corniculata\* \(C<sub>3</sub> plant\), \*Commelina communis\* \(C<sub>3</sub> plant\), \*Yoshinagella japonica\* \(Fungi\) at all. Similar phenomenon also occurred in the nature observations. Hatzioannou et al. \(1994\) and Iglesias and Castillejo \(1998\) observed that land snails do not eat plant species at random. Baldini et al. \(2007\) reported that the feces of land snails \(\*Cerion\*\) collected from \*Sporobolus domingensis\* \(C<sub>4</sub>\) plant exhibited  \$\delta^{13}\text{C}\$  values more typical of a C<sub>3</sub> plant, suggesting a preference of C<sub>3</sub> plant as food of these snails. Anyhow, the food selectivity of land snails increases the difficulty of their application in the paleo-environment reconstruction, especially for the accurate study of C<sub>3</sub>/C<sub>4</sub> vegetation distribution.](#)

Page 6573, Line 18,

‘Secondly, we found that snails discriminate in their choices of [different plant species](#) as food.’

**R1-S11. Page 6569. Lines 20. Actually, Balakrishnan and Yapp (2004) postulated that the atmospheric CO<sub>2</sub> had a minimal effect on the snail carbon isotope budget.**

Response: We thank the referee pointed out this issue. After we recalculating the contributions from each carbon source based on another assumption ([See the discussion in](#)

Page 13 of this document), the estimated values of atmospheric CO<sub>2</sub> decreased 4-6% for each snail individual (Table S1). However, the ignorable contributions are still existent.

As we discussed in R1-S6, we worried the flux model itself involved in the information from atmospheric CO<sub>2</sub>. And in Page 2015 (Balakrishnan and Yapp, 2004), the authors considered that a small effect of atmospheric CO<sub>2</sub> is existent, but there is no estimation of its real contribution. Therefore, the quote of Balakrishnan and Yapp (2004) in our manuscript may confuse the readers, so we will change it to another literature: Romaniello (2008) used combined tools of  $\delta^{13}\text{C}$  values and  $^{14}\text{C}$  values, calculating a contribution of atmospheric CO<sub>2</sub> ranging from 16% to 48% for living land snails.

By the way, besides the direct contribution as gaseous phase, another important pathway of atmospheric CO<sub>2</sub> is from the ingested water (dissolved and isotopic equilibrated CO<sub>2</sub>). The isotope fractionation has no difference between these two pathways. We added this information in Page 6585, Fig. 4, 'Respiration or dissolved into imbibed water'. From these two pathways, it is reasonable that the atmospheric CO<sub>2</sub> can contribute ~15% to the shell carbon. And we added this discussion in the revised manuscript.

Page 6569, Line 20-21,

~~'For instance, Balakrishnan and Yapp (2004) pointed out that pCO<sub>2</sub> ratio between the snail and its environment affects shell incorporation of respired CO<sub>2</sub>,'~~ according to a combination of  $\delta^{13}\text{C}$  and  $^{14}\text{C}$  values measured from modern land snail shells, Romaniello (2008) calculated the contribution of atmospheric CO<sub>2</sub> varying from 16% to 48%.'

Page 6570, Line 5,

'In this study, our calculation results revealed that atmospheric CO<sub>2</sub> can affect shell carbonate isotopic values, and our estimated contributions are, respectively, 16–24% (fed cabbage), 18–20% (fed corn). More clear evidence might be that, for snails growing up without CaCO<sub>3</sub> (Fig. 3), shell  $\delta^{13}\text{C}$  values became heavier than the expected values controlled by one end member (diet). Two pathways can be attributed to this contribution: 1) directly being introduced into the bicarbonate pool in the gaseous phase via respiration; 2) by means of the imbibed water with dissolved and isotopic equilibrated atmospheric CO<sub>2</sub>, while the carbon isotope fraction has no difference between these two pathways.'

R1-S12. Figure 4. Even though snails from cultured experiments here fed upon living plant matter only, in the wild snails feed upon both living and decayed plant matter, and other carbon sources like fungi, moss, etc. Consider that many snails are also omnivorous or carnivorous and consume other snails and arthropods.

Response: We agree. We will revise the words in the 'Food' box in Figure 4. Using the following text 'Food (living plants; other organic matter, e.g., fungi, lichens, etc.)'. And we added the following discussions. By the way, moss is a kind of C<sub>3</sub> plant.

Page 6569, Line 14-15,

~~'this food selectivity of land snails increases the difficulty of their application in the paleo-environment reconstruction, especially for the accurate study of C<sub>3</sub>/C<sub>4</sub> vegetation distribution. Therefore, future studies of food quality's influence on snail shell  $\delta^{13}\text{C}$  values might be helpful.'~~

In addition, although some literatures reported that the fresh plants are the main diet for some land snail species (e.g. Suzuki and Yamashita, 1967; Colonese et al., 2014), it might not be a common way to all the species. According to the literature, land snails can eat decayed plant matter (Richardson, 1975), fungi, animal tissue (Mason, 1970), lichens (Baur, 1994) and other organic matter in nature. The consumed proportions of these food sources vary among land snail species (Mason, 1970).



Consequently, we suggest that the pre-investigation of food preference on living snails is important before applying shell carbon isotopic values in the plaeo-environmental reconstruction of a certain species. And future studies of food quality's influence on snail shell  $\delta^{13}\text{C}$  values might be helpful, too.'

## Referee #2 G. Zanchetta (Referee)

Received and published: 29 May 2014

### General comments

R2-G1. -Section 4.1 for the calculation of the different carbon source the authors used isotopic composition of body tissues, the reason of that becomes clear later in section 4.2.2. I think a link should be found.

Discussion: We agree with the referee that the discussions in Sect 4.2.2 are important for understanding why to choose 'the isotopic composition of snail body tissue', but not food tissue for calculation, so that we use the words, 'see Sect. 4.2.2'. Does the referee mean that we need to use a hyperlink to the section 4.2.2 in the final revised manuscript?

R2-G2. -It seems to me that isotopic composition of atmospheric CO<sub>2</sub> is assumed instead to be measured directly in-situ. This can give some concerns, because in a closed environment respired CO<sub>2</sub> would be important (or other sources) component. This may also occur in nature (e.g. canopy conditions). I think a comment on this is necessary.

Discussion: We thank the two referees point out this important issue and we totally agree that to make a comment or clarification is necessary on this point. In this study, because of the limitation of our experimental apparatus, we didn't monitor the CO<sub>2</sub> concentration and isotopic values in-situ, but used an annual average value observed in the local urban air. The CO<sub>2</sub> from respiration (both snails and food) might more or less affect the composition of the atmospheric CO<sub>2</sub> in the half sealed system, and in further cause a probable overestimation of contribution from atmospheric CO<sub>2</sub>. Balakrishnan and Yapp (2004) predicted a similar condition that under forest canopy, the accumulated CO<sub>2</sub> produced by the respiration would contribute an insignificant variation of shell aragonite than for the case of only ambient CO<sub>2</sub>. However, we still consider that the accurate estimation on atmospheric CO<sub>2</sub> needs other well designed culturing experiments in the future. We will point out and discuss this issue in the revised manuscript. In addition, we think this will not significantly affect our estimation of diet and limestone, as discussed in Page 6566, Line 21-29 and Page 6567, Line 1-5.

Page 6570, Line 6-11.

'However, since we cultured the land snails in the semi-sealed system without monitoring the CO<sub>2</sub> concentration and its isotopic values in-situ, the gradually accumulated CO<sub>2</sub> from respiration of snails and plant tissues might more or less affect the composition and concentration of the ambient CO<sub>2</sub> in the incubator, and in further influence the accuracy of our estimations on atmospheric CO<sub>2</sub>. Although Balakrishnan and Yapp (2004) predicted a similar condition from flux balance model, that the accumulated CO<sub>2</sub> produced by the respiration under forest canopy would contribute an insignificant variation of shell aragonite than for the case of ambient average troposphere CO<sub>2</sub>, we suggest that additional studies based on experiments are needed. Besides, the limited accuracy of our assumption of similar diet/atmospheric CO<sub>2</sub> ratio at the same temperature must be acknowledged because the snails have individual differences of metabolic rates during their growth (Barnhart and McMahon,

1987). Therefore, to learn more accurate contributions of atmospheric CO<sub>2</sub>, further incubation experiments are necessary, which are expected to include several parallel groups with labeled  $\Delta^{14}\text{C}$  or  $\delta^{13}\text{C}$ -different CO<sub>2</sub> compositions, and also to record the concentration and isotopic composition variations of in-situ atmospheric CO<sub>2</sub> during cultivation.'

R2-G3. -Along the manuscript there are interesting points for paleo environmental researchers. I think it should be more stressed the relation found between shell weight and isotopic composition. This could be methodologically important: if in past populations we found differences this may depends on carbonate ingestions and not only depending on metabolic rate (both can depends on environmental stress, too). So methodologically would be important to stress that shells need to be well preserved and it is necessary to weight them.

Discussion: Yes, we found that the differences of land snails in shell carbon isotopic composition may not only depend on C<sub>3</sub>/C<sub>4</sub> plant distribution, but also amount of carbonate ingestion (affected by the availability of local limestone or variations of metabolic rate due to environmental stress). However, here we showed a positive relation between shell weight percentage (equal to dry shell weight divide by the total mass of land snail individual) and shell carbon isotopic composition. And this may be difficult to be applied directly in the paleo-environmental studies, because we could not get the total mass information from the preserved shells. By the way, we have also checked the relation of shell weight and isotopic composition, but the correlation is poor (R square is around 0.34).

R2-G4. -Snail shells usually show a very large variability both in experimental conditions and in nature. Do they authors argue that values dispersion can be used as a measure of environmental stress?

Discussion: Yes, that's what we want to show in Sect. 4.3. The global/ local temperature or other environmental parameters could suddenly change due to some known or unknown event (eg. the Younger Dryas event, ca. 1.3 ka BP), which would result in a large decrease of some species' populations or even cause extinction. Analysis of the carbon isotopic composition in the land snail shell fossils among ages can provide this kind of information. That means, for instance, in this study we find a 5 °C change of temperature will largely affect the carbon isotopic composition in land snail shells because metabolic rates changed significantly by environmental stress. This will be helpful when we combine it with other proxies in the paleo environment study, such as oxygen isotopic composition in shells. We added the discussion in the manuscript, and please check the response to R2-S9.

### Specific comments

R2-S1. Pag. 6557 line 26 laboratory

Response: We revised the 'lab' to 'laboratory'.

R2-S2. Pag. 6559 please can you give the species of the cabbage?

Response: The cabbage we used in this study is belonging to species *Brassica oleracea* var. *capitata*, and the cultivar is green cabbage. We added the information in the manuscript as 'were fed cabbage (*Brassica oleracea* var. *capitata*, green cabbage, C<sub>3</sub> plant,  $\delta^{13}\text{C} = -28.4 \pm 1.2\text{‰}$ ,  $n=12$ )'.

R2-S3. Pag. 6562 lines 15-18. Here are reported preliminary results. Why preliminary? You use only here the t test, why?

Answer:

**\*\*We use the phrase, ‘preliminary results’ here because our sample numbers of snail fed by corn are limited (n=3) compared with those fed by cabbage (n=26), and we hope some further culturing researches related to this point could be done in the future.**

**\*\*T test is to show the significance of the difference on shell weight proportion between two kinds of food sources. For other comparisons in this paragraph, we considered that the readers could easily find the differences, for instance, P6562 Line 11-12, shell weight proportion of snails fed with CaCO<sub>3</sub> vs. those fed without CaCO<sub>3</sub>.**

**R2-S4. Pag. 6566 lines 21-26. In these sentences is not clear when are used “no-carbonate” and “carbonate” conditions.**

Response: We revised the ‘observed under a no-CaCO<sub>3</sub> condition at different temperatures’ to ‘**observed from those snails fed without CaCO<sub>3</sub> at different temperatures**’.

**R2-S5. Pag. 6567 line18 BalakrishananandYapp, 2004.**

Response: Revised as suggested.

**R2-S6. Pag. 6569 lines 12-15. I think it would be very useful to use “natural” examples”: e.g. litter compared to shells and not only experimental conditions. Snails can eat a mixture of organic matter (e.g. fungi), which is difficult to be simulated in laboratory. This is quite different from simulating different condition using C3/C4 food, for the evident (now very clear!) selection of food by snails. In this respect along the discussion would be useful also to quote and consider the recent published paper by Colanese et al., 2014 P3, 394, 119-127.**

Response: We agree this comment and revised the manuscript as shown in R1-S12.

**R2-S7. Pag.6570 line 4. Heaver????**

Response: We revised the word ‘heaver’ to ‘**heavier**’.

**R2-S8. Pag. 6571 Lines 1-5. Be honest not very clear, please specify about Yanes et al., 2011 and make more clear the subject.**

Response: We revised the Page 6571, Line 1-5.

**‘Therefore, we suggest that the variations in metabolic rates attributable to the shift of environmental conditions, which can produce discrepancies of shell carbonate  $\delta^{13}\text{C}$  values, should be take into account in the paleo-environment studies. For example, Yanes et al. (2011) observed 3‰ higher moving average shell  $\delta^{13}\text{C}$  values during the glacial interval (~15 to ~50 ka) than today, and inferred a larger proportion of C<sub>4</sub> plant during that period. However, in this study, we found that shell carbonate  $\delta^{13}\text{C}$  values of land snails, for those fed same diet (cabbage) and carbonate but growing up at different temperatures, can also vary as large as 3.5‰ (Fig. 6a).’**

**R2-S9. Pag. 6571 lines6-12. The sentences are not very clear. For instance why radiocarbon is intended paleo environmental parameter. Please restructure all the sentences in a more simple and clear form.**

Response: The previous discussion is not necessary, and we revised it as, Page 6571, Line 6-12,

~~‘Moreover, these results suggest a lack of a common model for all land snail species. We must calculate the relative contribution of each source to each snail individual. Therefore, the~~

~~metabolic rates among individuals might prevent the reconstruction of the paleo-environment parameters (e.g. radiocarbon dating) precisely in quantitative analysis from shell carbonate  $\delta^{13}\text{C}$  values, but sometimes it might help us understand the environmental conditions better where and when a snail grew up, which should be addressed in a future study.~~ Consequently, the carbon isotopic composition in land snail fossils can be considered as an auxiliary tool to understand changes of paleo-environmental conditions, such as, a suddenly decreased temperature record during the Younger Dryas event.'

R2-S10. Pag. 6572 depletion: please be more specific e.g.  $^{13}\text{C}$ -depletion.

Response: Done, Page 6572, Line 3, 'showed depletion of 2.5‰' was revised to 'showed a  $\delta^{13}\text{C}$  depletion value of 2.5‰'; Page 6572, L7, 'obvious depletion' was revised to 'obvious  $\delta^{13}\text{C}$  depletion'.

R2-S11. Please check carefully the references. Zanchetta et al. 2005 is not reported in the list, some journals are not quoted correctly (e.g. P3 etc).

Response: We added this paper as suggested. For the journals, we quoted as the suggested abbreviated name of these journals (the requirement of Biogeosciences), for example, according to the 'ISI Journal Title Abbreviations Index', 'PALAEOGEOGRAPHY PALAEOCLIMATOLOGY PALAEOECOLOGY' is shorten to 'PALAEOGEOGR PALAEOCL', which was used in our manuscript.

Reference added:

Baldini, L. M., Walker, S. E., Railsback, L. B., Baldini, J. U., and Crowe, D. E.: Isotopic ecology of the modern land snail *Cerion*, San Salvador, Bahamas: Preliminary advances toward establishing a low-latitude island paleoenvironmental proxy, *Palaos*, 22, 174–187, 2007.

Colonese, A. C., Zanchetta, G., Fallick, A. E., Manganelli, G., Lo Cascio, P., Hausmann, N., Baneschi, I. and Regattieri, E.: Oxygen and carbon isotopic composition of modern terrestrial gastropod shells from Lipari Island, Aeolian Archipelago (Sicily), *Palaeogeogr. Palaeoclimatol.*, 394, 119–127, 2014.

Mason, C. F.: Food, feeding rates and assimilation in woodland snails, *Oecologia*, 4, 358–373, 1970.

Baur, A., Baur, B., and Fröberg, L.: Herbivory on calcicolous lichens: different food preferences and growth rates in two co-existing land snails, *Oecologia*, 98, 313–319, 1994.

Richardson, A. M. M.: Food, feeding rates and assimilation in the land snail *Cepaea nemoralis* L., *Oecologia*, 19, 59–70, 1975.

Hatzioannou, M., Eleutheriadis, N., and Lazaridou-Dimitriadou, M.: Food preferences and dietary overlap by terrestrial snails in Logos area (Edessa, Macedonia, Northern Greece), *J. Mollus. Stud.*, 60, 331–341, 1994.

Iglesias, J., & Castillejo, J.: Field observations on feeding of the land snail *Helix aspersa* Müller, *J. Mollus. Stud.*, 65, 411–423, 1999.

Zanchetta, G., Leone, G., Fallick, A. E., and Bonadonna, F. P.: Oxygen isotope composition of living land snail shells: Data from Italy, *Palaeogeogr. Palaeoclimatol.*, 223, 20–33, 2005.

Azuma, M.: Land Snails of Japan, Hoikusha Publishing Co., 1995.

Kohno, M.: Rearing of SIEBOLD's globular snail *Acusta despecta* (SOWERBY) on a semi-synthetic diet (in Japanese), *The Japanese Journal of Malacology*, 35, 50–62, 1976.

Lee, J-S. and Kwon, O-K.: Studies on the intraspecific variations on geological distributions of *Acusta despecta sieboldiana* in Korea, *The Korean Journal of Malacology*, 12, 19–31, 1996.

Okuma, R.: Mating season of two land snails, *Bradybaena similaris* and *Acusta sieboldiana* (in Japanese), Chiribotan, 13, 51–59, 1982.

Sumikawa, S.: The life cycle of *Acusta despecta sieboldiana* PFEIFFER observed in Northern Kyushu (in Japanese), Doubutsugaku Zasshi, 71, 365, 1962.

Suzuki, M. and Yamashita, Y.: On the Food Plants of Siebold's globular snail, *Acusta despecta* Gray (Gastropoda, Mollusca) (in Japanese), Memoirs of the Institute of Sciences and Technology, Meiji University, 6, 211–227, 1967.

Takahashi, K., Asakura, A. and Kurozumi, T.: Copulation frequency and mating system of the land snail *Acusta despecta sieboldiana* (Pfeiffer) (in Japanese), The Japanese Journal of Malacology, 51, 323–326, 1992.

Takeuchi, M. and Tamura, M.: Seasonal occurrence, daily activity and food utilization of three sympatric terrestrial Gastropods (in Japanese), House and Household Insect Pests, 17, 11–23, 1995.

Besides, we would like to revise the following quote:

Page 6565, Line 3, ‘The average value is  $-9.5\text{‰}$  (Zhang et al., in prep.,  $n=42$ , Zhang et al., unpublished data)’, and delete, ‘~~Zhang, N.Z., Yamada, K., and Yoshida, N.: Variations of  $^{13}\text{C}$ ,  $^{18}\text{O}$  and its controlling factors in the atmosphere of Yokohama, Japan based on the observation during 2007–2011, in prep.~~’

Finally, we have been aware of another possibility about one process in our calculation framework which was shown in Fig. 1. We would like to discuss this to the referees here and would like to add some clarifications in the manuscript. In our calculation (Page 6563, Line 16–25), we considered that all of the metabolic  $\text{CO}_2$  is gaseous status when produced and they would equilibrate with dissolved  $\text{CO}_2$  in snail body water, which can involve in a carbon isotopic depletion of 1–2‰ (depends on the temperature). However, another possibility should also be noticed, that the metabolic  $\text{CO}_2$  has already dissolved into body water (no isotopic fractionation) as molecular  $\text{CO}_2$  when produced. In this case, the carbon isotopic fractionation from metabolic  $\text{CO}_2$  to shell aragonite should be 12.4‰, 11.8‰, and 11.2‰, at 20 °C, 25 °C, and 30 °C, respectively. We could not negate either of these two possibilities. Thus we re-calculated the contributions of each carbon sources by the latter assumption and would like to show these results in the supplement (Table S1). The re-calculated  $x$ ,  $y$ ,  $z$  values for snails fed  $\text{C}_3$  plants reveal that the contributions of diet, atmospheric  $\text{CO}_2$ , and ingested limestone varied respectively as 70–85%, 13–19%, and 0–13%. For those fed  $\text{C}_4$  plants, they vary respectively as 64–73%, 15–17%, and 11–22%. Therefore, for snails fed  $\text{C}_3$  plant, the estimation of each carbon source has a shift of  $4.8 \pm 0.3\%$ ,  $-4.6 \pm 0.2\%$ ,  $-0.2 \pm 0.1\%$ , respectively. These differences compared with previous estimation reflect lower contribution of atmospheric  $\text{CO}_2$  and higher contribution of diet, with ignorable influence to the estimation of limestone. At the same time, for those fed  $\text{C}_4$  plant, the discrepancies of two estimations are  $8.2 \pm 0.8\%$ ,  $-2.9 \pm 0.1\%$ ,  $-5.3 \pm 0.7\%$ , respectively. Fortunately, all of these discrepancies from two estimation methods have no influence to the previous discussions in the manuscript, therefore, we don't want to change any discussions, but hope to add the following paragraph to show this possibility.

Page 6567, Line 6–7,

~~‘From the information presented above, we are confident that our calculation method and results are suitable and reasonable.~~ At the same time, we have been aware of another possibility about the carbon isotopic fractionation related to metabolic  $\text{CO}_2$ , which probably has already dissolved into snail body water when produced without any isotopic fractionation generated from gaseous status to aquatic status. In this case, the total carbon isotopic



fractionation from metabolic CO<sub>2</sub> to shell aragonite should be 12.4‰, 11.8‰, and 11.2‰, at 20 °C, 25 °C, and 30 °C, respectively. We have calculated the contributions of each carbon source by this assumption and shown the results in supplement (Table S1). In such circumstance, the contributions of diet, atmospheric CO<sub>2</sub>, and ingested limestone for snails fed C<sub>3</sub> plants varied respectively as 70–85%, 13–19%, and 0–13%, while those fed C<sub>4</sub> plants varied respectively as 64–73%, 15–17%, and 11–22%. This estimation shows lower contribution of atmospheric CO<sub>2</sub> and higher contribution of diet than the previous one. Although we can not eliminate either of these two possibilities with present knowledge, both of them implicate same discussions and conclusions. Consequently, in the following discussions, we will only show the data presented in Table 1.’

## Supplement

Table S1. Stable Isotope Results of Snails Cultured Under Different Conditions and the Estimated Contributions of Their Shell Carbon Sources\*.

Snail No.	Temp. (°C)	CaCO <sub>3</sub>	Diet	Diet $\delta^{13}\text{C}$ (‰)	Shell $\delta^{13}\text{C}$ (‰)	Tissue $\delta^{13}\text{C}$ (‰)	x (%)	y (%)	z(%)	Shell weight proportion (%)
S1	20	+	Cabbage	-28.4	-9.6	-28.4	72.2	16.8	11.0	30.1
S2	20	+	Cabbage	-28.4	-9.1	-28.6	70.4	16.4	13.3	28.3
S9	20	+	Cabbage	-28.4	-9.1	-28.5	70.6	16.4	13.0	29.7
S10	20	+	Cabbage	-28.4	-9.7	-28.8	71.7	16.7	11.6	27.0
S11	20	+	Cabbage	-28.4	-9.7	-27.6	74.3	17.3	8.5	32.7
S12	20	+	Cabbage	-28.4	-9.5	-27.9	73.0	17.0	10.1	25.4
S5	20	+	Cabbage	-28.4	-9.5	-27.7	73.4	17.1	9.5	25.9
S6	20	+	Cabbage	-28.4	-9.8	-27.4	75.0	17.4	7.6	26.4
S13	20	+	Cabbage	-28.4	-9.4	-28.1	72.4	16.8	10.8	28.2
S14	20	+	Cabbage	-28.4	-10.2	-27.7	75.5	17.5	7.0	23.6
S15	20	+	Cabbage	-28.4	-11.0	-26.9	79.7	18.5	1.8	24.8
S3	20	-	Cabbage	-28.4	-13.1	-29.4	79.1	20.9	0.0	4.6
S7	20	-	Cabbage	-28.4	-12.3	-27.7	82.4	17.6	0.0	10.1
S8	20	-	Cabbage	-28.4	-12.3	-27.8	82.0	18.0	0.0	8.8
S16	20	+	Corn	-12.0	4.2	-10.3	67.4	15.7	16.9	34.4
S17	20	+	Corn	-12.0	4.0	-11.6	63.6	14.8	21.6	34.9
S18	20	+	Corn	-12.0	3.3	-10.5	72.5	16.9	10.6	-

S19	25	+	Cabbage	-28.4	-9.7	-27.5	74.5	12.6	12.8	33.1
S21	25	+	Cabbage	-28.4	-11.0	-27.7	77.9	13.2	8.9	31.0
S25	25	+	Cabbage	-28.4	-12.1	-28.7	79.1	13.4	7.5	19.9
S26	25	+	Cabbage	-28.4	-10.1	-27.7	75.4	12.8	11.8	34.5
S29	25	+	Cabbage	-28.4	-10.2	-27.3	76.6	13.0	10.4	37.8
S31	25	+	Cabbage	-28.4	-9.8	-27.0	76.1	12.9	11.0	30.2
S33	25	+	Cabbage	-28.4	-9.9	-26.5	77.6	13.1	9.3	9.7
S22	25	-	Cabbage	-28.4	-14.9	-28.5	89.6	10.4	0.0	4.3
S23	25	-	Cabbage	-28.4	-13.7	-27.5	88.0	12.0	0.0	5.9
S27	25	-	Cabbage	-28.4	-13.6	-28.5	82.5	17.5	0.0	5.1
S28	25	-	Cabbage	-28.4	-13.4	-27.9	84.3	15.7	0.0	4.2
S32	25	-	Cabbage	-28.4	-13.0	-27.7	83.2	16.8	0.0	8.7
S34	30	+	Cabbage	-28.4	-13.7	-28.0	83.3	15.4	1.2	13.8
S35	30	+	Cabbage	-28.4	-13.5	-27.2	84.7	15.7	0.0	18.2
S36	30	+	Cabbage	-28.4	-13.1	-28.7	79.7	14.8	5.5	18.3
S37	30	+	Cabbage	-28.4	-13.3	-28.3	81.2	15.0	3.8	16.2
S38	30	+	Cabbage	-28.4	-13.4	-28.5	81.1	15.0	3.9	14.5
S39	30	+	Cabbage	-28.4	-11.4	-27.3	78.0	14.4	7.6	31.0
S40	30	+	Cabbage	-28.4	-13.8	-29.3	80.4	14.9	4.7	14.4
S41	30	+	Cabbage	-28.4	-13.5	-28.0	82.7	15.3	2.0	17.1
S42	30	-	Cabbage	-28.4	-14.2	-28.2 <sup>d</sup>	84.5	15.5	0.0	-

\*The data were calculated based on the assumption that metabolic CO<sub>2</sub> has already dissolved into snail body water when produced without any isotopic fractionation generated from gaseous status to aquatic status. All of the symbols in this table are same as in Table 1.

Table S2. Mass and Size Information of Each Snail Individual.

Sample	Temperature (°C)	Carbonate	Diet	Total Mass (g)	Dry Tissue (g)	Shell (g)	Length (mm)	Height (mm)	Spiral
S1	20	+	Cabbage	0.60	0.24	0.18	11.4	11.6	4.5
S2	20	+	Cabbage	0.42	0.17	0.12	10.7	9.9	4.5
S9	20	+	Cabbage	0.50	0.20	0.15	10.1	11.3	5.0
S10	20	+	Cabbage	0.48	0.18	0.13	10.3	10.8	4.5
S11	20	+	Cabbage	0.41	0.18	0.13	10.2	10.8	4.5

S12	20	+	Cabbage	0.40	0.15	0.10	10.2	9.8	4.5
S5	20	+	Cabbage	0.79	0.28	0.20	12.6	12.9	5.0
S6	20	+	Cabbage	0.66	0.24	0.18	12.0	11.6	5.0
S13	20	+	Cabbage	0.93	0.37	0.26	14.1	12.7	5.0
S14	20	+	Cabbage	0.78	0.28	0.19	14.9	13.0	5.0
S15	20	+	Cabbage	0.47	0.17	0.12	11.3	11.0	4.5
S3	20	-	Cabbage	0.42	0.09	0.02	-	-	-
S7	20	-	Cabbage	0.39	0.11	0.04	11.1	10.2	4.0
S8	20	-	Cabbage	0.33	0.08	0.03	10.2	9.7	4.0
S16	20	+	Corn	0.24	0.11	0.08	11.0	8.2	4.5
S17	20	+	Corn	0.15	0.07	0.05	7.7	6.5	3.5
S18	20	+	Corn	-	0.05	0.04	7.4	6.4	3.5
S19	25	+	Cabbage	1.39	0.63	0.46	16.4	15.5	5.5
S21	25	+	Cabbage	0.87	0.37	0.27	14.1	13.2	5.0
S25	25	+	Cabbage	1.11	0.39	0.22	16.8	15.0	5.5
S26	25	+	Cabbage	0.69	0.31	0.24	13.1	12.4	5.0
S29	25	+	Cabbage	1.05	0.49	0.40	14.0	14.0	5.0
S31	25	+	Cabbage	1.53	0.62	0.46	17.2	14.9	5.0
S33	25	+	Cabbage	0.93	0.26	0.09	15.0	14.7	5.0
S22	25	-	Cabbage	0.58	0.11	0.03	13.6	11.6	4.5
S23	25	-	Cabbage	0.42	0.08	0.03	11.5	10.8	4.5
S27	25	-	Cabbage	0.67	0.12	0.03	13.1	12.0	-
S28	25	-	Cabbage	0.38	0.06	0.02	-	-	-
S32	25	-	Cabbage	0.71	0.18	0.06	13.4	14.4	4.5
S34	30	+	Cabbage	0.61	0.16	0.08	12.9	12.6	5.5
S35	30	+	Cabbage	0.25	0.07	0.05	10.4	8.8	4.5
S36	30	+	Cabbage	0.25	0.08	0.05	9.7	8.9	4.5
S37	30	+	Cabbage	0.66	0.17	0.11	13.4	11.8	5.0
S38	30	+	Cabbage	0.53	0.15	0.08	12.4	11.0	5.0
S39	30	+	Cabbage	0.77	0.31	0.24	13.8	12.2	5.0
S40	30	+	Cabbage	0.49	0.13	0.07	11.4	10.5	4.5
S41	30	+	Cabbage	0.40	0.11	0.07	10.8	9.7	4.5
S42	30	-	Cabbage	-	-	0.01	-	-	-
S43*	25	+	Cabbage	-	-	-	10.1	9.7	4.5
S44**	-	-	-	-	-	0.29	18.2	17.0	5.5
S45**	-	-	-	0.79	0.20	0.08	15.2	13.0	4.5
S46**	-	-	-	-	-	0.06	13.8	12.5	4.5
S47**	-	-	-	-	-	0.06	13.1	11.8	-
S48**	-	-	-	-	-	0.21	14.8	13.9	5.0
S49**	-	-	-	-	-	0.08	17.0	14.9	-

\*This snail has been identified as an adult individual.

\*\*Parents of the snails from S1 to S43.