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## ***Interactive comment on “Factors controlling shell carbon isotopic composition of land snail *Acusta despecta sieboldiana* estimated from lab culturing experiment” by N. Zhang et al.***

### **Anonymous Referee #1**

Received and published: 16 May 2014

#### General comment:

The present study attempts to quantify the environmental controls upon the carbon isotope composition of land snail shells cultured under laboratory control. Only three previously published studies have monitored the carbon isotope composition of snails from culturing experiment. While previous studies have used the helicid gastropod *Helix aspersa*, the present study uses a gastropod species (*Acusta despecta sieboldiana*) from the family Bradybaenidae, commonly distributed in Asia. Published laboratory studies concluded that the carbon isotope composition of the shell is significantly influenced by the carbon isotope composition of the plant diet, whereas atmospheric CO<sub>2</sub> and carbonate ingestion had a negligible effect. In contrast, the present work con-

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cluded that apart from diet, other carbon sources like CO<sub>2</sub> and limestone, contribute to the shell carbon isotope values. The authors also conclude that the relative contribution of different carbon sources (diet, CO<sub>2</sub> and carbonate) vary significantly between snails that follow either a C<sub>3</sub> or C<sub>4</sub> plant diet. 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.

Specific comments:

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?

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.

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.

Page 6559. I have noticed that relevant information about the lifespan and lifecycle 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.

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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.

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 2 days), 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.

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?

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 C3-fed snails rather than C4-fed snails. Did you mean that snails fed with C4 plants were smaller but thicker?

Page 6567. Lines 20-25. Do you have any supporting references that postulate that C4 plant diet stresses snail growth rates? In nature, snails from the same species that inhabit a C3/C4-CAM mixed habitat would ingest different photosynthetic pathways indiscriminately, in relation to their apparent abundance (Baldini et al., 2007, Yanes et al., 2008, 2013).

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

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precision.

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 C3/C4-CAM mixed systems, some individuals would preferentially ingest C4 plant only, as demonstrated in published field studies (Baldini et al., 2007; Yanes et al., 2008, 2013).

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.

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.

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