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Interactive comment on “Experimental assessment of environmental influences on the stable isotopic composition of *Daphnia pulicaria* and their ephippia” by J. Schilder et al.

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Reply to the Reviewer comment by Anonymous Reviewer #1 (part 1)

We thank the reviewer for taking the time to assess our manuscript. The reviewer raises a number of technical issues regarding our work: Reviewer #1 commented on the relatively small amplitude of the difference in $\delta^{13}\text{C}$ of algae between treatment 1 and 2 in the experiments and indicates that a Bonferroni correction for multiple comparisons may be necessary in our experimental setup. Reviewer #1 also implies that an alternative title may be more appropriate for our study, asks for more details on how biochemical and physiological processes are expected to influence offsets between the

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isotopic composition of *Daphnia* and their ephippia, and asks whether a clone effect is apparent in our results. We will reply to these comments in detail in one of our following replies. We also noted that the reviewer considered our work is suitable for publication in a peer reviewed scientific journal, that the manuscript was well written and presented and that our experimental runs had been performed rigorously.

However, Reviewer #1 also criticized the focus of our work and the way the experimental results are presented. This led the reviewer to question whether the study is suitable for the journal *Biogeosciences*. The reviewer claimed that the main findings of our study, the offsets between the isotopic composition of *Daphnia* and their ephippia, and the influence of the isotopic composition of available food and water on the isotopic composition of these fossilizing structures, addresses only a 'very small community', does 'not connect to biogeosciences' and is not suitable for the journal *Biogeosciences*, since *Biogeosciences* is a 'generalist journal'. The reviewer also stated that 'everything is done to inflate and oversell the real content of the paper' by presenting data that do 'not represent any novelty' to increase the potential readership. We strongly disagree with this assessment and reply to these comments point by point below.

Regarding the suitability of the manuscript for the journal *Biogeosciences*: The main focus of our study is validation of whether changes in the isotopic composition of ambient water (in the case of O-18) or food (in the case of C-13) are recorded in fossilizing structures (ephippia) of *Daphnia*. Furthermore, we examine to what extent offsets exist between the isotopic composition of ephippia and the parent *Daphnia* population (for O-18, C-13, N-15). Information on the difference between the isotopic composition of the entire organism and its fossilizing remains is essential information for any application of isotope analyses on fossilizing structures for palaeoenvironmental reconstruction. Constraining how the stable isotopic composition of fossilizing structures reacts to changes in the isotopic composition of available water and food sources is essential for a correct interpretation of stable isotope measurements on fossil remains of aquatic organisms. We focus on a single invertebrate species. However, there are

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to date only very few other studies available which examine offsets between the stable isotopic composition of organic invertebrate cuticles and the entire organisms, or study how changes in the isotopic composition of water or available food influence these offsets. All of these studies focus on carbon or nitrogen isotopes and insect larvae (Heiri et al. 2012, Frossard et al. 2013), whereas none are available for oxygen isotopes or fossilizing chitinous structures of crustaceans such as Daphnia. Our study therefore presents results that will be relevant for interpreting the stable isotopic composition of chitinous invertebrate remains from a much wider range of aquatic invertebrate groups. We would like to point out that proxy development and validation, the main focus of our manuscript, is a key area of interest listed on the Biogeosciences website (www.biogeosciences.net/general_information/journal_subject_areas.html). As outlined in our manuscript, the results presented in our study form the basis for producing new records of past ecosystem changes, carbon cycling, and climatic changes based on ephippia preserved in lake sediment records. "Past ecosystem functioning" and "Terrestrial record" (consisting to a large extent of lake sediment records) are also listed as key area of interest on the Biogeosciences website. The topic of our study is therefore well within the scope of topics suitable for inclusion in the journal Biogeosciences.

Regarding the issue raised by Reviewer #1 concerning alleged "Over selling" and "inflating of results": We clearly stated in the first few sentences of our abstract and within the first two paragraphs of the introduction that the main focus of our manuscript is on the implications of our experiments for reconstruction of past carbon cycling and food web structure in lakes (C-13, N-15) and of past climatic influences on lake ecosystems (O-18). Similarly, the concluding section of our discussion (section 4.5) focused exclusively on the implications of our study for palaeoenvironmental reconstruction. It is therefore difficult to understand how the reviewer can conclude that we are attempting to inflate and oversell our results. Of course we also discuss isotopic offsets between food and organism ($\Delta^{13}\text{C}$, $\Delta^{15}\text{N}$) in our experiments. This is necessary to interpret the results. Moreover, since we were aware that our experiment was not set up to assess

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isotope fractionation and the biochemical mechanisms behind it, we carefully avoided use of terms such as fractionation, α and ε . While experiments on the isotopic composition of *Daphnia* in relation to that of their food have been performed before (and are acknowledged in our manuscript), the number of published experimental studies is still very low. Furthermore, there have been repeated calls from the isotope community for controlled feeding studies (see e.g. Martínez del Río, 2009) and controlled feeding studies are generally very well cited by the broader isotope community. Our experiment differs in a number of characteristics from earlier studies: We have used a fresh algal food source to rear *Daphnia pulicaria* and measured C, N and O-isotopes, whereas in previous studies frozen algae were offered to a range of *Daphnia* species (including *D. pulicaria*) to investigate N-isotopes (Matthews and Mazumder, 2008), fish food was provided to *D. magna* to investigate C and N isotopes (Power et al., 2003), and fresh algae was given to *D. magna* to investigate N isotopes (Adams and Sterner, 2000). Our work on $\delta^{18}\text{O}$ values of *Daphnia* is entirely novel, as the reviewer indicates. We maintain that our results will also be of interest to readers interested in the isotopic composition of ‘modern’ *Daphnia*, although, as is clearly indicated in the abstract and introduction of our manuscript, the focus of the study is on proxy development and validation. We therefore believe it is appropriate to also mention the relevance of our work for modern studies assessing the isotopic composition of *Daphnia* populations and do not believe this represents ‘overselling’ or ‘inflating of results’.

Regarding the issue of the alleged relevance for a ‘very small community’: It is true that isotope analysis of chitinous invertebrate remains is a developing field and that, at present, there are only few studies published which focus on the stable isotopic composition of fossil *Daphnia ephippia*. However, this argument can be made against any work focusing on the development of new and emerging proxy types. During initial phases of proxy development the respective research fields are always small. Since Biogeosciences specifically welcomes studies focusing on proxy development we do not consider this a valid argument against publication of our manuscript in Biogeosciences. Furthermore, since this is one of the first studies that examines offsets

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between the isotopic composition of aquatic invertebrates and their organic fossilizing structures in an experimental setting, our work presents data which will be of interest to the larger community of researchers within the palaeo-biogeosciences that use fossil-specific isotope analyses for palaeoenvironmental reconstruction based on sediment records. We also note that other experimental studies that, following the reviewer's comments, could be considered to address a specialist audience are regularly published in the journal *Biogeosciences* (e.g. Zhang et al., *Biogeosciences* 11: 5335–5348 on sources of carbon in the shells of a single terrestrial snail species, Hippler et al., *Biogeosciences* 9: 1765–1775 on the mineralization process of crayfish eggs).

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