

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

Anonymous Referee #2

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General Comments- Stratigraphic variability in the isotopic composition of endogenic and biogenic components preserved in lacustrine sediments can provide a valuable insight into palaeoenvironmental conditions. Recently attention has shifted towards the development of proxies capable of recording information of their biochemical heritage. Although, the chitinous remains of aquatic invertebrates are one of the most abundant components preserved in lacustrine sediments, they have received relatively little attention as a tool for inferring past environmental conditions. The adoption of this approach has been hampered by the absence of empirical data exploring the relationship between environmental parameters, the isotopic composition of the remains and the offsets between living organism and fossilising structures.

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In this manuscript the results from a series of controlled laboratory experiments investigating the influence of diet, habitat water and temperature on the isotopic composition of *Daphnia* and their chitinous fossilizing structures are presented. Although the range of variables covered in the investigation are rather limited (from personal experience I appreciate the amount of work that is required to successfully conduct a laboratory calibration study of this nature) this study represents a fledgling step towards improving our understanding of this proxy in the reconstruction of a wide range of past environmental conditions. I believe that this research has been conducted in a rigorous manner and that the findings may be of interest to the palaeo-biogeosciences community therefore support its publication in this journal. However, I also acknowledge that the manuscript may be more accessible to a more relevant readership in a publication specifically aimed at communities interested in palaeoenvironmental reconstructions.

Specific Comments- I think there should be a caveat early on in the manuscript or in the discussion acknowledging, despite their obvious merits, the limitations of laboratory studies (e.g. unable to truly simulate the complex interactions operating in nature).

I found it difficult to differentiate between the open and closed circles in Figure 2. Perhaps one line could be dashed and the other solid?

Methods: The stock water solution was stored at 12°C. Was this water allowed to acclimatise before refreshing in Treatment 4? Do you have any concerns regarding temperature stability with performing replacements twice a week in Treatment 4?

Although the evaluation of the influence of temperature on the stable isotope ratios in chitinous remains is novel, and much needed, it's frustrating that only two temperatures were looked at in this study. One of which, it could be argued, is largely irrelevant in the context of palaeoclimate reconstructions (i.e. 20°C). Was there a specific reason why 12°C and 20°C was chosen as study temperatures? Furthermore, was temperature (either water or air) accurately measured throughout the duration of the experiment? I know from personal experience that maintaining controlled temperatures can very

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difficult, even in supposedly controlled environments. I found that the original temperature controlled cabinets I was using in my experimentation varied by as much as $\pm 5^{\circ}\text{C}$ throughout the duration of a culture!

Results: It is encouraging to note that there is no statistical difference between $\delta^{18}\text{O}$ water in the "cold" and "warm" treatments. From what I could infer from your results it looks like the $\Delta^{18}\text{O}$ between stock and culture waters was pretty small, suggesting minimal evaporative enrichment. Is this a correct inference? I had been considering an elaborate condensing unit to combat the effects of evaporation in my own experimentation but in the end opted for a similar solution to you. However, $\delta^{18}\text{O}$ algae (Figure 2) jumped by approximately 4 per mill from day 10 to 20, any idea what caused this?

Mean isotope values are presented in this section, could you clarify how many samples were measured for each value (n=..).

Discussion: In the discussion section the authors state that the unexpected isotopic differences between similar treatments may represent inherent variability in individuals measured. This argument would certainly be valid in nature but given the controlled laboratory conditions in this investigation I suspect that analytical uncertainties and/or variability in the isotopic composition of diet, to be the primary sources of the unexpected variability observed between the similar treatments.

I think the conclusion that the isotopic composition of eppihia reflect Daphnia is fair but I feel more emphasis should be placed on the fact that relationship between the two appears not be completely straightforward with further laboratory and field based calibration studies required to accurately determine the fractionations involved during the incorporation of environmental isotopic signatures into both the living Daphnia and their fossilizing structures. In particular, given the results presented in this study greater attention must now be paid to the influence of temperature. My own experimentation with chitinous remains also supports the presence of temperature dependant fraction-

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ations, however as with this investigation the magnitude of this influence is similar to analytical uncertainties.

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