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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.

J. Schilder et al.

j.c.schilder@gmail.com

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We thank the reviewer for assessing our manuscript and providing us with comments.

General Comments.

Reviewer 2 comment: 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.

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.

Author reply: We are glad to see that Reviewer 2 recognizes the importance of this manuscript in the context of the development of an emerging proxy.

Reviewer 2 comment: 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.

Author reply: We are glad to see that the reviewer supports publication of our manuscript in Biogeosciences. Biogeosciences is an open access journal which is well indexed and widely read by palaeoecologists and palaeolimnologists. We therefore believe the article will be easy to find also for specialists working in lake sediment records and palaeoenvironmental reconstruction.

Specific Comments.

Reviewer 2 comment: 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).

Author reply: We agree and will add text on this topic in the discussion of the revised manuscript.

Reviewer 2 comment: 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?

Author reply: We thank Reviewer 2 for the suggestion, we will implement this in the revised manuscript.

Reviewer 2 comment: Methods: The stock water solution was stored at 12°C. Was this water allowed to acclimatised before refreshing in Treatment 4? Do you have any concerns regarding temperature stability with performing replacements twice a week in Treatment 4?

Author reply: On the days of water renewal the first step was to filter the water (at room temperature) before other preparations were made. Therefore, the fresh water was kept at room temperature for $\sim$4 hours (and up to 7 hours on days where all treatments were refreshed; the 12°C samples were refreshed first on those days) before refreshing the water in the 20°C treatment. We therefore have no concerns regarding the temperature of the fresh water in Treatment 4. We will provide more information in the revised manuscript on the amount of time the water was allowed to acclimatize before replacement.

Reviewer 2 comment: 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 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°C throughout the duration of a culture!

Author reply: We agree that it would have been useful to examine a range of temperature values instead of two temperatures only. However, due to logistic reasons we had to limit ourselves to two temperature values. Our experiment did not only focus on the effects of temperature on the C, N, and O stable isotopic composition of Daphnia ephippia and on the offset between Daphnia tissue and ephippia. It was also designed to simultaneously examine the effects of variable isotopic composition of food and water on the C and O stable isotopic composition of Daphnia ephippia. As the reviewer indicates the experiments presented in our manuscript already represent a considerable amount of work and measurements. Further expanding the experiment with more treatments was simply not feasible with our available resources. We agree that future investigations with more detailed attention to specific aspects, such as temperature, are needed for Daphnia, but also for other organisms producing chitinous microfossils that are analysed in palaeoecological studies focusing on stable isotopes.

Our main concern regarding temperature was that the difference between the two temperature values should be relatively large (in our experiments 8°C) to ensure that any potential effect on the offset between ephippia and Daphnia δ18O values due to temperature would become apparent. 20°C is on the higher end of temperature values that Daphnia are exposed to in nature. However, we do not agree with the statement that a temperature of 20°C is irrelevant in the context of palaeoclimate reconstruction. The temperature in the epilimnion of lakes in temperate climates regularly exceeds 20°C during late summer and early fall. In shallow unstratified lakes the entire water column may exceed 20°C during this period. Since in many lakes Daphnia ephippia are also produced during late summer and early fall, temperatures of 20°C are therefore not irrelevant in a palaeoclimate context, especially since past climates in many parts of the

world include not just periods of cooler temperature than at present, but also warmer intervals (e.g. in Europe in the early Holocene). Moreover, the D. pulicaria clones used in this study originate from Lower Lake Constance, where temperatures above 20°C in the upper 10 m of the water column are quite common during summer months (see e.g. green reports on www.igkb.org).

In previous experiments, using the same incubators, it was established that the temperature of the culturing waters can be maintained at a stable level. The temperature never deviated more than 1-2°C from the target temperature when lights (i.e. an extra heat source) were used to simulate a diurnal cycle, whereas in our experiments the lights were never on.

Reviewer 2 comment: Results: It is encouraging to note that there is no statistical difference between δ18Owater in the "cold" and "warm" treatments. From what I could infer from your results it looks like the Δ18O between stock and culture waters was pretty small, suggesting minimal evaporative enrichment. Is this a correct inference?

Author reply: Yes, this is correct, the difference was on average 0.6 ‰

Reviewer 2 comment: 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, δ18Oalgae (Figure 2) jumped by approximately 4 per mill from day 10 to 20, any idea what caused this?

Author reply: We'd like to first point out, for clarity, that the algae were not cultured in the lake water we used in the experiment. The stock solution used for this purpose was based on distilled water with added nutrients. The algae indeed show a peak in δ18O values around day 17 of the experiment. We also noticed that a peak is visible in the δ13C values and C:N ratio of the algae around this time, which may be indicative of a peak in the growth rate of the algae in the cultures. It may be that the peak in algae δ18O is related to a peak in oxygen production and/or consumption in the chemostats, although we cannot demonstrate this based on the available data.

Reviewer 2 comment: Mean isotope values are presented in this section, could you clarify how many samples were measured for each value (n=..).

Author reply: The means presented are based on the results of three replicate treatments, and for each replicate treatment three replicate measurements were undertaken. Therefore, every mean represents 9 measurements. We will clarify this in the text of the revised manuscript.

Reviewer 2 comment: 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.

Author reply: The Daphnia were given algae from the same stock in the different treatments for which no differences where expected. However, the period of maximum growth may have differed between treatments. Since the isotopic composition of algae did not remain entirely constant over the duration of the experiment, we therefore agree that the isotopic composition of the diet may have played a role for explaining these unexpected differences. In the submitted version of our manuscript we discuss this on p 2586, line 22-25. In the revised version of our manuscript we will move this section up so that it will immediately follow the statement on the inherent variability of individuals measured.

However, the isotopic composition of Daphnia was measured 9 times for each isotope in each treatment, and the minor observed differences were very consistent within the treatments. We therefore think that the analytical precision is unlikely to be the cause for the observed variability between the treatments.

Reviewer 2 comment: I think the conclusion that the isotopic composition of eppihia reflect Daphina is fair but I feel more emphasis should be placed on the fact that relation-

ship 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 Daphina 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 fractionations, however as with this investigation the magnitude of this influence is similar to analytical uncertainties

Author reply: We agree. Based on the reviewer's comments we will emphasize in the section Implications for palaeoecological studies that further studies in the laboratory and in the field are necessary to determine the fractionations involved during the incorporation of environmental isotopic signatures into Daphnia ephippia, and especially to further constrain the effects of temperature on the isotopic composition of Daphnia and their fossilizing structures.

Interactive comment on Biogeosciences Discuss., 12, 2573, 2015.