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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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We thank the reviewer for examining our manuscript and pointing out areas that could be improved. We replied to several of Reviewer 1's general comments in an earlier response (Biogeosciences Discussions 12, C359-363). Here we provide a point-by-point reply to the remaining comments from Reviewer 1.

General comments.

Reviewer 1 comment: As stated at the end of the introduction, this paper was intended 'to experimentally examine whether offsets in δ13C, δ15N and δ18O values exist between Daphnia and their ephippia'. If this a question of interest for those who are

working on past changes in Daphnia isotope composition using ephippia recovered from sediment archives. This is however a very small community and I do not think that this paper will touch a large readership.

Author Reply: As outlined in detail in our earlier reply (pages C362-C363) we do not agree with this assessment.

Reviewer 1 comment: It does not really connect either to biogeosciences, and, as a matter of fact, there is very few references to any biological or geochemical processes within the whole paper (although it could be relevant to specify the working hypotheses in the introduction, i.e. according to which physiological hypotheses ephippia should exhibit isotope composition that would be differ from those of the whole carapaces/body).

Author Reply: We outlined in detail in our earlier reply (pages C360-361) that the topic of our article fits well within the scope of topics covered and described for the journal Biogeosciences. It is correct that we did not address in detail the biochemical processes that may influence a potential offset in stable isotopic composition between Daphnia and their ephippia. This is because it was not the aim of this manuscript to review these processes. However, we agree with the reviewer that some examples of how the isotopic composition of different tissue types can differ within organisms would help the reader to understand the relevance of our work. In addition to the example of Daphnia exoskeleton versus Daphnia whole body tissue already mentioned in the manuscript, we will also briefly discuss examples of culturing experiments showing offsets between the isotopic composition of whole body and chitinous structures for chironomids and cephalopods.

We note that Reviewer 2 supports publication of our manuscript in Biogeosciences.

Reviewer 1 comment: Besides, the experiment that has been performed is of very limited scale, at a point that unexpected results (such as those obtained for δ13C at 20°C or very different Daphnia δ15N in spite for similar δ15N of the food sources)

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remain hard to explain

Author Reply: As a pioneering study, our experiment was designed to be broad and provide both the first laboratory based assessment of the effects of variable isotopic composition food and water on the C and O stable isotopes of the ephippia, as well as of the potential effects of temperature on C, N, and O stable isotopes of Daphnia ephippia. However, even controlling for the factors we describe in our manuscript required considerable resources and we were only able to investigate two temperature values and conduct experiments with two different δ13C values for algae and two different δ18O values for water. Nevertheless, the experiment allowed us to draw clear conclusions on the isotopic offsets between Daphnia and ephippia, the relevance of lake water δ18O values for determining Daphnia and ephippia δ18O values, and the response in Daphnia and ephippia δ13C values to changes in those of the diet.

The apparent temperature effect on δ13C values was indeed unexpected and could not be explained based on the data we produced, which has led us to conclude that this may be due to e.g. microbial activity or increased algal respiration rates in the cultures. We provide, however, a thorough discussion on what may have been the cause and indicate what can be done in future experiments to further investigate the effect of temperature on δ13C values of Daphnia and ephippia. Our results for N and O stable isotopes appear unaffected by these processes and therefore provide first indications in respect to how δ15N and δ18O values of Daphnia ephippia respond to changing temperatures.

The δ15N results are not unexpected as the reviewer implies: With an isotopically identical food source, all Daphnia had δ15N values within a 1 ‰ range. This range is not larger than reported in similar experiments (Power et al., 2003; Matthews and Mazumder, 2008).

Reviewer 1 comment: Because the range of tested conditions is narrow, the study does not provide any novelty as compared to previous papers on that topic, exception maybe

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for δ18O.

Author Reply: We do not agree with this comment. Our experiments provide the first study assessing oxygen isotopic offsets between Daphnia and environmental water, and carbon, oxygen and nitrogen isotopic offsets between Daphnia tissue and Daphnia ephippia under controlled laboratory conditions, as well as an assessment of how consistent these offsets are under two different temperature conditions, and for different isotopic compositions of food (for C) and lake water (for O). Earlier experiments were constrained to C and N isotopes and did not include any measurements on ephippia. To our knowledge, this represents the first experimental study on the relationship between δ18O values of lake water, body tissue and fossilizing remains for any aquatic or terrestrial invertebrate group that produces chitinous fossils. Our results are therefore relevant for a further development of isotopic analysis on Daphnia remains for environmental reconstruction, but also of wider relevance for the field of invertebrate palaeoecology. We discuss in detail in our previous reply to the reviewer (page C362) how our results differ from earlier studies. The statement that our study does not provide any novelty is, in our opinion, therefore incorrect.

Reviewer 1 comment: Actually, the very annoying point of this paper is that everything is done to inflate and oversell the real content of the paper and the reviewer feels he is getting duped. The title is somewhat catchy, but 'environmental influences' actually refers to (i) test of two δ13C food values, which differ by less than 1.8 per mil, (ii) two temperature conditions, one of which leading to conditions that 'may not affect Daphnia in their natural environment' and (ii) two δ18O water values. Even if the experimental setting was ideal, it would have been only two conditions for each factor, and this would not be enough to be called 'environmental conditions'.

Author Reply: When submitting the manuscript we selected a title that was wide enough to cover all the manipulations we did in our experiments (temperature, isotopic composition of food and water). We suspect this title led the reviewer to expect an investigation of environmental influences on modern Daphnia stable isotope ra-

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tios, whereas our main aim was investigating whether the stable isotopic composition of (fossil) ephippia is indicative of that of (once living) Daphnia, and consequently of changing conditions in the environment the Daphnia lived in. We maintain that we clearly set our aims and goals in the abstract, introduction and discussion (see first reply pages C361-262). We realize, based on the reviewer's comments, that the title may be misleading for some readers. We will therefore change the title to "The stable isotopic composition of Daphnia ephippia reflects changes in δ13C and δ18O values of food and water".

Reviewer 1 comment: It is even more dubious that the experimental design was not perfect. If the point was to test whether food δ13C affect the isotopic offset, we would expect that a much larger range in δ13C values for the food sources. My guess is that much more labelled sodium bicarbonate would have required in the algal growth medium to create such a range of δ13C but this is understandable flaw because this can be usually difficult to anticipate. The experiment had been already conducted by the time that authors realized that labelling was too small to really serve the working hypothesis. In a sense, it is interesting to see that even such a small range of δ13C values is detectable at the level of ephippia isotope composition, but this is not the way this is presented in the paper.

Author Reply: It is correct that we aimed for a larger difference in algal δ13C values. However, as the reviewer also indicates, this is difficult to achieve without extensive pre-testing. Our experiment is "well behaved" in the sense that a relatively minor shift in algal δ13C values (1.8 ‰ leads to a similar shift in Daphnia soft tissue (1.5 to 2.1 ‰ and ephippia (1.5 ‰, whereas offsets between Daphnia and ephippia δ13C values remain minor and not statistically significant at the temperature for which algae with different δ13C values were provided. Our results therefore confirm the expected behaviour of δ13C values of Daphnia and their ephippia to changes in food source δ13C values. This is exactly what the experiment has been set up to investigate. We agree that the small difference in algal δ13C values would have been a problem if we would have

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received unexpected results, e.g. if a manipulation of algal δ13C values would not have led to a corresponding shift in δ13C values of Daphnia and their ephippia. However, this was not the case. We do not claim anywhere in our manuscript that we covered the full range of δ13C values expected for Daphnia in nature.

Reviewer 1 comment: To remediate to the narrowness of the potential readership, authors try to increase the perspective of the experiment by relating to the need for the community of isotope ecologists to quantify trophic fractionation factors (p2577, from l 20). Yet, because the experiment has not been initially designed for such purposes, it does not provide any more information than those that have been specifically conducted some time ago (Impact of temperature by Power, 2003 ; food composition by Matthews and mazumder, 2008).

Author Reply: We strongly disagree with this assessment and replied in detail to this comment in our earlier reply (pages C361-C362).

Reviewer 1 comment: To conclude, authors have targetted a high-level, generalist journal but this experiment, even if everything had worked perfectly, does not have the potential to reach such a readership. Inflating artificially the purpose of the paper is not enough to fool the reader on the actual contribution of the research (may be just enough to upset him/her). This study has been designed for a very specific purpose, and therefore should be published in a very specialized journal. The experiment itself has been performed rigourously, and even though it has a small scale and produced sometimes unexplained results, I am very confident it could be published in the adequate journal (JOPL ?).

Author Reply: We are pleased to see that the reviewer recognizes the strengths of the study. However, we disagree with the reviewer's assessment regarding the target journal. We reply in detail to this comment in reply #1 (pages C360-361).

Specific comments.

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Reviewer 1 comment: Overall, the language is very understandable and the paper is clear. However, I found that the graphical representations of the results (fig 2 & 3) were not legible, and hampered the understanding. Fig 1 is not necessary, the text is clear enough. Fig. 5 also can be removed, as it presents very straightforward results.

Author Reply: We agree that Figure 1 and 5 are not strictly necessary and will remove them in the final revision. We will revise Figures 2 and 3 to make them easier to read.

Reviewer 1 comment: Table 1: Significance detection in multiple paired comparisons requires accounting for Bonferroni's corrections.

Author Reply: We have applied Bonferroni corrections to the analyses presented in Table 1. The results confirm our previous analyses and do not alter our interpretation. In two cases (comparing δ15N values between Treatment 3 and 4 and δ18O values between Treatment 1 and 2) the comparisons are now not significant whereas previously they were marked as significant. However, Treatment 3 and 4 are not discussed in terms of δ15N values and Treatment 1 and 2 not in terms of δ18O values because the δ15N values of the food were the same in Treatment 3 and 4, and the δ18O values of the water were the same in Treatment 1 and 2. We agree that a Bonferroni correction is appropriate, and have applied it to the results shown in Table 1.

Reviewer 1 comment: Three different clones were used and they apparently did not contribute equally to ephippia production. Any clone effect on the isotope results?

Author Reply: We chose to work with three clones because this would: 1) give the experiment more resilience in case of unexpected developments (e.g., if a particular clone would not perform well under the experimental conditions), and 2) to avoid the risk of working with a specific clone that exhibits different offsets between Daphnia and ephippia than most other clones (in case there is indeed a clone effect). Unfortunately, the amount of ephippia produced was just enough to meet the degree of replication we wanted to achieve, and it was not possible to investigate a clone effect. Therefore, we cannot make statements on this matter.

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References.

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Power, M., Guiguer, K. R. R. A., and Barton, D. R.: Effects of temperature on isotopic enrichment in Daphnia magna: implications for aquatic food-web studies, Rapid Commun. Mass Sp., 17, 1619–1625, doi:10.1002/rcm.1094, 2003.

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