Hydrol. Earth Syst. Sci. Discuss.,
https://doi.org/10.5194/hess-2020-490-AC1, 2020

Hydrology and Earth System Sciences
Discussions

Interactive comment on "Environmental DNA simultaneously informs hydrological and biodiversity characterization of an Alpine catchment" *by* Elvira Mächler et al.

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Received and published: 21 December 2020

Thank you for taking the time to read and comment on our manuscript, we appreciate your helpful and constructive feedback. Please find below a point by point response (in *italics*) to your comments. We feel confident that a clarification as response is sufficient to address the comments of the reviewers. These clarifications could be transferred to the manuscript in a more condensed manner, which we would be happy to implement.

Sincerely,

The Authors

Refree 1

This manuscript presents genetic data collected in an Alpine catchment at various hydrologically relevant spatially distributed locations and as a function of time. The idea to link aquatic diversity with hydrologic processes is interesting. Authors present a coherent, innovative, and I think rather labour intensive work, which starts answering questions related to hydrologic connectivity of sources and eDNA diversity.

> Thank you for appreciating our work and acknowledging the novelty of our approach.

I am not an expert in eDNA, but to me, the enormous variability seems an issue. I also see that authors recognize this. My main (minor) problem with the approach followed is that authors seem to lose sight of eDNA mass. Perhaps eDNA diversity weighed for mass could have been beneficial in order to reduce the diversity somewhat, and to focus more on a subset of most important ZOTUs or something like that. I understand that the various steps in determining DNA sequences prevent working quantitative. In the future work section they could perhaps devote some attention to this aspect.

> We see there would be a benefit to have an additional measure of eDNA concentration as an proxy for eDNA mass; Indeed it is currently highly debated whether quantitative measures can be obtained from an eDNA metabarcoding approach due to various biases introduced in the laboratory procedures. To accommodate for this, we equalized the eDNA concentration of each sample before sequencing in order to get similar numbers of reads per eDNA sample, which is a standard practice in this field and likely already leads to a reduced diversity. Based on what we learned with this approach, in a future sampling effort at this site, it might be feasible to target specific sequences known to be tracers for specific processes or sources and assess them quantitatively, but according to our current understanding this would have to happen site-by-site and would not be easily transferable. We would be happy to clarify this opportunity in our outlook for the future.

Two micro issues:

On page 2, Line 31 sodium. I don't get it why sodium all of a sudden is so important here. Usually chloride is more important as this behaves conservative in groundwater.

> Indeed, we should change this wording from sodium to include all ions and minerals that could be in the water and drive electrical conductivity.

Page 3 L18: stream): the opening parenthesis is missing.

> Thank you for pointing out this error. We will remove the parenthesis as it is a relic of an older version of the manuscript.

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