

Interactive comment on “Water tracing with environmental DNA in a high-Alpine catchment” by Elvira Mächler et al.

Anonymous Referee #1

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In this study, the authors proposed to analyse eDNA from water samples to identify water source in an alpine catchment. For this, they collected water at 11 sampling dates (March – August 2017) in 10 stream sites, located in springs, tributaries and along the main channel, as defined by the authors. At each sampling date and site, they also measured temperature, conductivity, and the stable isotopes of water. Their main objective was to identify the spatial variability of water sources within the catchment, characterise the hydrological connectivity of the stream network, and assess their temporal variability based on the three types of variables: environmental conditions (temp., conduc.), isotopes, and eDNA. In particular, the authors aim to compare the capacity of each type of variables to characterize the water sources, and in particular assess whether eDNA could be used as an hydrological tracers in alpine catchments.

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This study is undoubtedly very interesting, original, and timely (with the rise of eDNA approach). Indeed, I am convinced that it is necessary to develop new methods and/or improve existing tracers to better characterize the spatiotemporal variability in water source contribution to stream flow. Even though the sampling performed did not allow developing a new methodology to disentangle water sources in alpine catchments (new hydrological tracers) based on eDNA samples; this ecological survey could provide insights into the use of eDNA as hydrological tracers, and allow identifying potential benefit compared to classical tracers. However, there are many major issues, preventing the publication of this study in this current form. Below, you will find my major concerns. Details comments are provided in the pdf.

First, the manuscript is quite unclear. Terms, such as “habitats”, “hydrological heterogeneity”, “physical environment”, “flow paths”... need to be clearly defined. What do you mean exactly when mentioning them? Hypotheses are lacking. The sampling design is also unclear, and needs to be better detailed, in particular in the methods (e.g. pages 4 and 5, lines 27-28: sampling at each site?, each sampling date...)

Readers became aware you examined fauna communities only in results page 11. You should mention it since the introduction. On the contrary, you mentioned diatoms and bacteria, even though you mainly cited ecological studies examining aquatic invertebrates in glacier-fed streams in that paragraph. Besides, I highly recommend to better characterising / analysing the community composition, instead of only providing OTU richness. You assumed eDNA might be an appropriate hydrological tracers for alpine streams due to the strong relationships between environmental characteristics of the different alpine stream types, but you did not detail these relationships in the intro (which environmental variables, which biological metrics, which species, based on previous ecological analyses). You also neither describe the communities you observed in your eDNA samples, nor compare them among stream types and with previous studies. Before assuming eDNA could be used as an hydrological tracers to disentangle water sources, it would be worth verifying you obtained similar taxa assemblages in the dif-

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ferent water sources compared to previous studies in alpine streams. In addition, you mentioned an increase in richness during snow-melt period and rain events linked to terrestrial eDNA transported from the catchment slope to the stream. You could test it this with your data.

I also have a major concern with the stream clustering. To me the term "tributary" cannot represent a water source, as it could be rain-fed, spring, snow-fed, glacier-fed. . . all small/headwater streams flowing to the main channel are tributaries. In addition, I would separate glacier-fed streams and snow-fed streams. Finally, "main channel" can also not represent a specific water source. Just to be clear, I have no problem with the idea of comparing various types of alpine tributaries (snow-fed, glacier-fed, spring. . .) with the main channel, but it should be presented differently. As you used interchangeably the term 'water source' and 'flow path' throughout the ms, your main message / objective is not very clear. In addition, do not omit the glacier influence in your catchment.

If I understood well, discharge was only recorded at the MR/ER site. Thus, how did you calculate dq/dt for the other stream sites, and assess the impact of temporal variability in qd/dt on eDNA?

I do not understand your time series: dq/dt Do you want to detect high flow events? Assess the flow variability? Why dq/dt was calculated for a period of 48 hours? What does this dq/dt time series mean for you? I am not convinced about the meaning of this ts. Especially, for these alpine streams displaying high temporal variability at the diurnal time scale (snowmelt, ice melt). In addition, according to the timing (middle of the night or middle of the daily glacial or snow-melt flood) of the dq/dt calculation, you will obtain completely different values.

In results, you described seasonal patterns in environmental conditions, stable isotopes, and eDNA metrics, as well as dissimilarities among stream sites. To me, there were often on the contrary no temporal and spatial variability. Anyway, you need to

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perform statistical analyses to test it.

good luck with the revision

Please also note the supplement to this comment:

<https://www.hydrol-earth-syst-sci-discuss.net/hess-2019-551/hess-2019-551-RC1-supplement.pdf>

Interactive comment on Hydrol. Earth Syst. Sci. Discuss., <https://doi.org/10.5194/hess-2019-551>, 2019.

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