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Hydrology and Earth System Sciences
Discussions

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

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> We thank reviewer 1 for the detailed reading and constructive feedback, which will help to sharpen our manuscript. We quote hereafter the original comments (*italic*), followed by our reply (normal font) on how we plan to modify our manuscript.

In this study, the authors proposed to analyze 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,

characterize 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 variable to characterize the water sources, and in particular assess whether eDNA could be used as a hydrological tracer in alpine catchments.

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 spatio-temporal 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...)

> We thank the reviewer for pointing out some unclear terminology. We agree that when bringing two different field of research together, a concise language use is of prime importance. We will define the terms better (e.g., with habitats we mean the stream reach belonging to our corresponding water types) and will pay attention to a consistent use. We also observed that most of the comments in the provided pdf are along these lines. We will carefully implement them and clarify the sampling design according to the annotations in the pdf. In a subsequent answer, we clarify that we understand there to be a number of water types (groundwater, subsurface runoff, and

stream water, which accumulates all upstream water), which we sampled at our 10 points (spring, tributary, main channel). The main differentiating feature between main channel and tributary is the established Strahler order, which we will explain further below. It is important to note that all tributaries and main channel points will include water that flowed from all upstream springs and smaller channels or tributaries. We will go into more detail discussing the implications of these differentiations in a subsequent answer and in the text.

More specifically, we will state the hypothesis more prominently. As stated in the original text, we asked the following three questions (P3, L27): First, does eDNA discriminate between the three sampled water sources? Second, how do changes in physical water flow processes, reflected as hydrologic heterogeneity in space and time, affect the eDNA richness? And third, whether upstream tributaries and springs determine the eDNA signal in the main downstream channel? Correspondingly, we will specify the hypotheses underlying our research: 1) Different alpine water types (groundwater, subsurface runoff, streamflow) carry significantly different eDNA and this difference can be assessed by sampling from select sampling points at different moments throughout the warm season (i.e., outside the winter season, when there is a complete snow cover in the entire catchment). 2) The seasonality of streamflow and subsurface water flow in a high alpine catchment is reflected in the stream network expansion or contraction, which in turn influences the eDNA and accordingly results in different ZOTU richness according to the season. Traditional hydrologic tracers (such as stable isotopes of water and electrical conductivity) can be used to quantify the seasonality of stream network evolution. 3) eDNA of upstream sampling points determines the eDNA of streamflow at the catchment outlet. While eDNA does certainly not behave like a conservative tracer in the stream network (i.e., its value does not remain constant in time while water is flowing downstream due to decay and interactions along the flow path), we still assume that ZOTU richness of upstream locations is partially reflected at downstream network locations. Similarly, water temperature is non-conservative, as it interacts with the atmosphere and the streambed, but some aspects are neverthe-

less preserved (e.g., warm or cool water inflow, seasonal cycles etc.) and it is thus a valuable tracer.

Readers became aware you examined fauna communities only in results page 11. You should mention it since the introduction.

> Thank you for pointing this out. In our revised manuscript, we will mention in the introduction that our study deals with eukaryotic communities.

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.

> We will clarify that there are several studies based on classical ecological sampling methods of invertebrates and a few molecular studies on diatoms and bacteria. We will cite both type of studies accordingly.

Besides, I highly recommend to better characterizing / analyzing the community composition, instead of only providing OTU richness.

> We would need to have taxonomic names assigned to our ZOTUS to further describe our community. Only about 4% of our sequences have a taxonomic assignment to phylum level and about 3% to the genus level, indicating the lack of reference data. Even for well-studied indicator organisms, such as aquatic invertebrates, reference data for about 30% of the species in Europe are missing (Weigand et al., 2019) and this is very likely even higher for understudied areas such as alpine regions. Our analysis is therefore restricted to ZOTU level, which is limiting the comparison of taxa assemblages of classical studies. However, we highlighted, that we found several species in the eDNA samples that were known to be present in that area from previous studies. Indeed, richness is a relatively simple measure, but it is often used as a basic investigation for diversity analysis and thus seemed to be an ideal starting point for a first assessment of eDNA in an alpine catchment. We agree, that further investigations are needed and

therefore we did compare the eDNA samples among the three stream types (original manuscript Fig. 4, NDMS). This analysis is based on species identity (i.e., the dissimilarity of species at the individual sites) and not on richness. We will reformulate the text to clarify why the analysis mainly stays at the richness level and to clarify the comparison of species dissimilarity between the sites.

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).

> In the revised version, we will discuss what is known about the relationship between environmental variables and inhabiting communities. In fact, each water type (subsurface runoff, groundwater, streamflow) harbors to some extent individual and specific community (as shown with classical sampling methods), thus we expect that these community differences are also reflected in the eDNA collected in the different water types, and correspondingly at the sampling points.

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 hydrological tracers to disentangle water sources, it would be worth verifying you obtained similar taxa assemblages in the different water sources compared to previous studies in alpine streams.

> As mentioned above, due to poor taxonomic assignment we cannot compare our data to traditional studies. Further, to our knowledge, no other eDNA study for eukaryotic diversity is currently available for alpine areas, indicating the pioneering character of our study.

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.

> We could indeed investigate if the percentage of terrestrial taxa increased on days of snow melt or rain. However, we can only do this for the 3% of the ZOTUs that have a taxonomic name assigned to it and therefore can be linked to a habitat. In our opinion, it would be highly questionable whether these 290 ZOTU are representative for the whole data set (about 9600 ZOTUs).

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/head water 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.

> As pointed out by the reviewer, our "water type / flow path" terminology was not specific enough. Accordingly, we will use a new, specific terminology in the revised version, where we talk about "water types" (see above) rather than water sources and flow paths. Within this new terminology, we can state that springs are assumed to sample subsurface runoff and groundwater flow from hillslopes, tributaries are first order streams (see hereafter) that sample surface runoff, subsurface runoff and groundwater from the upstream area and the main channel corresponds to a higher order stream that collects all upstream water types. Regarding the terminology "first order stream" and "higher order stream", we will use the well-known Strahler order terminology: UT was classified as 1, TT and ST as 2, and all "main channel" sites as 4. It is also important to note that over the course of 2017, TT and ST were seasonally dry, and correspondingly are labeled as ephemeral by BAFU (2014). UT flowed for the entire season, but it is imaginable that it could be dry as well some years, and BAFU (2014)

has identified it as ephemeral. It is difficult to imagine that any of the "main channel" sites, with the possible exception of UR, would flow seasonally. Even in winter visits, which were not part of the eDNA sampling, main channel sites BR, HR, and ER/MR were always at least partially uncovered and flowing although sometimes inaccessible due to extreme avalanche risk. All tributaries were mainly snow-fed to our knowledge. If there was a contribution of glacier-melt, it was at the UR. All spring sites were places where water emerged from the underground and flowed continuously during the observation period. As shown in the results, there is some clustering according to these types in our data as well. The additional information regarding stream classification and typology has been referenced and will be added in a new, additional table (see Additional Figures and Tables, Table R1) to the text. We have standardized what we are calling "water source" and "water type". We will further specify that UR is the only possible place where glacier melt could enter the main channel.

In addition, do not omit the glacier influence in your catchment.

> We hoped that there would be an influence of the glacier. As we assume that the eDNA from glacier melt would probably have a unique signature, different from the water types. This would have been a unique advantage compared to other tracers. For example, though stable isotopes of water can in some settings show a unique signature for glacier melt water, in particular for glaciers enclosing relatively old water, small, dynamic glacier most likely contain ice that was formed during conditions similar to current snowfall and thus do not have a distinct signature. However, there is no evidence of any significant glacier melt in 2017, and particularly not on days when eDNA was sampled. According to satellite imagery, validated by observation (shown in Fig. 2 in the original manuscript), snow cover did not disappear until after the 199th day of the year and began again before the 239th day of the year. Any existing snow cover during the observation period was essentially over the glacier (due to site exposition). Thus, glacier melt could only be a contributor on the sampling days 199, 207, and 215. Examining the discharge time series, we see that all increases in discharge

during this period corresponded to precipitation events and not to high levels of solar radiation (Fig. 2). This is particularly visible on the 215th day, when there has been no precipitation and near the maximum levels of solar radiation on the preceding days, yet the discharge is very continuous. Furthermore, we would expect water temperature to decline as discharge increases in the case of glacier melt. Looking at plot of water temperature, air temperature, and discharge, we can see that this is not true on these days (see additional attached Fig. R1). If there is a contribution of glacier melt, it is not significant enough to affect the temperature. This result (very little glacier melt) is not surprising nor special for the year 2017. In fact, the small Martinets Glacier in this catchment has survived at a relatively low altitude because it is shaded (by the Dent de Morcles) and debris covered and does thus almost not melt. This is confirmed by data from GLAMOS, the change in size of the Martinets Glacier has been minimal from the last quarter of the last century (GLAMOS, 2018). In the site description, our knowledge and more detailed information regarding the state of the glacier will be added to the manuscript. The text describing the best of our knowledge assessment of glacier contributions to stream flow in the results section will be refined to include our observations of temperature and discharge fluctuations.

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

> Thank you for pointing out this omission. The rate of change of discharge (dq/dt) is a metric that describes a catchment's recession behavior, i.e., how it releases water in absence of precipitation or melt input. Its analysis dates back to fundamental work in hydrology as e.g. the work of Brutsaert and Nieber (1977). In other words, when dq/dt is negative, it describes at what rate water has been stored (in the soil and in the groundwater) is flowing into the stream. In places where we have a dynamic stream network, dq/dt during recessions correlates with the expansion and contraction of the network (Biswal and Marani 2010, and Mutzner et al. 2013). This recession behavior is

an integral measure at the catchment scale that can be assumed to reasonably scale with the (sub-)catchment area (Alexander 1972, and Pilgrim et al. 1982), a property that is namely used in hydrological modelling (Schaefli et al. 2014) (note: to our knowledge there is no formal theoretical explanation for this scaling). Rather than assuming a particular scaling relationship, we assume that dq/dt at the outlet is a good proxy for the recession behavior upstream. This assumption is empirical confirmed in the field, based on our observations while sampling.

During times of increasing flow (dq/dt > 0), things are slightly more complicated since precipitation input results in overland flow (surface runoff), fast subsurface flow and stream network expansion. But we can still assume that flow increase at the downstream outlet is a good proxy for upstream flow increase, in particular for the Vallon de Nant catchment where most significant hydrologic responses have been shown to correspond to precipitation events that cover large parts of the catchment (Michelon et al. 2020) (i.e., are not concentrated on some small subcatchment). We will include the above explanation in the revised version.

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 (snow melt, 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.

> We use dq/dt as a proxy for how the stream network contracts (recession) and expands in combination with overland flow. For recession analysis (the more classical use of dq/dt), the rate of flow change can be calculated in many ways (Santos et al. 2019), the most frequent being the calculation of dq/dt from a streamflow record on a daily time step. We decided to use a two-day window to get a more robust estimate of when we are in a period of increasing streamflow versus a period of decreasing

streamflow. Such a two-day window is namely also less sensitive to streamflow recording noise (Michelon et al. 2020). It also corresponds to the persistence of DNA in the environment, which can last from days to 1-2 weeks. Early in the process, we did explore different windows for this calculation; we provide the correlations between those in Table R2 in the new additional figures and tables (but do not plan to include them in the revised paper). The Fig. S2 in the original supplemental information shows how the value dq/dt can vary according to how it is calculated. We have included a more complete version of this figure (Fig. R2 in new addition) that shows the same information for all sampling days as well as a plot of the first and last calculation for every sampling day (Fig. R3) and a seasonal view along with variations in daily discharge and baseflow (Fig. R4). To avoid any misunderstanding: we compute our dq/dt values from time-average data, averaged with a moving window (i.e., from a streamflow time series that gives streamflow average over two days for each day). We do not compute this value from a series with hourly time steps, in which case the result would depend on the exact timing chosen. Because it is a two-day window, the diurnal cycle will not dominate. Peculiarities could influence the data. We could have adjusted the time to correspond to approximately when the water would arrive at the outlet. The greatest flow distance to the outlet is 4200 m, which means that based on the speed of flow at the outlet on the sampling days, this water would have been at most an hour and a half earlier at the uppermost sampling site.

In results, you described seasonal patterns in environmental conditions, stables isotopes, and eDNA metrics, as well as dissimilarities among stream sites. To me, there was often, on the contrary, no temporal and spatial variability. Anyway, you need to perform statistical analyses to test it.

> We described what we observed in the data. It is very likely that these variables vary seasonally because seasonal physical processes govern them. For example, enrichment and depletion of the stable isotope concentrations in streamflow is mainly determined by contributions of snow and snowmelt versus rainfall. As visible on Fig. 2

in the original manuscript, both the form that precipitation falls as and the contribution of snowmelt is very seasonal. The additionally supplied figures (Fig. R5 and R6) show these relationships more clearly but we considered it too redundant to include them in the manuscript since they show the same information as in Fig. 3 (original manuscript). The new additional Table R1 includes the average values and standard deviations of all variables.

To explore statistical significance, motivated by your comment, we used the Kruskal-Wallis test to compare the mean ranks between the time series of the different water types (R, T, and S). We found significance ($p < 0.05$) in mean rank difference of $\delta^{18}O$ between main channel and spring (R-S) and main channel and tributary (R-T) but not between spring and tributary (S-T). We found significance in mean difference of $\delta^{2}H$ also in those two comparisons, R-S and R-T; in one comparison of lc-ex, R-S; in 2 comparisons of E.C., R-S and R-S; in all comparisons of temperature, R-S, R-T, and S-T; and in all comparisons of rarefied ZOTU richness. Additionally, we performed a Mann-Kendall test to check for significant seasonality ($p<0.05$) in each variable at each site (Fatichi, 2020). We found significant seasonality in 6 sites for $\delta^{18}O$ (HR, HS, MR, TT, UR, UT), 7 sites for $\delta^{2}H$ (BR, ER, HS, MR, TT, UR, UT), 3 sites for lc-ex (BR, BS, MR), 1 site for E.C. (GS), 1 site for rarefied ZOTU richness (ER), and no sites for temperature according to the calendar year. The calendar year season makes sense for trends controlled by processes that peaked prior to our observation period and that didn't begin again until the end of our observations, such as snowmelt. We also cut the year differently, which would be the case for processes that were controlled by something that peaked in the middle of our observation period, such as air temperature. We found significant seasonality additionally for $\delta^{18}O$ (all except ST), $\delta^{2}H$ (all except ST), lc-ex (ER, HR, UT), and E.C. (BR, BS, ER, MR, UR, UT), and rarefied ZOTU richness (GS, HR, HS, UR, UT), but still not for temperature. It would have been impossible to detect seasonality at the ST site samples since it was dry during all but two days of the observation window. Thus, seasonality was widespread, even across our partial year from March to August found for 60% (lc-ex and rarefied ZOTU

richness) or 70% (E.C.) to all ($\delta^{18}O$ and $\delta^{2}H$) of the sites. We did not find significant seasonality in water temperature, but due to technical problems, it was unfortunately only measured starting from the sixth sampling day, July 13th, so "lack of seasonality" just means that for the 6 sampling days spanning from mid-July to late-August, it did not change according to a detectable seasonal pattern. In the text, the word "significantly" has been replaced so that it is only used when a statistical test has been performed.

Further detailed comments (see the pdf attached to the original review):

> We are thankful for the time that the reviewer took in editing our manuscript. They have identified many points in our text that were unclear, poorly explained, and had grammatical or typographic errors. We are happy to make the suggested improvements. However, we still hope to maintain its readability by an interdisciplinary audience.

For example:

P2 L4-14: These are helpful comments regarding how to situate our work in the broad problematic and we will benefit from all of them.

P2 L16: We will refine our simple situation of the problem in hydrology, but we will keep the goal of this sentence somehow for readers with little hydrology training

P3 L32 / P10 L21: Use of the *kryal*, *rhithral*, and *krenal* stream typology seems confusing and inappropriate. We would be happy to remove this and stick to the more appropriate typology as described in our answer to their earlier questions (see above).

P4 L8: We will make it clearer that the glacier influence is insignificant according to our previous answer.

P4 L15: We will add a table with the coordinates of sampling sites, including the one at the gauging station.

P4 L17: We can certainly add the meteorological stations to the map.

P4 L24: We can include the detailed metadata regarding temperature data collection method and even a plot comparing manual and logged measurements though this will be in a future publication. Not all temperatures were available from loggers because some were lost early in the study.

P4 L29: Water was sampled a single time following eDNA collection at each of the 11 sampling points using 12mL amber screw vials.

P5 L5: We can include the metadata of all our samples. Not all the points were accessible year-round and rain was not sampled during the winter. The surface of the glacier was sampled but it is not considered representative and so was not used. The melt from the glacier is not accessible. The snow collection was limited due to high avalanche risk at the site, so it was not as systematic as we planned. All metadata will certainly be include when data is published and in forthcoming publications where this data is more significant. The isotopes of precipitation play a very small role in the current analysis.

P10 L24: We did in fact measure temperature all year and do an informal assessment of clarity at the time of sampling (ranking 0-5). Additionally, suspended sediment in the water column slowed filtering DNA considerably, so we were very aware of its presence. Unfortunately, this information was excluded from this version of the manuscript, which is limited to the data that is relevant to the study at hand. Nevertheless, perhaps we should elaborate that we intensely studied this catchment from 2016-2018, but other work is still forthcoming.

P15 L18 / Fig. 8: We will improve this figure with patterns so that areas are not identified by colors alone, as this was obviously unclear.

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Please also note the supplement to this comment:
https://www.hydrol-earth-syst-sci-discuss.net/hess-2019-551/hess-2019-551-AC1-supplement.pdf

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