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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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Water tracing with environmental DNA in a high-Alpine catchment. Mächler et al. (in review)

General Comments

The motivation for this work was to investigate whether eDNA methods can be used to trace the flow path of water? The authors stated three questions they were pursuing.

1) Does eDNA discriminate between three sampled water sources?

Two methods were directed at this question. Non-metric multidimension scaling of species similarity. Dissimilarity measures fitted to environmental variable in what ap-

peared to be an approach similar to a discriminant analysis or principle components.

2) How do changes in physical water flow process, reflected as hydrologic heterogeneity. . ., affect eDNA composition?

A modelling approach was directed at this question. Species richness (i.e. rarefied of ZOTU counts) derived from eDNA given i) measure of variability in discharge or ii) an index of connectivity of water between surface and hyporheic zones.

3) Whether upstream tributaries and springs determine the eDNA signal in the main downstream channel?

A comparative approach was directed at this question. ZOTUs observed from all collection locations were assigned an origin given where the ZOTU was predominantly observed. Then, the numbers of non-mainstem ZOTUs (i.e. spring or tributary) were counted from within samples taken in the mainstem to estimate the source proportion in the mainstem.

The molecular biology conducted was satisfactory to obtain species communities measures within the collections. The environmental covariates associated with eDNA collections was quite detailed. In my opinion, the field collection configuration compromised using these data to determine water path given the biological communities observed. More detailed comments are provided below, but I would encourage the authors to de-emphasize this particular study's contribution to tracing water path and emphasis the considerable amount of community ecology data available to contemplate how eDNA could inform ecosystem processes. These data are quite useful and should be published, but as a reviewer, there was an expectation mismatch between what was promised and what was delivered. It was quite ambitious to suggest that researchers could peer into water collections from the base of a watershed and infer the mixture proportions (of different upstream habitat types) comprising those samples. While characterizing habitats by their species community (or even defining what an ecosystem might be) is a worthy endeavor, it is quite premature to suggest these

eDNA data may enable tracking a habitat's species community through space. Rather, these data provide an empirical source of information that can be used to hypothesize how community composition could be exploited to enhance knowledge about ecosystem processes.

Comments on Methods

For this review, I focused on the aspects of eDNA evaluation, and assumed that the hardware used to collect environmental covariates was operated correctly and included required quality assurance/quality control procedures.

The authors used standard and acceptable lab practices. The metabarcoding primers (Geller et al. 2013) are a set currently used to evaluate the presence of metazoans, although the portion of COI targeted by Geller et al. (2013) is somewhat arbitrary. The primers appear to work well for invertebrates. The Geller reverse primer has many degenerate base pairs, allowing detection of bacteria, invertebrates, herps, mammals, etc.

The authors' bioinformatics procedures look typical of approaches other labs have taken to metabarcoding. They only instance where the authors made a procedural decision not cited in published literature was the choice of removing any sample with sequence below 20,000 reads. They declared them "failed". This seems reasonable (2.4.2 Data Cleaning, last line in paragraph).

Comment on Results

1) Does eDNA discriminate between three sampled water sources?

Differences in species composition were observed by habitat type. This is useful information. Yet, it is unclear whether the differences were divergent enough to support mixture analysis.

Perhaps this following two examples will prove useful, I don't know. They relate to how I was intellectualizing tracing water. Mixture analysis is a common population genetics

application. Procedure is predicated on sources contributing to an unknown mixture being statistically distinctive (at population level of allele frequencies). Distinction enables a probability to be derived for each observation, with mean likelihood over all observations essentially being the estimated mixture proportion. A second example is decoding movement patterns using otolith microchemistry. If an individual moves through space and time being exposed to different isotopic ratios, these differences are reflected in the otolith. Again, this is predicated on differences in chemistry across space.

In the case here, what is the probability a group of species may be observed in a habitat type? If species (probabilistically) could occur in many or all habitats, then the power to identify a habitat using species is quite ambiguous. There are additional comments below in #3.

2) How do changes in physical water flow process, reflected as hydrologic heterogeneity. . ., affect eDNA composition?

I understand the interest in species composition. Further, the data provide by the authors provides direct evidence that both habitat complexity and connective will increase species richness. Yet, the number of species isn't indicative of a habitat type. Perhaps the exact timing and location of a subset of species could predict habitat type, but these data are not presented.

3) Whether upstream tributaries and springs determine the eDNA signal in the main downstream channel?

In this question is where it all went wrong for me regarding using eDNA to deduce water path.

The species detection distance is undetermined. Pronouncing a sample as "spring" because a species was observed in spring and mainstem, but not tributary, is dubious without accounting for distance (effect size) of DNA detection for metabarcoding given

your lab's procedures. The assumption is that each species has a 100% detection probability below its origination point, which isn't the case.

Habitat types are aggregated. Yet, each site is spatial explicit. For example, were all species from tributary UT observed at River UR, with a predictable reductions at BR, then HR, and subsequently MR/ER. Another example, spring GS is below every collection site expect River MR/ER, spring HS is below half of collection sites and spring BS is below two tributary and two river sites. I my view, aggregating data (i.e. species) by habitat type ignores that geographic location of sites precludes the movement of species across habitat types for many (most) study sites.

To trace water path, species from a habitat type (source) would have to be absent in receiving habitat type, with detection of that species in receiving habitat being indicative of source contribution. Saying there was a shift in species richness is inadequate. Perhaps specific species were diagnostic for richness changes from different sources? This was not presented, but if it exists could form the basis for a subsequent project developing a water path analysis.

Further, was there a seasonal shift in species detected? ZOTUs are a fairly liberal index of diversity. For species noted by name (i.e. unverified species of midge, short-horned grasshopper, alpine salamander, common European adder, ptarmigan, and the chamois) were each of these species present throughout entire study? (Could be any species though). Perhaps natural introduction and removal of eDNA source at (upstream) habitat types could be informative for water contribution from that site downstream to other sites connected by water flow. Again, if species are present throughout different habitat types and elevations, then using those species to identify water source is limited.

Data from this manuscript provides a means to explore these issues further. This is a beginning step in this endeavor and would benefit from adjusting the tone of the manuscript to reflect the infancy of this line of research. Toward the end of the dis-

cussion the authors note the study design did not capture the complexities involved with the overarching objective. While I agree, I would also stress that the spatially explicit information about species diversity will prove useful for the authors and other researchers to further explore ecosystem processes.

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