ID: 423

Evaluation and Optimization of DNA Metabarcoding of Aquatic Invertebrates

Vera Zizka, Bianca Peinert, Matthias Geiger & Florian Leese
University of Duisburg-Essen, Faculty of Biology, Aquatic Ecosystem Research

Background

Morphological identification of indicator organisms for river quality assessment is time consuming, expensive and often only possible at a coarse taxonomic level. DNA metabarcoding offers a potentially cheap, quick and accurate alternative. However, different methods for metabarcoding exist the advantages and disadvantages of which are rarely systematically compared. One question is, if sample tagging introduces a severe bias in the data. A recent study [1] reported that direct tagging via a one-step PCR creates unreliable taxa lists as compared to two-step PCR tagging. Another question is, if community composition can be inferred in a non-destructive way by only using the fixative of the sample [2]. This would potentially also improve the speed of environmental assessment as samples would not need to be sorted.

Aims

1) Compare the performance of three sample tagging methods, i.e. one-step, two-step PCR and ligation-based tagging via the TrueSeq kit (Illumina) using a standard mock community [3]

2) Test if taxa lists obtained through metabarcoding the ethanol of a stream macroinvertebrate sample differ substantially from the bulk sample metabarcoding data.

Results (1)

All three methods identified a high number of taxa in an artificially created, diverse macrozoobenthos community. Highest detection rate was observed with the TrueSeq Kit, which was however not significantly different to the two-step PCR approach (***Fig. 1***).

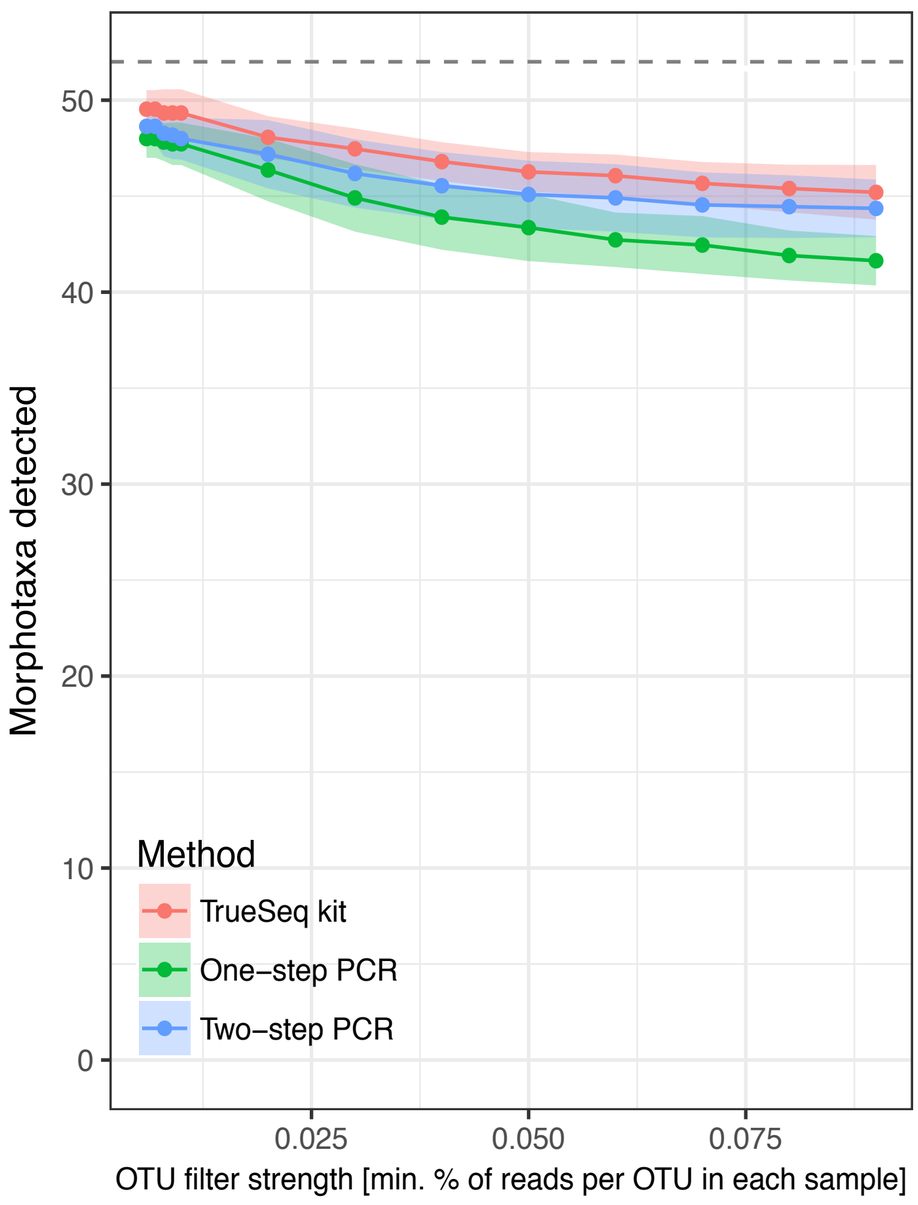

Fig. 1: Detection of morphotaxa depending on the OTU filtering threshold for the three different methods tested.

Results (2)

The greatest proportion of OTUs were identified with both methods (***Fig. 2***). This holds true in particular when only considering the sensitive bioindicator taxa Ephemeroptera, Plecoptera and Trichoptera (***Fig. 2B***). However, total OTU number was always slightly higher in the bulk samples.

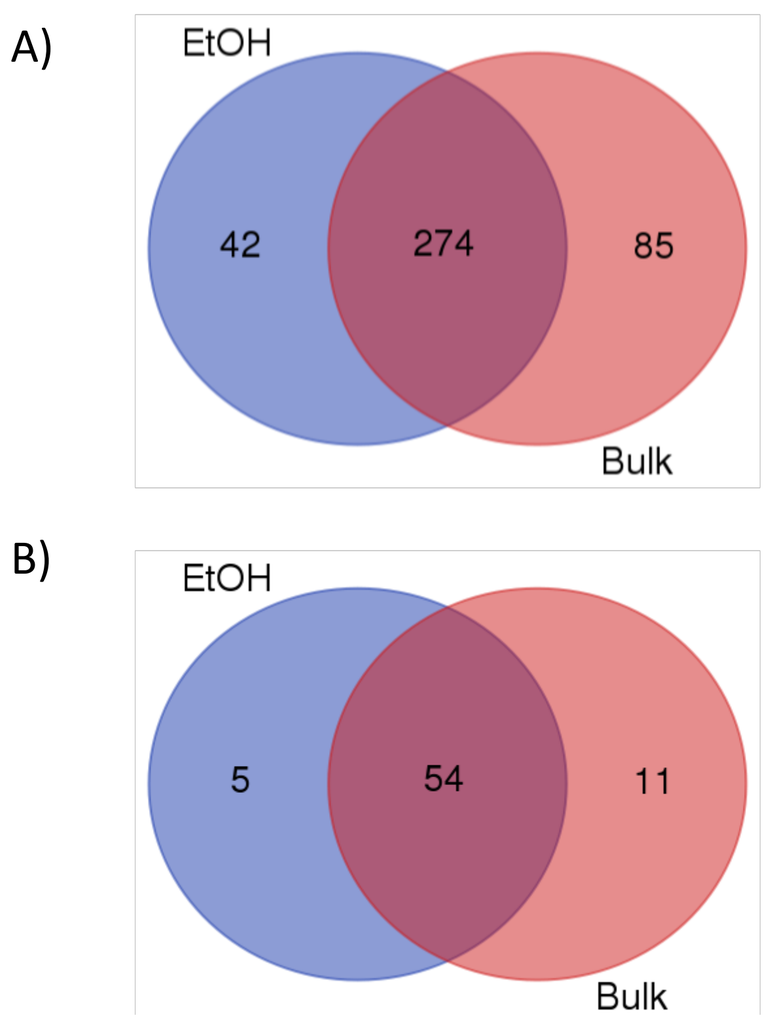

Fig. 2: OTUs identified only in the filtrate (blue) the bulk sample (red) or in both (intersection) for A) when considering the whole sample community, or B) when only comparing the EPT bioindicator taxa.

Conclusions

1) All tested methods showed high consistency and detected a high number of taxa of a diverse macrozoobenthic mock community. Due to its low susceptibility to inhibiting substances and the comparatively low costs and time effort we recommend the two-step PCR that labels samples via fusion primers in a second PCR step.

2) Isolation of DNA from ethanol was successful. Community composition inferred from ethanol metabarcoding was similar to the one inferred through bulk metabarcoding via sample homogenisation. Thus, DNA metabarcoding of only the fixative of bulk samples seems a promising, quick and non-destructive alternative

References
1) **O´Donnell J. L.**, Kelly R. P., Lowell N. C., & Port J. A (2016) Indexed PCR Primers Induce Template-Specific Bioas in Large-Scale DNA Sequencing Studies. PloS One, 11(3), e0148698.
2) **Hajibabaei M**., Spall J. L., Shokralla S., Konynenburg von S (2012) Assessing biodiversity of a freshwater benthic macroinvertebrate community through non-destructive environmental barcoding of DNA from preservative ethanol. BioMed Central, 12:28.
3) **Elbrecht V.**, & Leese F. (2015) Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass-sequence relationships with an innovative metabarcoding protocol. PLoS One, 10(7).

Funding Sources/Acknowledgements