

Conference Abstract

Species sensitivity analysis as a tool for interpreting diatom metabarcoding for WFD bioassessment

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Abstract

Recent metabarcoding work in European rivers, including our own studies of Mediterranean rivers in Catalonia (Pérez-Burillo et al. 2020), has shown promising agreement between morphology- (LM) and DNA-based (HTS) assessments of diatom periphyton for the Water Framework Directive. However, in 10 out of the 164 Catalan sites we analysed, the ecological status class was downgraded from "Good"/ "High" with LM to "Moderate"/ "Poor"/ "Bad"by HTS. We call these the "critical" sites and they are especially important because the WFD requires remedial action to be taken by water managers for any river stretch with Moderate or lower status. In order to discover the reasons for downgrading we investigated the contribution of each species to the Indice de Polluosensibilité Spécifique [Specific Pollution-sensitivity Index, IPS] using a "leave-one-out" sensitivity analysis, paying special attention to the critical sites. Discrepancies in IPS between LM and HTS were mainly due to the misidentification and overlooking in LM of a few species, which were better recovered by HTS. This bias was particularly important in the case of *Fistulifera saprophila*, whose clear underrepresentation in LM was important for

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explaining 8 out of the 10 critical sites and probably reflected destruction of its very weaklysilicified frustules during sample preparation for LM. Another important bias was brought about by differences between species in the *rbcL* copy number per cell, which for example affected the relative abundance obtained by HTS for *Nitzschia inconspicua* and *Ulnaria ulna*; these species were also identified by the sensitivity analysis as important for the WFD. However, blanket application of a correction factor based on cell volume did not lead to a clear improvement in the agreement between HTS and LM assessments. Only minor IPS discrepancies could be attributed to the incompleteness of the reference library, as most of the abundant and influential species (to the IPS) were well represented there. Finally, we propose that leave-one-out analysis is a valuable method for identifying priority species for isolation and barcoding that are currently not, or only poorly, represented in the DNA reference database.

Keywords

diatoms, *rbc*L, DNA metabarcoding, biological indicators, Freshwater ecosystems, Sensitivity analysis, Water Framework Directive

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References

 Pérez-Burillo J, Trobajo R, Vasselon V, Rimet F, Bouchez A, Mann DG, et al. (2020) Evaluation and sensitivity analysis of diatom DNA metabarcoding for WFD bioassessment of Mediterranean rivers. The Science of the total environment 727: 138445. https://doi.org/10.1016/j.scitotenv.2020.138445