Testing repeatability, testing repeatability, testing repeatability: How reproducible are DNA metabarcoding data for marine macrobenthos?

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Macrobenthos is worldwide accepted as a good indicator to evaluate the potential effects of human activities on the marine benthic ecosystems. In traditional environmental impact assessments (EIAs), macrobenthic species identification is based on morphological characteristics, a time-consuming and labor-intensive process for which specific taxonomic knowledge and experts are needed. DNA metabarcoding can circumvent most of these shortcomings. However, to be applicable in EIAs, a standardized protocol that allows for reproducible and reliable DNA metabarcoding results is a prerequisite. We already know that specific changes to the lab protocol, such as the choice of the DNA extraction kit, primer pair or PCR conditions, can influence macrobenthos diversity estimates. In this study, we investigated whether a certain 'fixed' DNA metabarcoding protocol is repeatable across different institutes, an important step to convince stakeholders that this new and quick method generates reliable and comparable results, regardless of who has conducted the work.

Within the international Interreg NSR project GEANS, we developed a ring test where subsamples of 12 bulk macrobenthos samples, originating from four different macrobenthic communities in the Belgian Part of the North Sea (differing in species density and diversity), were distributed to and further processed following the same standardized lab protocol by four different institutes located in Belgium, the Netherlands, Germany and Denmark. DNA was extracted from each subsample by each institute and part of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified and sequenced using Illumina Miseq. The resulting sequences were processed through the DADA2 pipeline to generate amplicon sequence variants (ASVs), and a custom-made macrobenthos DNA reference database was used to assign taxonomy to these ASVs. Both alpha and beta diversity patterns were compared between institutes. The number of ASVs and the number of species reflected the morphological diversity patterns, i.e. highest values for the replicates from the highly diverse macrobenthic community, lowest numbers in the low diversity replicates and intermediate values in the samples from the medium diversity community. These patterns were identical between the four institutes, showing high repeatability for alpha diversity when using the same protocol. In total, 100 macrobenthic species were detected through DNA metabarcoding, of which 60 species were picked up by all four institutes, while the number of species recorded by only one institute, ranged between zero and 14 species for the different institutes. Also beta diversity patterns were comparable between the four institutes, as the nMDS plot clearly showed clustering based on the different macrobenthic communities, independent of the institute that conducted the work.

This ring test shows for the first time that DNA metabarcoding offers a highly repeatable assessment of alpha and beta macrobenthos diversity patterns, which supports the suitability of DNA metabarcoding of marine macrobenthos in monitoring studies. In a next step, we tested the robustness of DNA metabarcoding, by changing some steps in the lab protocol, and by using different bioinformatics pipelines to estimate macrobenthos diversity. Together with the ring test, these results are highly valuable to establish a harmonized and uniform DNA metabarcoding protocol, to be used by all institutions in Europe when implemented as a new standard method in EIAs of the benthic ecosystem.

Keywords: Environmental Impact Assessment; North Sea; Macrobenthos; Metabarcoding; COI; Standardized operational protocol; Harmonization