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Conference Abstract

Testing repeatability, testing repeatability, testing repeatability: harmonization of the DNA metabarcoding protocol for macrobenthos across Europe

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Abstract

Macrobenthos is a good indicator to evaluate the potential effects of human activities on the marine benthic ecosystem. In environmental impact assessments (EIAs), macrobenthic species identification is typically 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 and to be adapted by policy, a standardized protocol that allows for reproducible and reliable DNA metabarcoding results is a prerequisite. Here, three research questions were investigated as part of the international Interreg NSR project GEANS: 1) “How many replicates of DNA extractions and PCR products are needed to capture most of the macrobenthic species in a sample?”, 2) “Is a ‘fixed’ DNA

metabarcoding protocol repeatable across different institutes?" and 3) "What is the impact of small changes in this DNA metabarcoding protocol on alpha diversity?". These are important steps to convince stakeholders that this efficient and quick method generates reliable and comparable results.

First, variation in macrobenthic species across technical replicates was investigated in three biological replicates from three macrobenthic communities in the Belgian Part of the North Sea (BPNS) with high, medium and low diversity. For each biological replicate, six DNA replicates were taken and one of these DNA replicates was used to assess variation between three replicates for PCR amplification. Three DNA replicates were needed in locations with a high and medium diversity to pick up at least 80% of the species diversity present in the six replicates, while four DNA replicates were needed in the location with low diversity. Variation in the detected species between PCR replicates was high, illustrating the importance of including at least three PCR replicates in the lab protocol. Second, we conducted a ring test where subsamples of 12 bulk macrobenthos samples, originating from four different macrobenthic communities in the BPNS (differing in species density and diversity), were distributed to four institutes located in Belgium, the Netherlands, Germany and Denmark. Samples were processed using the same standardized lab protocol and the resulting datasets were processed bioinformatically by one institute. 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 0-14 species were recorded by only one of the four institutes. Beta diversity patterns were also comparable between the four institutes, as the nMDS plot clearly showed clustering based on the macrobenthic communities, independent of the institute that conducted the work. Finally, small changes to the lab protocol (different DNA extraction kit, different high fidelity polymerases for PCR amplification, different reagents for clean-up) resulted in only minor changes in alpha diversity: similar number of species were detected as with the fixed protocol in all samples and 70% - 75% of the species were shared between the 'fixed' and adjusted protocols.

This study shows for the first time that DNA metabarcoding offers a highly repeatable assessment of alpha and beta diversity patterns, which supports the suitability of DNA metabarcoding for monitoring of marine macrobenthos. 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

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