

## Implementation of DNA metabarcoding in environmental impact assessments

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Environmental impact assessments (EIAs) are conducted to investigate whether human activities; like offshore wind energy production or sand extraction; are carried out in a sustainable way. Such assessments evaluate the quality status of the environment by using biotic indices based on macrobenthos communities; which are considered as sensitive impact indicators due to their sessile and sedentary life. Up till now; the characterization of macrobenthic communities is based on morphological species identification and density and biomass measures. This method is time-consuming; labor-intensive and demands specific taxonomic knowledge. Faster and cost-effective identification methods such as DNA metabarcoding; exist; but are not yet implemented in EIAs. The EU funded Interreg North Sea Region project GEANS (Genetic tools for Ecosystem health Assessment in the North Sea region) strives to implement DNA metabarcoding in routine environmental impact monitoring programs. A first aim of the project is to produce a high-quality DNA reference database for macrobenthos species; as many DNA sequences are not yet available; while public databases (still) contain many sequences linked to wrong species names. Every vouchered species in our database is accompanied by a picture of the specimen; the taxonomically identified specimen itself; the extracted DNA sample and the DNA (COI) sequence. The GEANS reference database of the North Sea targets 800 different species; of which 553 species are already collected; with a corresponding sequence for 339 species already available. A second aim of the GEANS project is to standardise genetic monitoring protocols across labs. Currently; different primer pairs and different numbers of DNA and PCR replicates are used between labs. We designed a lab experiment to test five commonly used primer sets for macrobenthos; along with mixtures containing DNA of one to six DNA extractions per bulk macrobenthos sample. We also tested whether PCR replication is needed to obtain an accurate diversity estimate of the bulk samples. The resulting sequence data will be presented. A third aim of the GEANS project is the application of DNA metabarcoding in different pilot studies. A pilot project targeting the impact of sand extraction in the North Sea is performed to compare traditional benthic monitoring (morphological analyses) and genetic monitoring (metabarcoding). Therefore; Van Veen grabs were taken at the Thorntonbank in the Belgian part of the North Sea; covering a gradient in sand extraction intensity from zero (reference locations without extraction) to high intensity (daily extracted). Biotic indices based on traditional morphological macrobenthos data gave a moderate; poor and bad status; respectively for the low; medium and high impact zones. Multivariate analysis further showed distinct groups between the high impact and reference samples; while the group of low impact samples was plotted close to the reference samples; indicating that low intensity of sand extraction only has a limited effect on the macrobenthos community composition. In a following step; we will investigate whether the same patterns can be found based on the DNA metabarcoding. To sum up; the project GEANS can show the added value of DNA based methods in environmental impact assessments.

Keywords: Metabarcoding; Reference sequence database; COI; Macrobenthos; Environmental impact assessments; North Sea