Paving the way for macrobenthic diversity assessment in impacted areas of the Belgian part of the North Sea using metabarcoding

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Human activities such as marine aggregate dredging, construction of wind turbines and dredge disposal induce habitat changes which alter species composition and structure of the seafloor. Macrobenthos is used as an indicator for environmental quality, where species are identified using morphological characteristics. DNA based approaches such as metabarcoding may complement, and speed up the characterisation of these communities. However, the taxonomic resolution of the method is currently hampered by a lack of reliable reference sequence data to which the metabarcode sequences can be compared. In addition, methodological issues such as homogenization of bulk specimens versus eDNA present in the ethanol preservative and the choice of primers for amplification may influence the resulting diversity estimates.

We compiled long-term monitoring data from soft sediment macrobenthic communities in the Belgian part of the North Sea to determine the taxa that are expected to be found in this area. Taxon names were cross validated against the WoRMS database. Next, ethanol preserved specimens were identified, morphologically diagnostic characters photographed and specimens stored as reference material for DNA sequencing. Partial or whole specimens were subjected to DNA extraction and COI Sanger sequences have been generated. In total, 334 species belonging to nine phyla were identified in soft sediments of the BPNS over the last 15 years. The classes Polychaeta, Malacostraca and Bivalvia are the most species rich and harbour 40%, 37% and 13% of all species, respectively. At present, 90 species are in the reference database, which reflect the expected phyla and classes. Collection of additional species is an ongoing effort. The reference specimens provide a taxonomic link of metabarcode sequence data which is indispensable when using metabarcoding for the assessment of environmental quality and health.

In addition to the generation of the reference database, we collected three replicate grap samples from three impacted and one control area in the Belgian part of the North Sea. Species composition in these areas is well known. Specimens from one replicate per area were morphologically identified before homogenization and DNA extraction. We optimized the amplicon library preparation for both the ethanol fixative and the bulk specimens using two primersets for the mitochondrial COI gene and one primer set for the ribosomal 18S rRNA gene. Metabarcoding data of mock communities, field samples and the ethanol fixative will be compared with morphological identification of the communities to verify the applicability of the method for monitoring.

Keywords: reference database; macrobenthos; voucher specimens; DNA barcoding; metabarcoding