## **ABSTRACT**

## DNA BARCODING OF BENTHIC SPECIES FROM THE BELGIAN PART OF THE NORTH SEA FOR DIVERSITY IMPACT ASSESSMENTS

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Species living in or near the seafloor fulfill a variety of ecosystem functions. Understanding how benthic communities respond to human activities is crucial to determine how such activities impact the functioning of the benthic environment. In the Belgian part of the North Sea, ILVO is extensively monitoring different benthic ecosystem components, and explores new ways to characterise the structural and functional benthic diversity. DNA-based approaches such as metabarcoding may complement and speed up the characterisation of changes noted in these communities during impact assessments. Linking sequences to taxonomic data is indispensable when information on ecosystem functioning is the key concern. However, the taxonomic resolution of metabarcoding is still hampered by a lack of reliable reference sequence data to which the metabarcode sequences can be compared. We compiled longterm monitoring data from soft sediment macrobenthic, epibenthic and pelagic fish communities in the Belgian part of the North Sea. For macrobenthos, 334 species belonging to nine phyla have been registered in the past 15 years. The classes Polychaeta, Malacostraca and Bivalvia are the most species rich and include 40%, 37% and 13% of all species, respectively. ILVO aims to barcode as many benthic species as possible to validate and - when proven to be reliable - to implement DNA-based methods for biodiversity impact assessments. To this end, ethanol preserved voucher specimens are morphologically identified, photographed and stored as reference material for DNA sequencing. Partial or whole specimens are subjected to DNA extraction and COI Sanger sequencing. At present, the genetic reference database contains 95 macrobenthic species, representing the aforementioned classes and phyla. In addition, 85 epibenthic species and demersal/benthopelagic fish have been barcoded. Collection and sequencing of additional species is an ongoing effort. Next to DNA barcoding, we also explore new library preparation methods for high throughput sequencing of benthic communities by using different COI primer sets and primer free approaches such as long read 18S rRNA sequencing, on mock communities, ethanol fixatives and field samples. These methods will be compared to morphologically identified samples to assess whether comparable patterns in species and functional diversity patterns in impacted versus non-impacted sediments are found by the different approaches.