DNA-based monitoring in the North Sea region: The future for environmental benthic monitoring?

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Human activities such as marine aggregate extraction, construction of wind turbines and dredge deposition induce habitat changes that alter both abiotic and biotic components of the seafloor ecosystem. Macrobenthos is used as an essential indicator for environmental quality, but up till now based on morphological species identification. DNA-based approaches such as metabarcoding may speed up or at least complement the characterisation of the different macrobenthos communities. However, diversity assessment by means of metabarcoding strongly depends on the chosen primers, the bioinformatic pipeline and the availability of reliable reference sequence data. Furthermore, standard routine application and comparison across areas is hampered due to differences in sampling strategies, laboratory protocols and data analyses used in different countries. To accommodate this, the EU funded Interreg NSR project GEANS (Genetic tools for Ecosystem health Assessment in the North Sea region), aims to harmonize and consolidate existing genetic tools and methods across the North Sea to improve and enhance genetic monitoring.

The objective of this study is to compare DNA-based versus traditional morphological species identification for macrobenthic communities. To that end, the set-up of a reliable genetic reference library for the Belgian part of the North Sea (BPNS) is needed. In the last 15 years, ILVO researchers have listed 334 macrobenthic species from nine phyla for the BPNS. Polychaeta, Malacostraca and Bivalvia are typically the most species rich classes and harbour 40%, 37% and 13% of all species, respectively. Ethanol preserved specimens have been morphologically identified, diagnostic characters photographed and stored as reference material for DNA-based identification. To date, the ILVO genetic reference database contains 101 vouchered macrobenthos species and 119 epibenthos and demersal fish species. Completing the reference database on a North Sea scale, i.e. collecting and sequencing the remaining species is one of the main goals within the GEANS project.

To further evaluate whether DNA-based approaches describe the macrobenthic diversity in a comparable way as the morphology-based approach, and to highlight the added value of both approaches, we applied both methodologies on field samples from the four prevailing habitat types in the BPNS, each characterized by a different level of diversity and macrobenthic species community. Three replicate Van Veen grabs were collected in each of the four habitats. One replicate per habitat was processed according to standard monitoring protocols, resulting in the morphological identification of 45 families. All replicates were further used for genetic analyses, using DNA from both the bulk samples and from the ethanol fixative of the sample. We compared the performance of four primer sets (respectively amplifying 420, 580, 510 and 350bp of the COI barcode region) in terms of macrobenthic taxon detection. Despite substantial differences in the total number of amplicon sequence variants (ASVs) generated by each primerset (2139, 22151, 14813, 15211, respectively), the number of detected families was comparable to the morphology-based analysis (64, 51, 56 or 43, respectively). However, nearly 50% of the families were not similar between both methods (27, 26, 27 or 22, respectively). This can be explained by: 1) the fact that not all replicates were morphologically identified (4 samples), leading to a underestimation compared to the DNA-based analyses (12 samples); 2)

some families detected through genetic analyses are normally not accounted for as 'macrobenthos' in morphological assessments, and should be omitted when comparing both methods; and 3) the genetic (COI) reference database is far from complete: combining our own generated barcode sequences with those available in the public Barcode Of Life Database (BOLD) showed that so far only 33 out of the 45 morphologically identified families have been sequenced and voucher referenced. Our results further showed substantial differences in taxonomic diversity depending on the primer set used, and on the source of DNA (bulk versus ethanol). The results contribute to a thorough evaluation of the pros and cons of DNA-based identification of macrobenthic communities in relation to human impacts.