

eDNA metabarcoding reveals distinct fish and invertebrate diversity patterns in the shallow and well-mixed Belgian part of the North Sea

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Recently, environmental DNA (eDNA) metabarcoding is gaining success as a novel tool for biomonitoring marine ecosystems. In deeper (> 50 m) and calmer marine environments eDNA metabarcoding can detect spatial patterns in fish community structures. However, the knowledge about the use of eDNA metabarcoding to determine spatial patterns in well mixed shallow waters, such as the Belgian Part of the North Sea (BPNS), is still scarce. Long-term epibenthos and fish monitoring data using morphology-based identification have shown the existence of clear coastal and offshore communities in the BPNS, and at smaller spatial scale secondary effects of offshore windfarms (OWFs) could be detected as well. These were more pronounced for C-Power than for Belwind when comparing locations inside and outside the OWF.

As part of the ZEROimpact project, we investigated whether eDNA metabarcoding would reveal similar diversity patterns at these two spatial scales in the BPNS: between the coastal and offshore area on the one hand and between locations within and outside the OWFs C-power and Belwind on the other hand. Water samples were taken in 12 coastal (x 3 biological replicates) and 18 offshore (x 5 biological replicates) locations in autumn 2021. Two markers were targeted to characterize species level diversity: the mitochondrial 12S ribosomal DNA marker to assess fish diversity and the cytochrome c oxidase I (COI) marker to assess invertebrate diversity. For both markers, the species richness between the coastal and offshore waters did not differ significantly ($p > 0.1$), but the species community composition did ($p < 0.001$). This was, for both markers, linked to a difference in the relative read abundance of the species in the two habitats. In the 12S (fish) data, the offshore samples were dominated by two species, *Merlangius merlangus* and *Limanda limanda*, whereas the relative read abundances in the coastal waters were more evenly distributed among the different fish species. For COI, some marine invertebrates that were common in the coastal samples were less frequently detected offshore and other species that were common in the offshore samples were less frequently detected in the coastal samples. At a smaller spatial distance (< 10 km), eDNA metabarcoding could capture differences in the species community compositions as well. In the OWF area, the fish and invertebrate community assemblage differed between both OWFs ($p < 0.002$). Furthermore, species community assemblage inside C-power differed significantly from those in the reference areas ($p < 0.02$), while no such difference was observed for Belwind ($p > 0.05$). These patterns broadly correspond with the ones observed in long-term morphology-based data.

Limitations of eDNA metabarcoding were the low sequencing depth for COI due to the amplification of non-target Chromista, and the lack of taxonomic resolution to species level for 16 fishes from six distinct families. Nevertheless, eDNA metabarcoding with the 12S and COI marker was able to detect more species than the morphology-based method.

Keywords

eDNA metabarcoding; Fish and Marine Invertebrate Community Monitoring; Offshore Windfarms; Belgian Part of the North Sea