# eDNA metabarcoding reveals horizontal and vertical patterns of the fish communities in a shallow and well-mixed marine environment

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While human-induced stressors such as climate change, habitat disruption and overfishing continue to pose a substantial threat to the health of our oceans, monitoring efforts became critically essential for the sustainable management and conservation of marine environments. EU marine policy centered on the Marine Strategy Framework Directive (MSFD) highlights the importance of establishing monitoring and assessment activities for achieving the goals of maintaining biodiversity and good environmental status. Traditionally, biodiversity assessment has relied on methods such as catch-based surveys and visual surveillance. Catch-based surveys, in particular, have detrimental effects on benthic habitats leading to mortality of the benthos, physical disturbance of the seabed, and changes in biogeochemical characteristics. Additionally, identification of the species by these techniques generally requires taxonomic expertise and can be time consuming. In contrast to the conventional methods, environmental DNA (eDNA) metabarcoding emerged as a non-invasive and cost- effective approach for assessing biodiversity that utilizes DNA extracted from the environmental samples. eDNA metabarcoding substantially contributed to the monitoring efforts by detecting elusive and low abundance species that would otherwise go unnoticed.

eDNA metabarcoding has also been proven effective to describe the horizontal spatial patterns of the fish communities with a fine-scale resolution, ranging from a few meters to a few kilometers. However, research on the vertical eDNA profile has been predominantly focused on stratified open ocean waters or regions with a distinct halocline, where vertical eDNA dispersal is limited. The studies about the vertical eDNA signatures in shallow and well-mixed areas are very scarce. To understand the spatial fish eDNA patterns and how these patterns correspond to traditional beam trawl data in such hydrodynamically active areas, we collected water samples from 17 stations within the Belgian Part of the North Sea (BPNS). The samples covered both surface and bottom depths across three zones (coastal, transition and offshore) associated with distinct fish communities established through long-term trawl monitoring.

Our results showed that eDNA was able to detect the majority of the fishes found in the catch data (81%) and detected 23 additional species. While a few species persisted in being exclusively identified through trawl catch data, eDNA metabarcoding significantly contributed to the detection of the species-level taxonomic diversity by 53%. The species richness and Shannon diversity index were not significantly different between the two depths ($p$-value > 0.05), but showed significant differences between the zones ($p$-value < 0.0001). Both alpha diversity measurements were significantly lower for the coastal zone compared to the other zones. Community composition was not significantly affected by the depth factor (pseudo F= 1.3, p=0.25). Zone, on the other hand, had a significant effect on fish community composition (pseudo-F= 32.19, p=0.0001). Post hoc analysis showed that communities of coastal, transition and offshore zones were significantly different from each other ($p$-value = 0.0001).

These results demonstrate the effectiveness of eDNA metabarcoding in reconstructing the fish community patterns that are closely aligned with the long-term beam trawl data within the BPNS. Moreover, we showed that shallow and well-mixed waters of the BPNS lack distinct vertical eDNA patterns corresponding to the depth preferences of the fish species. Sampling at different depths has no impact on the alpha and beta diversity assessment within the area, suggesting that sampling at one depth should be adequate to capture the majority of fish diversity. Our study represents one of the first efforts to elucidate the vertical eDNA profiles in shallow and well-mixed marine environments.

**Keywords**
Monitoring; EDNA Metabarcoding; Beam Trawling; Belgian Part Of The North Sea; Spatial EDNA Patterns; Fish Communities