Accurate long-read eDNA metabarcoding of North Sea fish using Oxford Nanopore sequencing

Karlijn Doorenspleet‡, Lara Jansen‡, Saskia Oosterbroek‡, Reindert Nijland‡
‡ Wageningen University, Wageningen, Netherlands

Corresponding author: Karlijn Doorenspleet (karlijn.doortje@hotmail.com)
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Abstract

To halt North Sea ecosystem degradation, accurate and intensive monitoring of the North Sea ecosystem and its fish is vital to correctly inform management decisions. DNA based techniques and especially the use of environmental (e)DNA from seawater can become a powerful monitoring tool. However, current eDNA based metabarcoding approaches are based on genetic target regions of <500 nucleotides which offers only limited taxonomic resolution.

We tested sensitivity and applicability for field samples of newly designed universal fish primer targeting a 2kb region covering mitochondrial 12S and 16S genes in eDNA samples. Samples were processed using long read nanopore sequencing in combination with the consensus builder Decona and retrieved accurate read identities of up to 99.9%. To test accuracy of the primer, eDNA was analyzed from a tropical aquarium with a known species composition of bony fish and elasmobranchs. This showed that over 50% of species present can be identified. The majority of remaining reads are identified as -in aquarium present- genera and can be explained by an incomplete reference database for the fish present in the aquarium. Primers were also applied in North sea eDNA field samples. Distinct species compositions between different locations could be observed and consisted of ecological relevant species and shows the applicability for long-read eDNA metabarcoding in field studies.
Incomplete reference databases currently form the main bottleneck to further develop high resolution nanopore based long read sequencing as metabarcoding strategy. Nevertheless, this study shows that long read nanopore sequencing of eDNA can be used to obtain accurate information on the fish and elasmobranch species composition in the North Sea and beyond.

Keywords

environmental DNA, eDNA, long-read metabarcoding, nanopore sequencing, fish, elasmobranch, North Sea

Presenting author

Karlijn Doorenspleet

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