Chasing Tuna: Using Nanopore sequencing of environmental DNA to assess fish biodiversity in the North Sea

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Using environmental DNA (eDNA) analysis to monitor fish biodiversity in the North Sea

The North Sea ecosystem is degraded. Compared to a century ago, the higher trophic levels are missing. Large predators such as bluefin tuna, halibut, sharks and rays used to be common, and are now rare or absent.

Construction of many large offshore wind farms are planned in the North Sea. Wind farms will be closed for fishing, and can play a positive role in restoring the North Sea food web. Biodiversity monitoring in wind farms is essentials to measure their effects on the North Sea ecosystem.

Monitoring supports artificial reef design

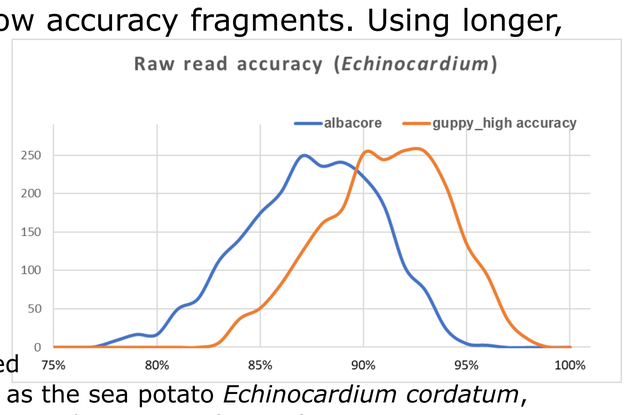
We can evaluate vertebrate biodiversity through nanopore eDNA sequencing 'in the field' at the North Sea. This enables biodiversity assessment of a diversity of reef structures in the North Sea, supporting optimal design of artificial structures such as wind farm foundations and scour protection. When managed properly, these optimal designs can assist in recovery of the North Sea ecosystem.

Scuba diver monitoring artificial reefs at protected area in the North Sea (Borkumse stenen).

At this site we also collected water samples for eDNA analysis on board of the diving vessel.

Raw read accuracy of 85% identity is sufficient for (most) species classification

Low accuracy, medium length nanopore reads (650nt) are sufficient to identify most fish to species level. Some species, e.g. flounder/plaice or cod/whiting/poor cod, are genetically very similar and cannot be distinguished based on the 650nt, low accuracy fragments. Using longer, 2kb fragments mitigates this problem. Re-basecalling of older fast5 data improves raw read accuracy, but, surprisingly, does not improve species level classifications.

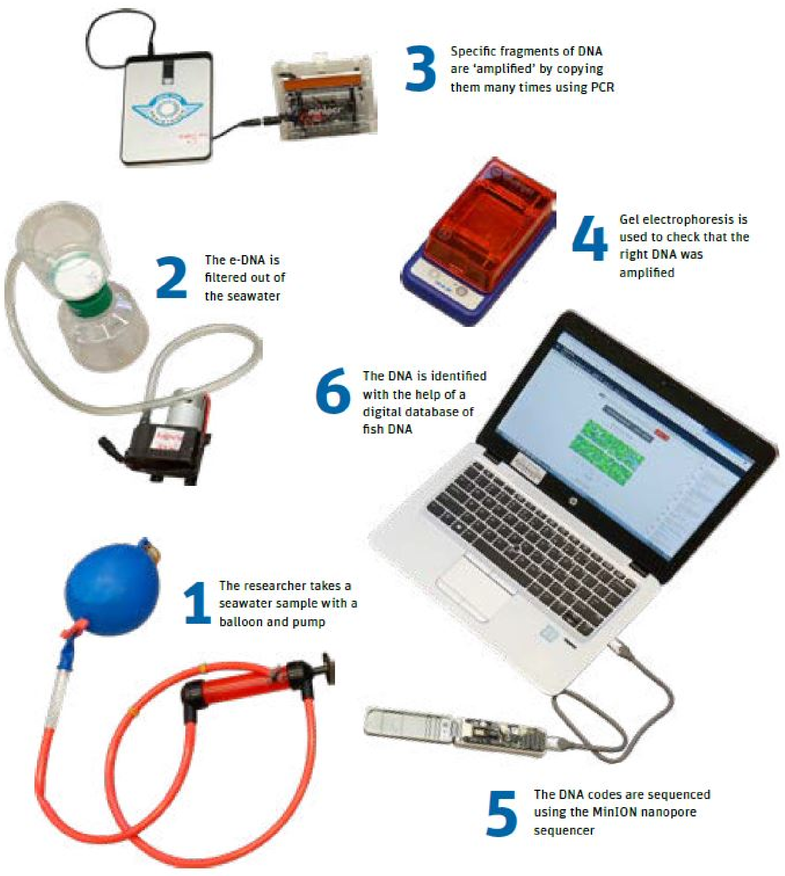


Raw read identity of sequences obtained during eDNA metabarcoding, classified as the sea potato *Echinocardium cordatum*, basecalled with either Albacore or Guppy High Accuracy (v3.0.1)

Mobile eDNA sequencing workflow

Above:
Field kit packed in a backpack for mobility

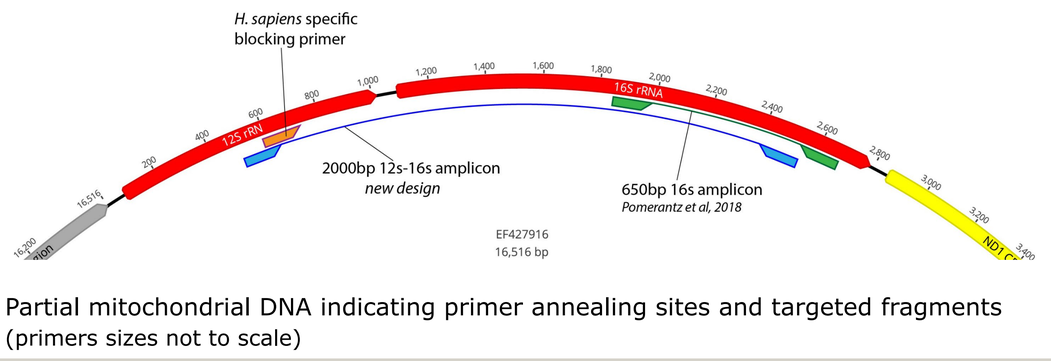
Right:
fieldkit equipment and workflow. Currently the laptop+ MinION Mk1B (5) has been replaced by a Mk1C



eDNA metabarcoding consensus read accuracy: >99%

Read polishing: We now use a workflow where raw reads are clustered followed by consensus generation and polishing using Racon en Medaka. This results in eDNA metabarcoding consensus read accuracy of >99% (based on database references), which allows differentiation to species level and often also identifies separate haplotypes.

To improve identification of closely related species, we designed eDNA primers amplifying a 2kb fragment of 12s and 16s mitochondrial DNA, optimized for fish, and able to detect all vertebrates, including marine mammals (below). These longer fragments allow better classification resolution to species level already using the raw reads directly.



Partial mitochondrial DNA indicating primer annealing sites and targeted fragments (primers sizes not to scale)

Outlook: temporal resolution in eDNA?

- Nanopore sequencing allows us to monitor biodiversity through eDNA sequencing 'in the field' *and* in the laboratory. This will supporting optimal eco-friendly design of artificial structures such as wind farms. When managed properly, these optimal designs can assist in recovery the of the North Sea ecosystem.

- Using the MinION platform we are not limited by DNA fragment size. As eDNA rapidly degrades, using short and long DNA fragments could allow for the addition of a temporal resolution to eDNA monitoring

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Acknowledgements

We like to thank "Stichting Duik de Noordzee schoon" for enabling the sample collection. Part of this work was supported by the NWO Domain Applied and Engineering Sciences under Grant 14494; the Nederlandse Aardolie Maatschappij BV, Wintershall Holding GmbH, Energiebeheer Nederland B.V and Neptune Energy